TURUN YLIOPISTON JULKAISUJA
ANNALES UNIVERSITATIS TURKUENSIS

SARJA - SER. D OSA - TOM. 944

MEDICA - ODONTOLOGICA

EARLY NUTRITIONAL DETERMINANTS IN CARDIO-METABOLIC PROGRAMMING

A Prospective Randomized Controlled Dietary Intervention Study

by

Jonna Aaltonen

TURUN YLIOPISTO
UNIVERSITY OF TURKU
Turku 2010

From the Department of Pediatrics, University of Turku and Turku University Hospital, and Functional Foods Forum, University of Turku, Turku, Finland

Supervised by

Doctor Tiina Ojala, MD, PhD
Hospital for Children and Adolescents,
University of Helsinki,
Helsinki, Finland

Professor Erika Isolauri, MD, PhD
Department of Pediatrics
University of Turku,
Turku, Finland

Reviewed by

Professor Johan Eriksson, MD, PhD
Department of General Practice and Primary Health Care
University of Helsinki, Helsinki, Finland

Adjunct Professor Ursula Schwab, PhD
Department of Clinical Nutrition
University of Eastern Finland, Kuopio, Finland

Dissertation opponent

Adjunct Professor Mikael Fogelholm, Sc.D.
Health Research Unit
Academy of Finland, Helsinki, Finland

ISBN 978-951-29-4507-8 (PRINT)
ISBN 978-951-29-4508-5 (PDF)
ISSN 0355-9483
Painosalama Oy – Turku, Finland 2010

ABSTRACT

Jonna Aaltonen

Early nutritional determinants in cardio-metabolic programming – A prospective randomized controlled dietary intervention study

Department of Pediatrics and Functional Foods Forum, University of Turku, Finland. Annales Universitatis Turkuensis, Medica-Odontologica, Turku, 2010.

Western societies have been faced with the fact that overweight, impaired glucose regulation and elevated blood pressure are already prevalent in pediatric populations. This will inevitably mean an increase in later manifestations of cardio-metabolic diseases. The dilemma has been suggested to stem from fetal life and it is surmised that the early nutritional environment plays an important role in the process called programming. The aim of the present study was to characterize early nutritional determinants associating with cardio-metabolic risk factors in fetuses, infants and children. Further, the study was designated to establish whether dietary counseling initiated in early pregnancy can modify this cascade.

Healthy mother-child pairs (n=256) participating in a dietary intervention study were followed from early pregnancy to childhood. The intervention included detailed dietary counseling by a nutritionist targeting saturated fat intake in excess of recommendations and fiber consumption below recommendations. Cardio-metabolic programming was studied by characterizing the offspring's cardio-metabolic risk factors such as over-activation of the autonomic nervous system, elevated blood pressure and adverse metabolic status (e.g. serum high split proinsulin concentration). Fetal cardiac sympathovagal activation was measured during labor. Postnatally, children's blood pressure was measured at six-month and four-year follow-up visits. Further, infants' metabolic status was assessed by means of growth and serum biomarkers (32-33 split proinsulin, leptin and adiponectin) at the age of six months.

This study proved that fetal cardiac sympathovagal activity was positively associated with maternal pre-pregnancy body mass index indicating adverse cardio-metabolic programming in the offspring. Further, a reduced risk of high split proinsulin in infancy and lower blood pressure in childhood were found in those offspring whose mothers' weight gain and amount and type of fats in the diet during pregnancy were as recommended. Of note, maternal dietary counseling from early pregnancy onwards could ameliorate the offspring's metabolic status by reducing the risk of high split proinsulin concentration, although it had no effect on the other cardio-metabolic markers in the offspring. At postnatal period breastfeeding proved to entail benefits in cardio-metabolic programming. Finally, the recommended dietary protein and total fat content in the child's diet were important nutritional determinants reducing blood pressure at the age of four years.

The intrauterine and immediate postnatal period comprise a window of opportunity for interventions aiming to reduce the risk of cardio-metabolic disorders and brings the prospect of achieving health benefits over one generation.

Keywords: blood pressure, breastfeeding, cardiac sympathovagal activation, dietary counseling, glucose metabolism, growth, nutrition, pregnancy, programming

TIIVISTELMÄ

Jonna Aaltonen

Varhaisen ravitsemusympäristön yhteys sydän- ja verenkiertoelimistön ohjelmoitumiseen – Satunnaistettu ja kontrolloitu ravitsemusinterventioseurantatutkimus

Lastentautioppi ja Funktionaalisten elintarvikkeiden kehittämiskeskus, Turun yliopisto. Annales Universitatis Turkuensis, Medica-Odontologica, Turku, 2010.

Länsimaissa lasten ylipaino, heikentynyt sokerinsieto ja kohonnut verenpaine ovat yleistyviä trendejä, jotka tulevat vääjäämättä lisäämään sydän- ja verisuonitautien myöhempää ilmenemistä. Ongelman on esitetty juontuvan sikiökaudelta, ja varhaisen ravitsemusympäristön on epäilty vaikuttavan tähän niin kutsuttuun ohjelmoitumisprosessiin. Tämän tutkimuksen tavoitteena oli selvittää tarkemmin varhaisen ravitsemusympäristön ja -tekijöiden yhteyksiä sikiö-, imeväis- ja lapsuusiän sydän- ja verisuonitautien riskitekijöihin. Lisäksi tutkimuksella pyrittiin selvittämään, voidaanko varhaisraskaudessa aloitetulla ravitsemusneuvonnalla vaikuttaa näiden riskitekijöiden kehittymiseen.

Tutkimuksessa seurattiin 256 tervettä ravitsemusinterventiotutkimukseen osallistuvaa äiti-lapsiparia alkuraskaudesta leikki-ikään saakka. Interventio sisälsi yksityiskohtaista ravitsemusneuvontaa, joka kohdistui suositukset ylittävään tyydyttyneiden rasvojen sekä suosituksia alhaisempaan kuitujen määrään ruokavaliossa. Sydän- ja verisuonitautien ohjelmoitumista tutkittiin jälkeläisiltä mittaamalla varhaisia riskitekijöitä kuten autonomisen hermoston yliaktiivisuutta, kohonnutta verenpainetta sekä epäsuotuisaa aineenvaihdunnallista tilaa (esimerkiksi seerumin korkeaa split proinsuliini tasoa). Sikiön sydämen autonomisen hermoston aktiivisuutta tutkittiin synnytyksen yhteydessä. Syntymän jälkeen lapsilta mitattiin verenpaine sekä kuuden kuukauden että neljän vuoden seurantakäynneillä. Lisäksi imeväisten aineenvaihdunnallista tilaa arvioitiin kuuden kuukauden iässä kasvun ja seerumista mitattavien markkereiden avulla (split proinsuliini, leptiini ja adiponektiini).

Tutkimuksessa todettiin sikiön sydämen autonomisen hermotoiminnan aktiivisuuden olevan suorassa yhteydessä äidin raskautta edeltävään painoindeksiin, mikä viittaa jälkeläisen epäedulliseen sydän- ja verenkiertoelimistön ohjelmoitumiseen äidin painoindeksin noustessa. Korkean split proinsuliinin riski imeväisiässä oli pienempi ja verenpaine neljän vuoden iässä matalampi lapsilla, joiden äidin raskaudenaikainen painonnousu ja ruokavalion rasvojen laatu sekä määrä olivat suosituksien mukaista. Huomionarvoista on, että äidille varhaisraskaudessa aloitetulla ravitsemusneuvonnalla pystyttiin parantamaan lapsen aineenvaihdunnallista tilaa vähentämällä seerumin korkean split proinsuliinitason riskiä. Neuvonnalla ei kuitenkaan ollut vaikutusta muihin varhaisiin sydän- ja verenkiertoelimistön sairauksien riskitekijöihin. Syntymän jälkeen lapsen sydän- ja verisuonitautien ohjelmoitumisprosessiin voitiin vaikuttaa suotuisasti imettämällä lasta. Lisäksi lapsen oman ruokavalion ravitsemussuositusten mukaiset proteiini- ja rasvasisällöt varhaislapsuudessa olivat tärkeitä verenpainetta laskevia tekijöitä.

Sydän- ja verisuonitautien riskitekijöiden kehitystä voidaan ehkäistä vaikuttamalla sikiö-, imeväis- ja lapsuusiän ravitsemusympäristöön. Tämän kriittisen ajanjakson luomia mahdollisuuksia tulisi terveysvalistuksessa jatkossa hyödyntää kattavammin.

Avainsanat: imetys, kasvu, ohjelmoituminen, raskaus, ravitsemus, ravitsemusneuvonta, sokeriaineenvaihdunta, sydämen autonomisen hermoston aktiivisuus, verenpaine

TABLE OF CONTENTS

ABBREVIATIONS

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BMI	Body mass index
BSA	Body surface area
CI	Confidence interval
DIPP	Type 1 Diabetes Prediction and Prevention project
DOHaD	Developmental Origins of Health and Disease
ECG	Electrocardiogram
E%	% of the total energy intake
GDM	Gestational diabetes mellitus
HR	Heart rate
LF/HF	Low-to-high frequency ratio of fetal HR variability
MUFA	Monounsaturated fatty acid
NAMI	Nutrition, Allergy, Mucosal immunology and Intestinal microbiota project
NNR	Nordic Nutritional Recommendations
OR	Odds ratio
PUFA	Polyunsaturated fatty acid
Q	Quartile
SD	Standard deviation
SFA	Saturated fatty acid
T	Tertile
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following original publications, referred to in the text by the Roman numerals I-IV. Some previously unpublished data are also presented.

I Ojala T, Aaltonen J, Siira S, Jalonen J, Ekholm E, Ekblad U, Laitinen K. Fetal cardiac sympathetic activation is linked with maternal body mass index. Early Hum Dev 2009 Sep;85(9):557-60.

II Aaltonen J, Ojala T, Laitinen K, Poussa T, Ozanne S, Isolauri E. Impact of maternal diet during pregnancy on infant metabolic programming: a prospective randomized controlled study. Eur J Clin Nutr Epub 2010;doi:10.1038/ejcn.2010.225.

III Aaltonen J, Ojala T, Laitinen K, Piirainen T, Poussa T, Isolauri E. Evidence of infant blood pressure programming by maternal nutrition during pregnancy: a prospective randomized controlled intervention study. J Pediatr 2008;152:79-84.

IV Aaltonen J, Laitinen K, Isolauri E, Poussa T, Ilmonen J, Ojala T. Impact of intrauterine and postnatal nutritional determinants on blood pressure in early childhood**.** Submitted

1. INTRODUCTION

Industrialized countries worldwide are experiencing an epidemic of overweight and obesity, elevated blood pressure and impaired glucose regulation. Alarmingly these manifestations are already seen in pediatric populations (Kaufman 2002, Hedley et al. 2004, Muntner et al. 2004) and tend to pass on from childhood to adulthood (Bao et al. 1996, Vogels et al. 2006), which augments further concern for later health and the need for more efficient means of prevention.

The predicament in these adverse cardio-metabolic conditions is linked to the increased availability of food and a decrease in physical activity, i.e. to consequences of changed lifestyle and demographic patterns during the past decades. Additionally, it has been surmised that the risk factors involved may originate in fetal life and early infancy. Indeed, evidence from epidemiological and experimental studies suggests that maternal nutrition and nutritional status during pregnancy can program or imprint the developing fetus and influence later body size, performance and health, and also cardio-metabolic programming (Barker 2004). This statement, however, contains a number of important definitions and unresolved aspects comprising an integral part of the present thesis, as listed below:

Programming – The process whereby a stimulus or insult at a critical period of development has lasting or lifelong effects (Lucas 1991).

Critical period or critical window – In the critical period of development the tissues and organs of the body undergo rapid cell division. If this process slows, for example as a direct consequence of undernutrition or through altered concentrations of growth factors or hormones, it may permanently reduce the number of cells in that particular organ (Widdowson and McCance 1975).

The International Life Sciences Institute North American Branch has recently defined the critical window as "sensitive time periods of development where diet and other environmental influences induce lasting effects on physiology, function, health and disease risks". The windows occur as a developmental continuum which includes gestation, infancy and early childhood; it runs from placental implantation and embryonic cell differentiation through to five years of age (Field 2009).

Barker's hypothesis – According to Barker's hypothesis, or the developmental origins of health and disease (DOHaD) hypothesis, undernutrition in utero and during infancy can permanently change, i.e. program the organ structure and function, and thus lead to diseases later in life. Low weight or small body size at birth and delayed growth in infancy have been used as manifestations of undernutrition and predictors of higher subsequent disease risk (Barker 1990, 1994 and 2007).

Catch-up growth – Epidemiological studies have more recently suggested that not only poor growth during intrauterine life but rapid growth in early childhood is more influential for later disease risk (Eriksson et al. 1999). Additionally, the accelerated postnatal growth proves to be especially detrimental for later health after stunted intrauterine growth, which is mostly compensatory after a period of nutritional deficiency (Metcalfe and Monaghan 2001). Such newborns can be small for gestational age and predisposed to catch-up growth, a rapid growth during the first two years of life, which plays a significant role in the risk of developing diseases (Ibanez et al 2006, Vaag 2009).

Thrifty phenotype hypothesis – The thrifty phenotype hypothesis was proposed as an explanation for epidemiological findings linking reduced fetal growth to impaired glucose metabolism in adulthood, but it also explains the long-term adverse effects of exaggerated postnatal growth on later health. The thrifty offspring phenotype is adapted to survive in poor nutritional conditions as poor fetal nutrition has altered its organ structure and function. Supranormal nutrition in later life, i.e. mismatch between fetal and later nutrition, will then have adverse consequences such as type 2 diabetes (Hales and Barker 1992).

The theories focusing on programming are important for an understanding of the impact of the early nutritional environment on later health. However, the validity of the hypotheses among well-nourished women is debatable. Indeed, many previous studies have concentrated on an undernourished environment, while in developed societies overnutrition, a form of malnutrition, is a more frequent problem also among pregnant and lactating women. Further, previous studies have mostly evaluated either intrauterine or postnatal periods, whereas studies encompassing both periods allowing dissection of the effects of these separate nutritional environments, are scant. To improve our understanding of cardio-metabolic programming this insufficiently investigated area calls for further studies conducted in more a comprehensive manner during the critical windows of development.

2. REVIEW OF THE LITERATURE

2.1. Cardio-metabolic programming – theoretical background

It was long suspected that the mother may suffer on behalf of the fetus and that the fetus takes everything needed for growth and development. This is not however the case. As far back as the 1960s Geoffrey Rose noted an increased risk of ischemic heart disease in individuals who had siblings who were stillborn or had died in early infancy (Rose 1964). The conception that adverse early environment may increase susceptibility to later diseases, and that coronary heart disease risk is related to intrauterine and postnatal environment arose from studies by Barker and Osmond in the 1980s. They observed geographical relationships between high neonatal mortality and high coronary heart disease mortality about 50 years later in specific areas in the United Kingdom (Barker and Osmond 1986). These findings suggest that the same factors which affected the health of the newborn were also reflected in the coronary heart disease risk.

According to the original Barker's hypothesis (Barker 1990) undernutrition in utero and during infancy leads to increased susceptibility to chronic diseases later in life (**Figure 1**). Further nutrient deficiency in particular stages of gestation has varying impact on fetal growth and different birth outcomes result in different morbidities (Barker 1994). Human and animal studies suggest that chronic nutritional deprivation sustained from early pregnancy induces symmetrical growth disturbance and susceptibility to neurodevelopmental impairment in the subject. An inadequate nutritional environment in mid- or late pregnancy causes asymmetrical or disproportionate fetal growth, seen in larger head circumference compared to abdominal circumference. Among the consequences of undernutrition in later stages of pregnancy are disturbed glucose-insulin and lipid metabolisms, elevated blood pressure and inadequate immune function.

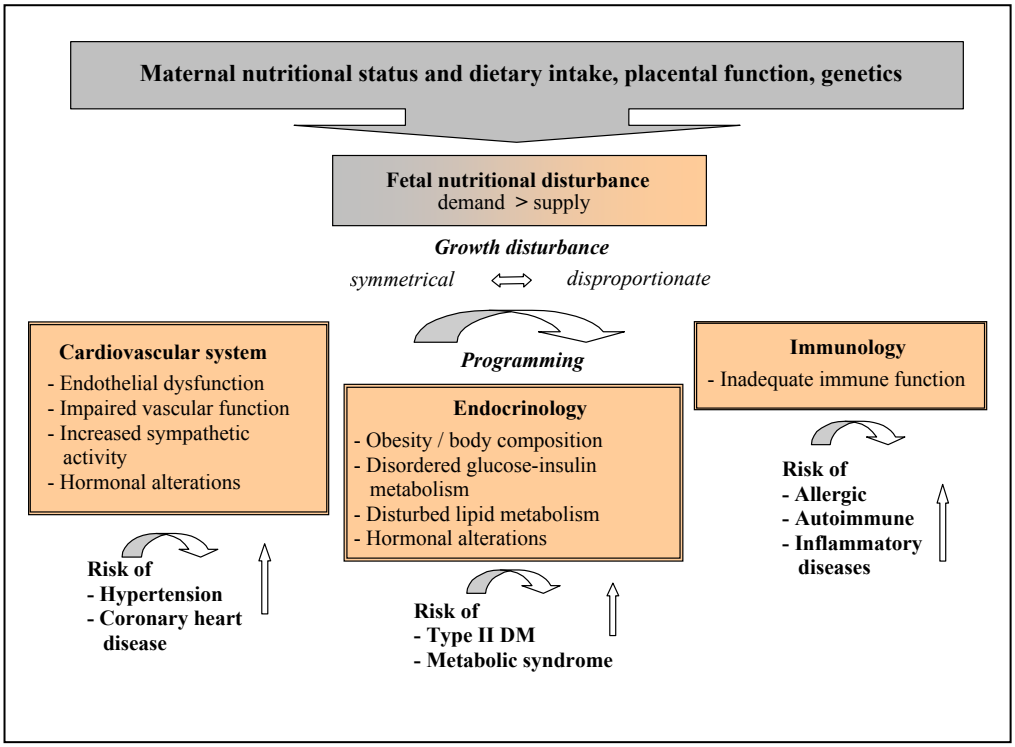


Figure 1. Factors associated with cardio-metabolic programming

The nutritional programming theory described by Alan Lucas (1991) explained the physiological mechanisms underlying the hypothesis: under conditions of poor intrauterine or postnatal nutrition the blood flow and nutrients are redistributed to the most important organs such as the brain, while the energy uptake of other organs is reduced. This programs organ structure and function and has lasting or lifelong effects on the control of tissue physiology and homeostasis.

Results from the Helsinki Birth Cohort Study have since emphasized the significance of rapid childhood growth, especially after stunted intrauterine growth, as a risk factor for subsequent cardio-metabolic diseases (Eriksson et al. 1999 and 2007, Forsen et al. 2000), and the thrifty phenotype hypothesis serves as an explanation for the link between low birth weight, obesity and impaired glucose metabolism in adulthood (Hales et al 1991, Hales and Barker 1992 and 2001). “Thrifty phenotype” was related to poor fetal nutrition altering the organ function of the offspring. The fetus had adapted to living in a poor nutritional environment, while supranormal nutrition in later life was followed by adverse metabolic consequences such as type 2 diabetes. The effects of programming may thus be vital for the survival of some organ at the particular moment but profound and irreversible maladaption may result in detriment to organ function or longevity.

2.2. Early life determinants of cardio-metabolic programming

2.2.1. Maternal metabolic status

Barker's hypothesis describes the wide range of consequences of maternal undernourishment (section 2.1.), but genetic factors and maternal constraints (Gluckman and Hanson 2004a), for example small body size and placental insufficiency, may also disturb intrauterine growth. Further, maternal obesity represents the mirror form of nutritional or metabolic imbalance affecting fetal development. Its consequences have recently been reviewed by Vasudevan and associates (2010) and for example structural anomalies such as neural tube and structural cardiac defects are more common in infants of obese compared to infants of non-obese mothers. Furthermore, overweight and obese women have an increased risk of preeclampsia (Mbah et al. 2010) and preterm delivery (McDonald et al. 2010). Possibly as a consequence of macrosomia (Bodnar et al. 2010) the risk of stillbirth and perinatal mortality are also increased. Outcomes are also related to altered placental function, although the pathogenic mechanisms involved are not fully understood (Higgins et al 2010).

In a Swedish birth cohort from 1992 to 2001 the most important risk factors associated with large for gestational age newborn were heightened maternal body mass index (BMI) and decreased smoking (Surkan et al. 2004). A high maternal pre-pregnancy BMI may also predict child weight and body fat percentage at the age of nine years (Catalano et al. 2009) and impair the offspring's later glucose metabolism (Thomas et al. 2007). Maternal obesity is a well-known contributor to gestational diabetes mellitus, which further predisposes offspring to metabolic disorders such as insulin resistance (Catalano et al. 2009) and type 2 diabetes (Damm 2009) later in life.

Gestational diabetes mellitus and excess maternal weight gain during pregnancy go hand in hand and are both possibly harmful for fetal development. According to results from the Project Viva study cohort, higher gestational weight gain is associated with higher child BMI, skinfold thickness, blood pressure and risk of overweight at the age of three (Taveras et al. 2009). The impact of gestational weight gain on offspring BMI, blood pressure (Mamun et al. 2009) and risk of overweight (Wrotniak et al. 2008) have been shown to extend into later childhood and even into adulthood.

The mother's dietary intake determines her serum cholesterol concentrations and metabolic status, and influences fetal vascular health. Maternal hypercholesterolemia may even initiate a cascade of fetal atherogenesis, manifested as fatty streak formation in children whose mothers were hypercholesterolemic during pregnancy (Napoli et al. 1997).

2.2.2. Growth of the fetus / infant / child

Intrauterine growth

According to Barker's studies low birth weight, as a marker of poor intrauterine environment and growth, has traditionally determined the subsequent risk of chronic diseases (Barker 2004). In previous meta-analyses low birth weight has increased the risk of ischemic heart disease (Huxley et al. 2007), elevated blood cholesterol concentrations (Lawlor et al. 2006) and blood pressure (Gamborg et al. 2007) as well as the risk of type 2 diabetes mellitus later in life (Whincup et al. 2008). One kilogram increase in birth weight is estimated to be associated with a 10 to 20% lower risk of ischemic heart disease, with a 1.5 to 2.8 mmHg decrease in systolic blood pressure and the odds ratio for type 2 diabetes is 0.75. However, these inverse associations proved to be graded. Among females of birth weight over four kilograms the higher birth weight increased subsequent blood pressure (Gamborg et al. 2007). In contrast, for type 2 diabetes the inverse association was graded at birth weights of three kilograms or less (Whincup et al 2008). In Pima Indians a U-shaped relationship has been demonstrated between birth weight and later glucose tolerance; the prevalence of diabetes was greatest in persons of low (<2500g) or high (≥ 4500g) birth weight (McCance et al. 1994). This is largely explained by the high prevalence of type 2 as well as gestational diabetes mellitus among the mothers or by the family history and genetic susceptibility. The same pattern of association between birth weight and type 2 diabetes mellitus risk may also apply in children nowadays (Wei et al. 2003).

Postnatal growth

Epidemiological studies suggest that decelerated growth during infancy predisposes to obesity (Law et al. 1992), impaired glucose metabolism (Hales et al. 1991) and cardiovascular disease (Barker et al. 2005) later in life. On the other hand, according to findings from the Helsinki Birth Cohort Study the combination of low birth weight and rapid subsequent growth in childhood may enhance the predisposition to type 2 diabetes (Forsen et al. 2000) and hypertension (Eriksson et al. 2007) in adulthood. However, especially in the case of increased risks of obesity there would appear to be more than one critical period of growth (Dietz 1994), and excessive weight gain may be detrimental even as early as in infancy (Rolland-Cachera 2005, Ekelund et al 2006).

According to large cross-sectional data on over 15 000 children from a series of annual surveys in England between 1995 and 2002, birth weight was negatively related to blood pressure at ages of five to fifteen, but advanced analyses pointed to the importance of later weight gain; in a sub-cohort of 3 900 children a one standard deviation (SD) increase in weight from birth to thirteen or to fifteen years of age increased systolic blood pressure by 0.8 mmHg (Primatesta et al. 2005). Other cardio-metabolic consequences of high postnatal weight gain, i.e. obesity, insulin resistance, and serum cholesterol concentrations, have also been seen in children (Ong et al. 2000, Gardner et al. 2009).

2.2.3. Nutrition

Intrauterine nutrition

The implications of the direct influence of intrauterine undernutrition on the offspring's subsequent risk of diseases emerged in epidemiological studies. Subjects conceived during the Dutch famine of World War II had a higher cumulative incidence of coronary artery disease (Painter et al. 2006), they were more obese (Ravelli et al. 1999), and their glucose tolerance was decreased (Ravelli et al. 1998, de Rooij et al. 2006) compared to persons born before or conceived after the famine.

Both experimental and human evidence has suggested that specific components in the maternal diet during pregnancy have an impact on the offspring's cardio-metabolic risk factors (Symonds et al 2009 a, b). In rodents a low protein proportion in the dam's diet during pregnancy has been shown to restrict the offspring's intrauterine growth (Fernandez-Twinn et al. 2003), and these rats manifested elevated blood pressure, endothelial dysfunction (Brawley et al. 2003) and insulin resistance (Ozanne and Hales 1999) later in life. Exposure to a high-fat diet in utero can further alter the offspring's susceptibility to future metabolic challenges. A wide range of risk factors, including abdominal obesity, abnormal glucose homeostasis and dyslipidemia, elevated blood pressure and endothelial dysfunction, have been reported in rodents after maternal high-fat dieting during gestation (Armitage et al. 2005).

In human studies, where the recorded protein intake as a proportion of energy intake (E%) and the ratio of protein to carbohydrates (20 to 33%) met the current dietary recommendations (described in section 2.3), especially unbalanced protein content in the maternal diet during pregnancy has proved to be associated with restricted fetal growth (Godfrey et al. 1996 a) and a predisposition to elevated blood pressure later in life (Campbell et al. 1996). For example, in the mentioned observational study by Godfrey and associates (1996a) the unbalanced protein content equaled a low ratio of protein to carbohydrates. In this case the offspring mean birth weight was lower (3312g vs. 3529g) in women whose carbohydrate intake was high (>340g/day) in early pregnancy and meat protein intake low (23.5g/day) in late pregnancy compared to the offspring of mothers whose carbohydrate intake was lower (<256g/day) and meat protein intake higher (>34g/day). Similarly, a low maternal animal protein intake (<50g) combined with increasing carbohydrate intake was associated with elevating blood pressure in adult offspring (Campbell et al.1996). Further, according to the other study of Godfrey and colleagues (1996 b) the low maternal protein intake after a period of high energy intake during pregnancy may have implications for the offspring's risk of impaired glucose metabolism. The cord plasma concentrations of insulin, split proinsulin and C-peptide fell significantly with increasing maternal energy intake during the first trimester and with decreasing protein intake later in pregnancy. Contrary to these findings, higher protein intake may also be detrimental as shown in the study of Shiell and co-authors (2000), where a higher maternal protein intake

during pregnancy was associated with lower insulin increment in adult offspring. The offspring's insulin increment was lowest if the mother's protein intake was >80g/day and the insulin increment was highest if the intake was ≤ 60g/day.

Prospective cohort studies have suggested that dietary fat quality during pregnancy may also entail long-term effects on the child. Dietary *n*-3 polyunsaturated fatty acid (PUFA, source not specified) intake during pregnancy in well nourished English women was inversely associated with the blood pressure of the seven-year-old offspring (Leary et al. 2005). Further, a study by Olsen and Secher (2002) showed that high maternal fish consumption during pregnancy could reduce susceptibility to preterm delivery and low birth weight in the newborn. Here the odds ratio for a preterm (<37 gestational weeks) or a low birth weight baby (<2500g) was about 3.6 in mothers who ate no fish in relation to the babies of women eating fish more than once a week. Maternal weight may however contribute to the associations, as demonstrated in a large French cohort study (Drouillet et al. 2009 a, b). They showed that in overweight women (BMI≥25 kg/m^2) higher seafood consumption (divided into groups of <1 time/week, 1-2 times/week and >2times/week) and *n*-3 PUFA intake one year prior to pregnancy were positively associated with the child's birth weight without inducing macrosomia. However, the maternal pre-pregnancy seafood consumption or *n*-3 PUFA intake were not related to newborn's size at birth in normal weight women.

Postnatal nutrition

Low saturated fatty acid (SFA) and high unsaturated fatty acid content in the diet, along with high consumption of fruits and vegetables, seems to be important from the perspective of a low cardio-metabolic risk profile (Appel et al. 2006). However, the efficacy of dieting in reducing cardio-metabolic risk factors is best proved in adults. In Finland the North Karelia Project was the first to show that a low-fat diet with a high ratio of PUFA to SFA could lower blood pressure and reduce cholesterol and low-density-lipoprotein cholesterol (Enholm et al. 1982, Puska et al. 1983). The diet of participants was modified to resemble that maintained in Mediterranean countries, and special attention was paid to the amount of saturated fat and cholesterol in the diet and the dietary content of vegetables. The total fat intake was about 39 E% at baseline and about 24E% during the six weeks' intervention period. The ratios of PUFA to SFA were 0.15 at baseline and 1.2 during the intervention.

A comparable healthier diet seems to regulate the individuals' coronary heart disease risk factor profile from early life onwards (Viikari et al. 2004). Intervention studies in children and adolescents have proved that a low-saturated fat diet from infancy onwards inhibits excess weight gain in girls (Hakanen et al. 2006), lowers blood pressure (Niinikoski et al. 2009), and reduces the clustering of other obesity-related cardio-metabolic risk factors (Hakanen et al. 2010). Here the interventions focused mainly on restricted dietary intakes of total fat and SFA, but ample use of vegetables, fruits, berries and whole-grain products was also encouraged. During the intervention

period from 13 months to 15 years of age the intake of total fat and SFA as E% were lower in the intervention group (26.3 to 30.5 E% and 9.5 to 11.4E% respectively) than in the control group (27.8 to 32.7 E% and 12.8 to 14.6 E%). On the other hand supplementation of PUFA in the early postnatal diet has not been found to affect children's growth at the age of 18 months (Rosenfeld et al. 2009), whereas similar manipulation has lowered blood pressure in the children at the age of six years (Forsyth et al. 2003).

In most studies in adults a greater protein intake, especially substituting carbohydrates with proteins, has been associated with lower blood pressure (Appel et al. 2006). Studies among children are scant but have obtained relatively congruent results. A greater dietary protein intake in 2.5 years old children has been associated with lower current blood pressure in a Danish population (Ulbak et al. 2004). However in extreme groups such as preterm infants, those randomized before weaning to an enriched-nutrient diet, i.e. preterm formula, containing a relatively high amount of proteins (2g/100ml) and fats (4.9g/100ml) manifested higher blood pressure later in childhood compared to children randomized to a low-nutrient diet, i.e. term formula or breast milk, (containing proteins 1.1-1.5g/100ml and fat totally 2.0-3.8g/100ml) (Singhal et al. 2007). The highlighted explanation for the deleterious effect of an enriched-nutrient diet was that it induced excessive postnatal weight gain. According to the investigators' assessments the enriched-nutrient diet was also suggested to impair glucose-insulin metabolism (Singhal et al. 2003 a) and lipid profile (Singhal et al.2004) especially in children born preterm.

Breastfeeding

Breast milk is generally accepted as an optimal nutrition for infants with both short- and long-term health benefits. Breastfeeding protects against infections as well as against non-communicable diseases such as obesity, elevated blood pressure and dyslipidemia, and it could be especially important in allergy prevention in genetically susceptible children (Agostoni et al. 2009).

Human milk contains nutrients but also a complex mixture of bioactive compounds. Breast milk hormones, growth factors and immunological components provide passive protection against inflammatory agents, and promote gastrointestinal mucosal maturation, and possess immunomodulatory and metabolic functions which link breastfeeding to the health benefits of the child (Walker 2010).

Lipids, carbohydrates, i.e. lactose, and proteins, comprise the energy source of breast milk. The breast milk fatty acid content, reflecting the diet of the mother (Vuori et al. 1982), may promote beneficial programming of glucose-insulin metabolism (Singhal et al. 2003), lipid profile (Singhal et al. 2004) and blood pressure (Singhal et al. 2001). Of the carbohydrates, oligosaccarides have prebiotic effects, meaning that these non-digestible food ingredients can beneficially affect the host by stimulating the growth and/or activity of specific bacteria in the colon (Gibson and Roberfroid 1995). These

can thus alter the gut microflora by stimulating the growth of Bifidobacteria and Lactobacilli, which typify the gut microbiota of the healthy breastfed infant (Fanaro et al. 2003). The colonization pattern in the gastrointestinal tract of the newborn is of significance in that it involves important and specific trophic, protective and metabolic functions (Guarner and Malagelada 2003). Previous demonstrations further suggest that the gut microbiota composition is linked to weight status in childhood (Kalliomäki et al. 2008).

2.3. Nutritional recommendations in Finland

The current Nordic Nutritional Recommendations (NNR 4th edition) were issued in 2004 (Becker et al. 2004). Compared to the previous version (Nordic Working Group on Diet and Nutrition 1996) the latest also takes into account the interaction between physical activity and the individual nutrient recommendations whenever appropriate. Regarding the proportions of energy-yielding nutrients the recommendations have remained practically unchanged. **Table 1** presents the recommended daily intakes of energy-yielding nutrients for infants, children and adults. In Finland the Ministry for Social Affairs and Health has also published nutritional recommendations for pregnant women and infants/children based on the NNR (Hasunen et al. 2004), and these recommendations for energy-yielding nutrients are same for pregnant women and children over two years old as for adults. In infants (from six to eleven months of age) the guidelines do not apply to breastfed children. For infants under six months old there are no given recommendations for total fat, carbohydrates and protein intake, as an exclusive breastfeeding is the preferable source of nutrition. The population target of salt intake is 6g/day for women and 7g/day for men. The infants' foods should be unsalted, the salt intake should not exceed 0.5g/MJ in one to three years old children and 5g/day in three years old and older children. A minimum of 30 minutes' physical activity of moderate and/or vigorous intensity daily is recommended for adults, and a minimum of 60 minutes for children and adolescents.

Breastfeeding

The Word Health Organization recommends exclusive breastfeeding for the first six months of life and the introduction of complementary foods thereafter (WHO 2001). Partial breastfeeding should be continued for the first two years. In Western countries partial breastfeeding is recommended to continue until one year of age in view of an increased risk of iron deficiency anemia in the breastfed child thereafter (Hasunen et al. 2004).

Current situation

According to the National FINDIET 2007 survey conducted in the adult Finnish population the total fat intake was 33E% in men and 31E% in women, which meets the recommendations. However the intake of SFA was over that recommended (<10E%)

in both men and women (13E% and 12E%, respectively) as was the intake of salt (9.3g in men and 6.8g in women) (Pietinen et al 2010).

In pregnant Finnish women the daily intakes of energy-yielding nutrients have been recently studied in the cohort of Type 1 Diabetes Prediction and Prevention project (DIPP) (Prasad et al. 2010). The nutrient intakes between years 2003 and 2004 are collected in Table 1, which shows an excessive SFA intake and insufficient PUFA intake in relation to the recommendations in pregnant women.

Among infants the feeding recommendations have not been achieved in Finland according to the DIPP birth cohort study (Erkkola et al. 2010). The median exclusive breastfeeding duration was only 1.4 months (range 0.8) and total breastfeeding duration 7.0 months (0-25) between the study years 1996 and 2004. Further, in the same birth cohort 22% of girls and 18% of boys were breastfed at the age of one year (Kyttälä et al. 2010). In this study by Kyttälä and associates (2010) the consumption of fresh vegetables, fruits and berries, vegetable-oil-based fats and fish was low among one- to six-year-old children and they consumed too little PUFA. The intake of saturated fat was considered to be too high although there are recommendations for types of dietary fat in children under two years of age only for *n*-3 PUFA and linoleic acid.

Table 1. Recommended daily intakes of energy-yielding nutrients and the intakes in children and in pregnant women in Finland.

	NNR, 6-11 months	1-year-olds* †, mean	NNR, 12-23 months	4-years-old †, mean	Pregnant women 2003-2004 ‡, mean	NNR, > 2 years and adults
Protein, E%	7-15	15.3	10-15	15.3	16.9	10-20
Carbohydrate, E%	45-60	55	50-55	53	50.5	50-60
Fat, E%	30-45	29	30-35	31	32.5	25-35
SFA, E%	-	11.6	-	13.7	13.6	<10
MUFA, E%	-	10.5	-	10.6	11.2	10-15
PUFA, E%	n-6 PUFA: ≥4 n-3 PUFA: ≥1	5.1	n-6 PUFA: ≥3 n-3 PUFA: ≥0.5	4.0	4.6	5-10
Dietary fiber, g/MJ	-	2.3	-	1.7	2.5	3

NNR, Nordic Nutrition Recommendations; E%, proportion of energy intake; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

*Breastfed infants not included, † Kyttälä et al. 2010, ‡ Prasad et al. 2010

2.4. Probiotics

Colonization in the newborn's gastrointestinal tract is important in that it constitutes trophic effects on intestinal epithelia and immune structure and function. Further, the colonization pattern on the gastrointestinal tract involves specific protective and metabolic functions (Guarner and Malagelada 2003). Experimental studies have pointed to the role of the gut microbial composition in energy acquisition and storage, and that this contributes to the development of obesity (Bäckhed et al. 2004, Turnbaugh et al. 2006). Childhood overweight has since been linked to aberrant gut microbiota composition (Kalliomäki 2008). Fecal samples from infants who became overweight contained lower levels of *Bifidobacteria* and higher levels of *Staphylococcus aureus* compared to samples from infants remaining normal-weight.

Probiotics, defined as "living micro-organisms which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition" (Guarner and Schaafsma 1998), are one tool to normalize detrimental deviations in microbiota composition (Rautava and Isolauri 2002). The most traditional uses of probiotics pertain to the treatment of infectious and antibiotic-associated diarrhea but these may also play a role in the management of inflammatory, allergic and atopic diseases in infants (Salminen et al. 2005, Gareau et al. 2010). Recently, it has been suggested that these agents may have potential applications even in the prevention of obesity and obesity-related conditions (Isolauri et al. 2009) linked with subclinical inflammation (Pradhan 2007). Indeed, maternal perinatal use of them has been shown to modify the early growth pattern of the child in a randomized, double-blind, prospective follow-up study of probiotics in allergic diseases (Luoto et al. 2010). Administration of *Lactobacillus rhamnosus* GG has restrained excessive weight gain from the fetal period to the age of two to four years when compared to a control group. In another randomized and controlled trial probiotics (*Lactobacillus rhamnosus* GG and *Bifidobacterium Lactis* Bb*12*) benefited women's glucose metabolism and weight management during and after pregnancy when accompanied by dietary counseling (Laitinen et al. 2008, Ilmonen et al. 2010). The combined effects of counseling, paying particular attention to the quality of dietary fat, and probiotic administration were explained among other things by the immunoregulatory properties of probiotics (Isolauri et al. 2001) and by similar signaling pathways of microbes and dietary fatty acids in immune responses (Laitinen et al. 2006).

2.5. Assessment of cardio-metabolic status in early life

2.5.1. Cardiac sympathovagal activation

Over-activation of the autonomic nervous system is one denominator of cardiovascular disease (Mancia et al. 2007). The vagal and sympathetic functions of the autonomic nervous system are manifested in the magnitude of short- and long-term variability in the heart rate (HR), respectively. Therefore heart rate variability can be used as a

monitoring tool in clinical conditions with altered function of the autonomic nervous system.

The impulses from the parasympathetic nervous system are conveyed much faster than those from the sympathetic nervous system, which leads to slow and fast oscillation of the HR by parasympathetic modulation and slow HR oscillation by sympathetic modulation. Fetal autonomic cardiac control can be characterized by frequency–specific assessment of fetal heart rate variability under power spectral analysis, which detects and quantifies changes in HR objectively (Akselrod et al. 1981, van Ravenswaaij-Arts et al. 1993, Siira et al. 2005 and 2007). Spectral power in the high-frequency (HF) range is associated with parasympathetic modulation and that in the low-frequency (LF) range with sympathetic and parasympathetic nervous system modulation (Akselrod et al. 1981, Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). Consequently, the low to high frequency (LF/HF) ratio in HR variability has been considered to mirror sympathovagal balance (Metsälä et al. 1995, Siira et al. 2007). During labor for example, fetal distress due to uterine contractions or hypoxemia can activate the fetal autonomic nervous system. When these conditions have been eliminated, it is possible to evaluate the basal sympathetic activity of the fetus (Siira et al. 2005).

2.5.2. Blood pressure

Elevated blood pressure already in childhood is one of the most important risk factors for subsequent cardiovascular diseases (National High Blood Pressure Education Program Working Group 2004). The blood pressure level programmed in early infancy has been shown to extend into childhood (Fuentes et al. 2002) and up to adult ages (Chen and Wang 2008), and may even predict hypertension and metabolic syndrome risks later in life (Sun et al. 2007). In normal circumstances the growth of height modifies the increase in blood pressure, as previously noted by Rosner and colleagues (2008). Further, obesity and overweight have already impact on blood pressure progression in childhood, and, as recently reviewed, the blood pressure is linked to body adiposity (Ben-Dov and Bursztyn 2009).

2.5.3. Metabolic status

Body mass index, waist circumference and skinfold thicknesses have been highly intercorrelated and used as markers of cardio-metabolic status in early life along with leptin and adiponectin (Shea et al. 2003, Valle et al. 2003, Darendeliler et al. 2009, Corvalan et al. 2010). Leptin and adiponectin are adipokines, hormones secreted by the adipocytes, the concentrations of which already correlate with the amount of adipose tissue in newborns and children and contribute to early growth patterns (Schubring et al. 1997, Mantzoros et al. 2009). Further, these participate in immunity and metabolic development (Ronti et al. 2006).

Leptin is a proinflammatory cytokine (Matarese et al. 2005). It may impair vascular function (Singhal et al. 2002) and has been associated with the progression of insulin resistance even in children (Steinberger et al. 2003). In overweight adults leptin further increases sympathetic activity (Collins et al. 1996) and may thus be linked to cardiovascular diseases in the context of metabolic disorders (Mancia et al. 2007). Although both of these cytokines are primarily expressed in adipose tissue, leptin and adiponectin are also breast milk hormones (Savino et al. 2009). Leptin is transferred from the maternal circulation to breast milk and is produced in the mammary epithelial cells (Smith-Kirwin et al. 1998). Leptin concentrations are higher in breastfed than in formula-fed infants (Savino et al. 2002) and the breast milk leptin composition could contribute to short-term control of food intake and exert a long-term effect on energy balance and body weight regulation (Stocker and Cawthorne 2008).

The origins of breast milk adiponectin remain to be established, but it contributes to neonatal weight regulation (Newburg et al. 2010). The relevance of adiponectin in human physiology and pathophysiology has recently reviewed by Guerre-Millo (2008). In plasma, adiponectin circulates in three multimeric forms, trimers, hexamers and high-molecular-weight multimers, of which the high-molecular-weight form appears to be clinically the most relevant. Unlike leptin the concentrations of adiponectin decrease with increasing adiposity mass. This has been linked to the low-grade inflammation stage in obesity, where proinflammatory cytokines lower adiponectin release of adipose tissue. Adiponectin sensitizes tissues to insulin, and high adiponectin levels may even protect from the development of type 2 diabetes mellitus (Guerre-Millo 2008). The anti-inflammatory actions of adiponectin (Fantuzzi 2008) have been associated positively with measures of endothelium-dependent and independent vasodilatation (Goldstein and Scalia 2004) and may have antiatherogenic actions (Kubota et al. 2002).

Instead of direct measurement of insulin resistance or glucose tolerance, elevated serum or plasma 32-33 split proinsulin concentrations have been used as a marker of insulin resistance in adults as well as in children (Temple et al. 1989, Mykkänen et al. 1997, Singhal et al. 2003a). The 32-33 split proinsulin is a conversion intermediate of intact proinsulin, which is secreted together with insulin and C-peptide by the pancreatic beta-cells (Melani et al. 1970) its concentrations being increased in type 2 diabetes (Temple et al. 1989). In nondiabetic adults the split proinsulin concentration has been found to be positively associated with markers of subclinical inflammation, i.e. C reactive protein and measures of body fat (Festa et al. 2000). Further, in adult men 32-33 split proinsulin concentrations have tended to be associated negatively with birth weight (Hales et al. 1991). In this study in question the split proinsulin concentrations were also lowest (geometric mean the 2.1pmol/l) in men of the highest weight group at the age of one year (>10.7kg) and the lowest adult BMI group ($\geq$25.4kg/m^2), and highest (4.8pmol/l) in men of the lowest weight group at 1 year ($\geq$9.8kg) and highest BMI group in adulthood (>28 kg/m^2). Furthermore, 32-33 split

proinsulin levels have been shown to be higher in children of seven years of age who experienced catch-up growth (deviation from the 25th to 50th centile, 50th to 75th centile ect.) between birth and the current age, although the levels remained within normal range (Crowther et al. 2008). The schematic diagram below (**Figure 2**) shows the associations between obesity, adipokines and split proinsulin.

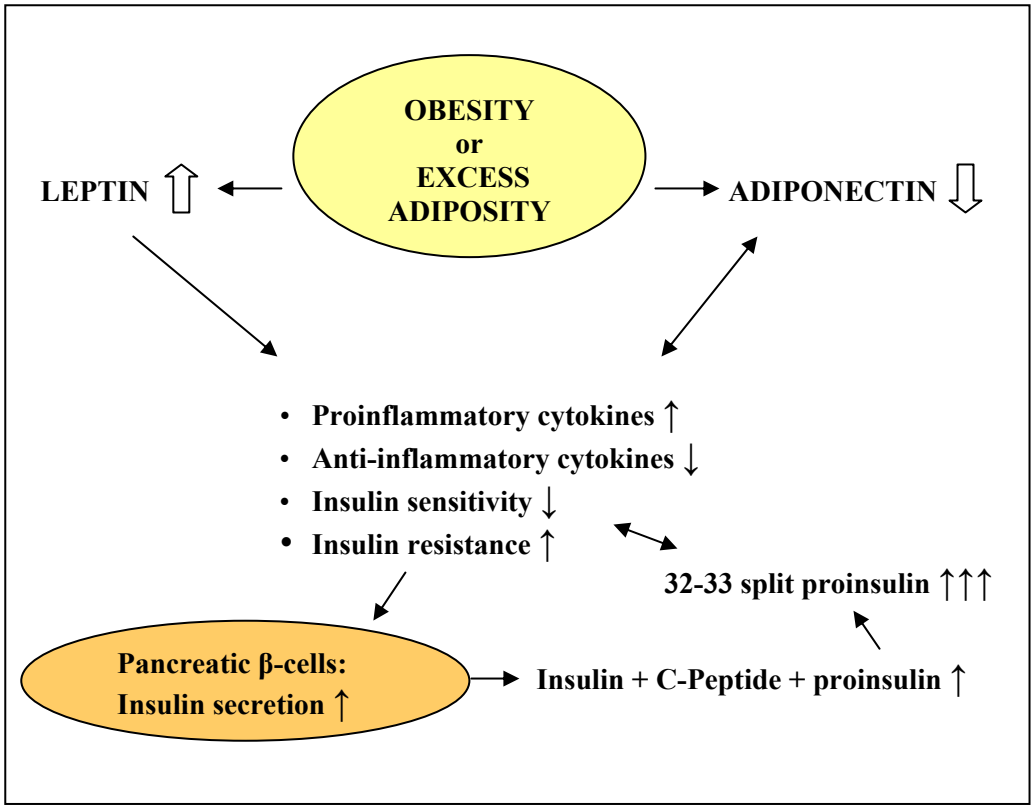


Figure 2. Adipokines and split proinsulin in obesity

3. AIMS OF THE STUDY

The objective in the present project was to evaluate the intrauterine and early postnatal nutritional determinants influencing early cardio-metabolic programming. Specifically, fetal cardiac sympatovagal activation, infant and child blood pressure and glucose-insulin metabolism in infancy were assessed:

1. to evaluate the impact of maternal metabolic status and dietary intake during pregnancy on infants' cardio-metabolic programming

2. to evaluate the impact of child postnatal dietary intake and growth on cardio-metabolic programming

3. to determine whether dietary intervention from early pregnancy onwards may benefit infants' cardio-metabolic programming

4. SUBJECTS AND METHODS

4.1. Subjects and study design

A total of 256 pregnant women in the first trimester of pregnancy were recruited from maternal welfare clinics in the area of Turku, Southwest Finland, between April 2002 and November 2004 to participate in a prospective, randomized mother-infant nutrition and probiotic study (NCT00167000; section 3, http://www.clinicaltrials.gov) (Laitinen et al. 2009). Exclusion criteria were any chronic diseases except for allergy. At the first study visit, representing the baseline, the participants were randomized into three study groups (**Figure 3**), two dietary intervention groups (Diet/probiotics and Diet/placebo) and one control group, according to computer-generated block randomization of six women. The study statistician, who was not involved in the study visits, generated the randomization list. Figure 3 also presents the number of children with recorded outcome measures, i.e. cardiac sympathovagal activation (LF/HF), 32-33 split proinsulin analysis (Split) and blood pressure measurements (BP) in each study group. **Table 2** gives reasons for discontinuation in the dietary intervention groups with probiotics and placebo and in the control group.

4.2. Inclusion criteria for studies I to IV

Study I: Fetuses from normal pregnancies born after 36 gestational weeks and whose parents permitted the use of a fetal scalp electrode for continuous fetal ECG recording were included (n=41).

Study II: All singleton infants whose venous blood sample was collected at the age of six months were included (n=194).

Study III: All 256 recruited women were included in the study. Dietary intake was estimated during pregnancy in 239 women who had filled the food diaries. Blood pressure was recorded in 171 six-month-old infants.

Study IV: All children who were born full-term from singleton pregnancies and had yielded three reliable resting blood pressure measurements at the age of four years were included in the study (n=109).

4.3. Maternal dietary and probiotic intervention

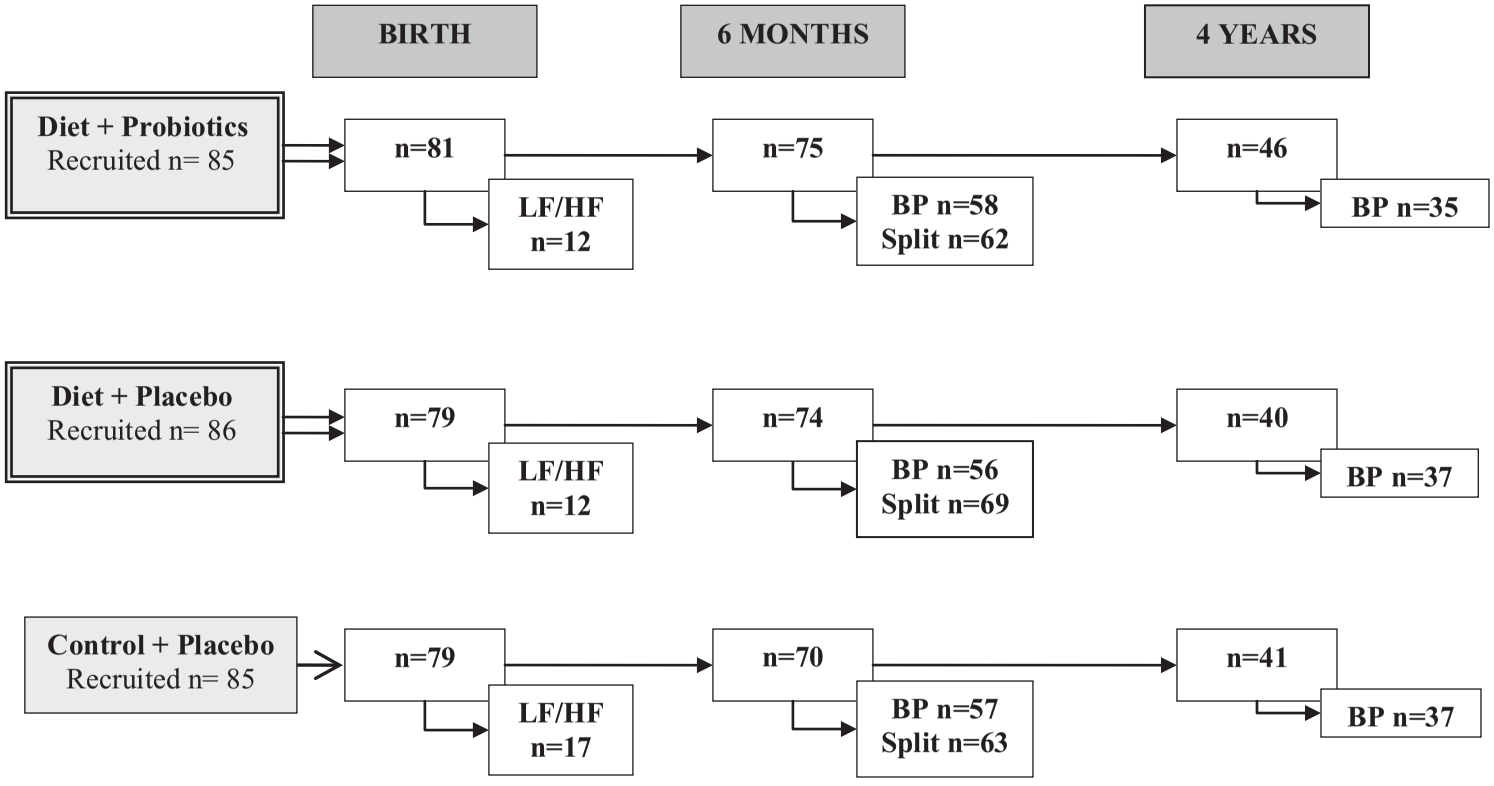
The pregnant women in the control group received standard dietary counseling in well-women clinics according to a national program. At each study visit, women in the dietary intervention groups (diet/probiotics and diet/placebo) received additional intensive dietary counseling in accordance with that recommended during the study years (Nordic Working Group on Diet and Nutrition 1996, Becker et al. 2004). The

target in counseling was to ensure dietary consumption containing carbohydrates 55-60E%, proteins 10-15E% and total fats 30E%, including MUFA 10-15E%, PUFA 5-10E% and SFA 10E% or less. The counseling was given in layman terms by a nutritionist who encouraged the participants to pay attention to the amount and type of fat and to the amount of fiber in their diet (Piirainen et al. 2006). They were encouraged to consume leaner meat products and low-fat cheese and fat-free/low-fat milk products, to increase their consumption of vegetables, fruits, berries and whole-grain cereals and bread, and to use vegetable-oil-based spreads and vegetable oil. Further, to strengthen the dietary course and to demonstrate sources of favorable fat and fiber content, various food products available on the market, for example vegetable-oil-based spreads and salad dressings and fiber-enriched pasta, breakfast muesli, and porridge cereals, were given to the families to use at home. The products were donated by the Raisio Group, Raisio, Finland.

The dietary intervention groups (diet/probiotics and diet/placebo) received capsules of probiotics (*Lactobacillus rhamnosus GG*, American type culture collection 53103, Valio Ltd., Helsinki, Finland and *Bifidobacterium lactis*, Chr. Hansen, Hoersholm, Denmark, 10^{10} colony-forming units each/day) or placebo (microcrystalline cellulose and dextrose anhydrate; Chr. Hansen, Hoersholm, Denmark) in double-blind manner, while the control group (control/placebo) received placebo in single-blind manner (Figure 3). The intensive maternal dietary and probiotic intervention continued from early pregnancy until the end of exclusive breastfeeding, a maximum six months postpartum.

4.4. Schedule for clinical assessments, dietary recording and blood sampling

The women visited the study clinic three times during pregnancy, at a median of 14 (range, 7 to 18), 24 (20 to 27) and 34 weeks of gestation (30 to 37), respectively. As shown in **Figure 4**, women's dietary intake was assessed in the context of all study visits during pregnancy and breastfeeding, and fasting blood samples were collected at the first and third trimester of pregnancy and six months postpartum. Prior to labor women with more than 36 completed gestational weeks who permitted the use of a fetal scalp electrode were asked to consent to continuous fetal electrocardiogram (ECG) recording at the time of labor. At birth, cord blood samples were collected and the offspring measured in the delivery hospital. About 97% of them were born in Turku University Hospital, and the remaining in central or regional hospitals nearby. Subsequent venous blood samples from infants were drawn in conjunction with the six months study visit (range 5.0 to 7.7). For obvious ethical reasons overnight fasting was not in fact possible at the age of six months, and sampling was performed before noon. The second postnatal study visit was made at the child age of four years (3.9 to 4.2). The children's nutritional status, growth, continuation of breastfeeding (exclusive, partial or ended) and blood pressure were assessed at postpartum visits, and dietary intake at the age of four years.



Recruited women total n= 256. The number of participating children is presented from delivery onwards.
Randomization: Open to Diet or Control group, ⟹ Double-blinded to Probiotics or Placebo, → Single-blinded to Placebo
LF/HF: Cardiac sympathovagal activation, BP: Blood pressure measurement, Split: 32-33 split proinsulin analysis

Figure 3. Study design and subject flow

Table 2. Reasons for discontinuing the study at different time points

	Diet/probiotics	Diet/placebo	Control
Before birth	4	7	6
Miscarriage	3	2	0
Illness in mother	0	2	2
Illness in child	0	0	0
Moved	0	1	0
Unwilling to continue	1	2	4
Before 6 mo	6*	5	9
Illness in mother	2	0	0
Illness in child	4	1	1
Moved	1	1	0
Blood sampling	0	0	5
Unwilling to continue	1	3	3
Before 4 years	29	36	29
Illness in mother	0	3	1
Illness in child	1	1	2
Moved	2	3	1
Unwilling to continue	26	29	25

Data presented as number of subjects

* multiple reasons in two cases

No statistically significant differences were found in numbers of women or children among the study groups by χ^2 test.

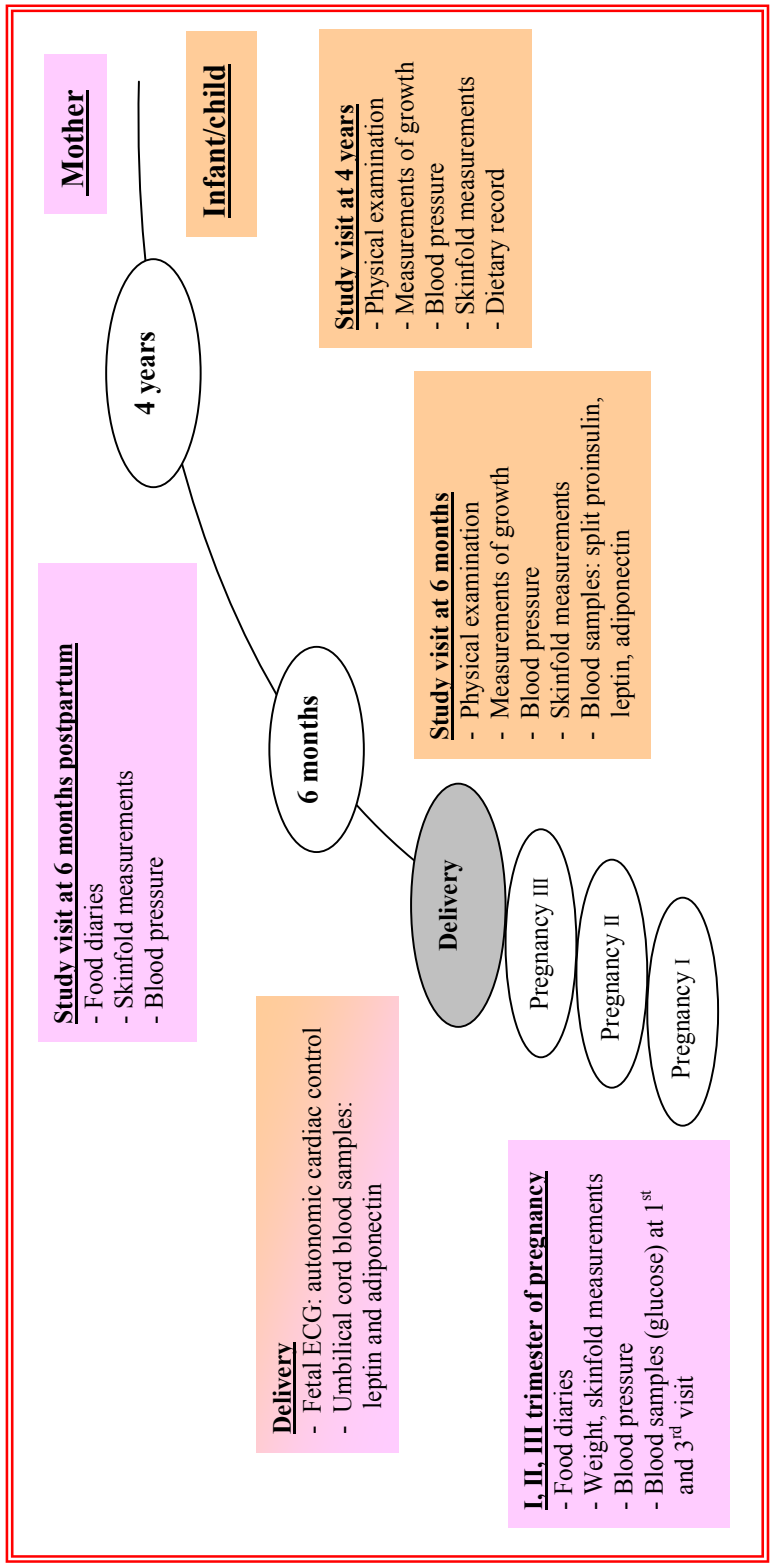


Figure 4. Conduct of study

4.5. Evaluation of maternal clinical characteristics

Background information on the pregnant women was obtained by interview at study entry. The research nurse measured the women's height, weight and blood pressure according to standard procedures (Perloff et al. 1993, WHO 1995). Fasting blood glucose was evaluated at first and third trimester of pregnancy. Maternal weight gain during pregnancy was calculated from self-reported pre-pregnancy weights and measurements recorded in hospital or at a prenatal study visit within one week prior to delivery. Appropriate gestational weight gain was evaluated according to the pre-pregnancy BMI (Institute of Medicine 1990). Pregnancy duration was calculated from the last menstruation. Diagnoses of gestational diabetes mellitus (GDM) were obtained from welfare clinic or hospital records. Specifically the diagnosis of GDM was based on modified criteria of American Diabetes Association (2004) according to recommendations implemented in Finland. The results of a 75g oral glucose tolerance test were considered pathological when one value exceeded $\geq$ 5.3 mmol/L at baseline, $\geq$ 10.0 mmol/L at 1h or $\geq$ 8.6 mmol/L at 2h.

4.6. Evaluation of offspring anthropometrics

At birth the midwifery team measured the infants with instruments comparable to those used at the study visits. They also measured the placental weight with a mechanical scale (Monilaite Dayton Oy, Soehnle, Vantaa, Finland) to establish the relative weight of the placenta (placental weight divided by birth weight). The research nurse measured weight in infancy with a Data baby scale 930 (Oriola, Espoo, Finland) and at the age of four years with a Seca digital column scale (Hamburg, Germany). Recumbent length was measured by Infantometer (Pedihealth, Oulu, Finland) and standing heights with a pull-down metal measuring tape (person-check REF 44 444; Medizintechnik KaWe, Kirchener & Wilhelm, Asberg, Germany). Body surface area (BSA) was calculated as the square root of height in centimeters multiplied by weight in kilograms divided by 3600. The occipitofrontal head circumference from maximum circumference between the supraorbital ridge and the occiput and waist circumference at the narrowest were determined with a measuring tape. Supra-iliac skinfold thickness was measured horizontally two centimeters above the anterior superior iliac spine from the line between spine and umbilicus with a Holtain Tanner/Whitehouse Skinfold caliper (Marsden Weighing Group, Hanley-on-Thames, Oxfordshire, United Kingdom), mean of three measurements being used in analyses.

4.7. Evaluation of fetal cardiac sympathovagal activation (Study I)

Fetal cardiac sympathovagal activation during delivery was performed by fetal ECG (Siira et al. 2005). The intrauterine fetal single-helix scalp electrode and a maternal skin electrode constituted the fetal unipolar ECG lead configuration. The fetal ECG

was continuously recorded for one hour in the first phase of labor using a STAN® S 21 monitor (Neoventa Medical, Gothenburg, Sweden).

R-peaks were detected and R-R intervals measured and digitalized (sampling rate 500Hz). The R-R interval data sets were stored on a PC hard disc and the intervals analyzed off-line by a signal analyst (Jarmo Jalonen, MSc), who remained blinded to the clinical details of the fetuses studied. Two-minute continuous signal segments at a stable fetal heart rate (HR) were required for spectral analysis. The quality of these segments was visually controlled by our signal analyst (JJ). On average, 19 segments (range from 5 to 29) were obtained from each fetus and the mean time lag from commencement of recording to delivery was 246 (± 217) minutes.

Spectral analysis decomposes sequential R-R interval series into a sum of sinusoids of different amplitudes and frequencies. The reciprocal of each R-R interval was computed to obtain the respective instantaneous HR reading. After linear interpolation of consecutive heart beats, the event series were resampled at a rate of 16Hz. Fast-Fourier-Transformed power spectra were then computed for the fetal HR signal segments (MATLAB®-oriented tailor-made signal analysis program, MARAPS, Tampere, Finland (Välimäki and Rantonen 1999). The fetal HR variability spectrum was integrated over the low-frequency (LF) band from 0.04Hz to 0.15Hz (from 2.4 to 7.8 cycles/min) and over the high-frequency (HF) band from 0.15Hz to 1.0Hz (from 7.8 to 60 cycles /min). The LF/HF ratio was calculated to display the balance of sympathetic and parasympathetic controls (Metsälä et al. 1995, Siira et al. 2007). The spectral variability data was given in arbitary units (AU).

4.8. Evaluation of infants' metabolic status (Study II)

Serum 32-33 split proinsulin and intact proinsulin concentrations were chosen as primary markers of infant metabolic status. The concentrations were dichotomized for analysis, as the clinically important difference in mean levels is not known and differences in proportions of higher values were considered more relevant than those in overall mean levels. Any 32-33 split and intact proinsulin concentrations above the 85^{th} percentile of the concentrations (7.9 pmol/l and 6.64 pmol/l, respectively) were considered high. As the agreement between high concentrations was good (kappa-coefficient κ=0.80), only the high 32-33 split proinsulin was analyzed as a final outcome variable in infant metabolic status. Measurements of supra-iliac skinfold and waist circumference were used to define adiposity and nutritional status, and serum leptin and adiponectin concentrations were measured as surrogate markers of early adiposity and nutritional stage (Yannakoulia et al. 2003, Tsai et al. 2004).

4.9. Methods to assess markers of glucose-insulin metabolism and nutritional stage

Fasting blood glucose in the pregnant women was analyzed at the laboratory of Turku University Hospital by an enzymatic method utilizing hexokinase in a Modular P800 automatic analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

The NIHR Cambridge Biomedical Research Centre, Core Biochemical Assay Laboratory (Cambridge, UK) analyzed the infants' blood samples. Serum was separated immediately and the samples initially stored at −20°C and then at −70°C. Serum 32-33 split proinsulin and intact proinsulin, leptin and adiponectin concentrations were assayed on a 1235 AutoDELFIA immunoassay system (Perkin Elmer Life Sciences, Boston, MA). All assays were in-house, two-step time-resolved fluorometric assays as previously described (Temple et al. 1989, Hales et al. 1991, Semple et al. 2006), and samples were analyzed in duplicate. Samples in which the coefficient of variation in duplicates was greater than 10% were repeated. Quality control samples with concentrations spanning the working range of the assay were run each day. The between-batch imprecision for the Quality Control samples was less than 8% for all assays and analyte concentrations.

4.10. Blood pressure measurements in infancy and childhood (Studies III and IV)

Children's blood pressure and HR were measured according to standard procedures using an appropriate-sized cuff with automated oscillometric recorders; with DINAMAP R (Criticon, Tampa, Florida, USA) at infant age of six months and DINAMAP ProCare 100 at child age of four years (National High Blood Pressure Education Program Working Group 1996 and 2004). The subjects were at rest, in sitting position appropriate for age, during the measurements. Blood pressure was determined as an average of three measurements, and the procedure was discontinued if the child became restless.

4.11. Evaluation of dietary intake of mothers and children

Maternal and child dietary food and nutrient intakes were evaluated by means of three consecutive days' food diaries, including one weekend day. The consumptions were to be reported as household measures (e.g. one glass of milk), in natural units (e.g. one apple or potato) or portions (e.g. one portion of lasagne). At the study visit the women were asked to compare the portion sizes to reference pictures of different size portions (Haapa et al. 1985, Paturi et al. 2006). The daily dietary intake of energy, foods and nutrients was calculated by the computerized program Micro-Nutrica, nutrient analyzing software based on Finnish nutrient analysis and international food composition tables, version 2.5 (Research Centre of the Social Insurance Institution, Turku, Finland). The foods consumed were combined into groups (grain, meat, fish

and dairy products, fruits and berries, vegetable-oil-based margarine and vegetable oil, sugar and sweets) in statistical analyses, but milk, cheese, sour milk products, vegetables and butter consumption was also analyzed separately. Grain products included for example bread, porridge, cereal, pasta and rice. The dairy products were not separated according to fat content (e.g. skimmed milk vs. full milk) in the statistical analyses of foods.

The duration of exclusive and partial breastfeeding and breastfeeding status at six months (exclusive, partial or ended) were recorded. Breastfeeding was considered exclusive when the infant received no solids or liquids, other than vitamins and medication, besides human milk, and partial when these or one of these was introduced into the diet (Kramer and Kakuma 2002, edited 2009).

4.12. Statistical methods

Characteristics are given as means (SD), medians (range) or number of subjects (percentage) and analyses as means or odds ratios (OR) with 95% confidence interval (CI). The impact of intervention on outcome measures was studied and if no differences were found assessments were made in combined study groups. The impact of dietary counseling on maternal and child dietary intake was studied between the combined dietary intervention group (Diet/probiotics and Diet/placebo) and Control/placebo group.

Study I: The effects of intervention and maternal and infant clinical characteristics on the fetal LF/HF ratio of HR variability were analyzed by multivariable generalized linear model procedure. Pearson's or Spearman's correlations, as appropriate, were used for further analysis of significant associations. The data were logarithmically transformed to normalize the distributions, if necessary. The data were analyzed using the SAS System for Windows, release 8.01 (SAS Institute, Cary, North Carolina, USA).

Study II: Comparisons amongst the three study groups in categorized outcome variables and the effects of maternal and infant clinical characteristics on the infants' high 32-33 split proinsulin concentration were analyzed by univariate logistic regression. Prior to these analyses the explaining factors were categorized according to median, tertiles (T) or quartiles (Q) (T1/Q1=lowest and T3/Q4=highest), as most associations were nonlinear in preliminary analyses.

The χ^2 test was used to evaluate associations between maternal dietary intakes during and after pregnancy and infants' high 32-33 split proinsulin concentration. The dietary intakes at each trimester and six months postpartum were divided into tertiles prior to the analyses. Statistical analyses were performed with SPSS version 15.0 (SPSS Inc. Chicago, IL, USA)

Study III: Analysis of variance (ANOVA) was used to compare the intervention groups. The effect of dietary counseling on maternal food and nutrient intakes during pregnancy was analyzed using analysis of covariance (ANCOVA), where the first trimester intake was included as a continuous covariate. The effects of maternal and infant clinical characteristics on the infants' blood pressure were analyzed by Pearson's correlations or the χ^2 test, as appropriate.

Maternal dietary intakes during pregnancy were divided into quartiles Q1 to Q4 (Q1 represented the lowest intake and Q4 the highest) to compare infant blood pressure between the intake quartiles by ANOVA. The significant and almost significant ($p<0.10$) variables were introduced into the model. The models were reduced stepwise to obtain a model with only significant nutrients. Statistical analyses were performed with SPSS version 14.0 (SPSS Inc. Chicago, IL, USA)

Study IV: ANOVA was used to compare child blood pressures between the study groups and the tertiles of explaining factors. Maternal and child characteristics and dietary intakes were categorized according to tertiles (T1=lowest, T2=middle, T3=highest), as many of them were not linearly associated with child's blood pressure. Pearson's correlation was used to study linear associations, after logarithmical transformation when needed. A stepwise multivariable regression model was used to obtain a model with only significant explaining factors. Maternal characteristics and dietary components as well as child anthropometrics and dietary components were analyzed separately in respective models in order to manage the large number of potential explaining factors. Statistical analyses were performed with SPSS version 17.0 (SPSS Inc. Chicago, IL, USA).

P-values <0.05 were considered statistically significant in all studies.

4.13. Ethical aspects

The study complied with the Declaration of Helsinki, as revised in 2000. Written informed consent was obtained from the women and the Ethics Committee of the Hospital District of Southwest Finland approved the study.

5. RESULTS

5.1. The clinical characteristics of participating mothers and children

The clinical characteristics of the study subjects are presented in **Table 3**. No statistically significant differences were found among the study groups. In general, all participants were Caucasians and in good health. The majority of the women had higher college or university education (75%), and gave birth after a normal pregnancy. **Table 4** shows detailed data on cardio-metabolic determinants of the fetuses, infants and children in combined study groups.

5.2. Maternal characteristics and child cardio-metabolic determinants

Fetal sympathetic activation: The LF/HF ratio of fetal HR variability was positively associated only with maternal pre-pregnancy BMI (correlation coefficient r=0.3, p=0.03).

32-33 split proinsulin: Maternal smoking prior to pregnancy tended to be associated with an increased risk of high 32-33 split proinsulin concentration in the infant (OR 2.07, 95% CI 0.95; 4.55, p=0.069 vs. not smoking). There was also a trend to high split proinsulin in infants whose mothers' weight gain during pregnancy was more than recommended (OR 2.12, 0.81; 5.58, p=0.128 vs. as recommended) or less than recommended (OR 3.33, 1.02; 10.17, p=0.030 vs. as recommended, global p=0.098). Maternal gestational diabetes mellitus did not explain increased infant risk of high split proinsulin statistically significantly (OR 2.51, 0.73; 8.67, p=0.114).

Blood pressure: The maternal clinical characteristics did not predict the children's blood pressure at the age of six months. In contrast, maternal systolic blood pressure at the first trimester and weight gain during pregnancy were non-linearly associated with the children's systolic blood pressure at the age of four years according to multivariate model; those from the lowest and highest tertiles had children with higher systolic blood pressure compared to the middle tertile (p=0.015 and p=0.010, respectively). The child systolic blood pressure means were 99, 95 and 99 mmHg in tertiles T1, T2 and T3 of maternal blood pressure and 97, 95 and 100 mmHg in tertiles T1, T2 and T3 of weight gain.

Table 3. Clinical characteristics of the study population in Studies I to IV.								
	Study I n=41		**Study II n=194**		**Study III n=256**		**Study IV n=109**	
Mother								
Age, y	30.0	(4.6)	30.1	(4.8)	30.0	(4.8)	29.6	(4.5)
Pre-pregnancy BMI, kg/m^2	22.7	(18–35)	23.7	(3.6)	23.6	(3.7)	23.4	(3.2)
Pregnancy weight gain, kg	15.5	(4.4)	15.0	(4.8)	14.9	(4.9)	14.7	(4.6)
GDM	14	(33%)	14/185	(7.6%)	14/225	(6.2%)	10	(9.2%)
Child					**n=239**			
Sex, male	23	(56%)	100	(51.5%)	126	(52.7%)	53	(48.6%)
Birth								
Gestational age, wk	40	(36–43)	40	(33–43)	40	(30–43)	40	(37–42)
Weight, kg	3.6	(0.40)	3.6	(0.43)	3.6	(0.46)	3.6	(0.42)
Length, cm	51	(1.7)	51	(1.8)	51	(1.9)	51	(1.8)
Relative weight of placenta	0.16	(0.02)	0.16	(0.03)	0.16	(0.03)	0.16	(0.02)
Exclusively breastfed, mo	-	-	4.0	(0–6.5)	4.0	(0–6.5)	4.0	(0–6.5)
Partially breastfed, mo	-	-	9.0	(0–39)	6.5	(0–39)	8.3	(1–39)

Results are given as means (SD), medians (range) or number of subjects (percentage). Total number indicated if all are not included. GDM = Gestational diabetes mellitus. Relative weight of placenta was estimated by dividing placental weight by birth weight.

Table 4. Child cardio-metabolic determinants in combined study groups			
	number	Mean/ Median	SD / Range
Fetal sympathetic activation	41		
Heart rate, beat/min		135	10.2
LF/HF ratio, AU		5.0	1.6 to 13.4
32-33 split proinsulin, pmol/l	194	3.3	1.2 to 21.9
Intact proinsulin, pmol/l	194	3.7	1.2 to 20.0
Systolic blood pressure, mmHg			
6 months	171	97	9.1
4 years	109	97	8.1
Diastolic blood pressure, mmHg			
6 months	171	63	8.9
4 years	109	57	7.1

Results are given as means (SD) or medians (range)

5.3. Maternal dietary intake during pregnancy and child cardio-metabolic determinants

The maternal intakes of energy, fiber, energy-yielding nutrients and sodium in the combined dietary intervention group (Diet) and in the Control group during pregnancy are shown in **Table 5.**

Fetal sympathetic activation: The maternal intakes of energy or energy nutrients during pregnancy were not associated with fetal HR variability measured in LF/HF ratios.

32-33 split proinsulin: To evaluate the impact of maternal nutrition in each trimester of pregnancy on infant 32-33 split proinsulin levels, maternal dietary consumptions were divided into tertiles (T1-T3: T1= lowest intake, T3=highest intake). The foods and nutrients significantly associated with infants' high split proinsulin are presented in **Table 6** as well as the mean intakes of foods and nutrients (range) in each tertile of intake. The tertile closest representing the recommended intake is shown in bold.

The recommended, highest tertile of grain product intake in the maternal diet, independent of fiber content, was associated with the most abundant number of infants with high split proinsulin concentration. The T1 and T3 consumptions of fruits and berries in the maternal diet resulted in contrast in lower infant risk of high split proinsulin compared to the middle tertile (T2), which is the recommended intake of fruits and berries. Infants whose mothers' total intake of fat was as recommended (T2) were protected against a high split proinsulin concentration compared to the other infants. In contrast, a maternal cheese intake about 1.5 times higher as recommended was associated with the lowest percentage of infants with high split proinsulin level (6.1%), while about 20% of infants whose mothers' cheese intake was as recommended or about 3 times above the recommendations had high split proinsulin at six months. The use of butter is not nowadays recommended, and the infant risk of high split proinsulin was lowest in the lowest tertile of maternal intake (T1). Mother's intake of vegetable-oil-based margarine and vegetable oil in the highest tertiles (T3), representing the lowest limit of the recommendation, resulted in high split proinsulin in 15% of infants in that tertile. In contrast, those infants whose mothers' intake was insufficient, i.e. T1 and T2, 22.7% and 6.8%, had high split proinsulin, respectively. The recommended intake of milk (T3) protected the infants against high split proinsulin concentrations.

Blood pressure at six months of age: Maternal dietary consumption was divided into quartiles (Q1-Q4, **Table 7**) in evaluating the impact of maternal nutrition during pregnancy on infant blood pressure at six months. Only maternal total carbohydrate intake during pregnancy was statistically significantly associated with both systolic ($p=0.006$) and diastolic blood pressure ($p=0.015$) at the age of six months. Maternal intake of MUFA had a significant effect on infant diastolic ($p=0.029$), but not on systolic blood pressure. As seen in **Figure 5** the association showed a U-shaped dose dependency; the highest and lowest quartiles of nutrient intakes resulted in higher infant blood pressure compared with the middle quartiles.

Blood pressure at four years of age: Maternal dietary consumption was divided into tertiles (T1-T3, Table 7) in evaluating the impact of maternal nutrition during

pregnancy on child blood pressure at the age of four. Of maternal nutrient intakes during pregnancy, those of carbohydrates overall (p=0.029) and total fat (p=0.004) explained child systolic blood pressure at the age of four in a final multivariate model. Intakes of total fat (E%), carbohydrates (g) and SFA (g) were introduced to the initial model according to univariate analysis. The association between fat intake and child blood pressure was V-shaped (**Figure 6**), the child blood pressure being lowest in children whose mothers' fat intake was as recommended. In contrast, child blood pressure at four years was positively associated with maternal carbohydrate intake during pregnancy.

Table 5. Maternal intake of energy, fiber, energy-yielding nutrients and sodium at baseline (1st study visit) and during intervention period (2nd and 3rd study visits) in combined dietary intervention group and in Control group. (Modified from Study III)

	Diet (n=160)		**Control** (n=79)		**D vs C**
Mother	1st visit Mean	2nd to 3rd visit Mean (SD)	1st visit Mean	2nd to 3rd visit Mean (SD)	Mean (P-value)*
Energy (kcal)	1979	2033 (401)	1936	1997 (427)	9.1 (0.843)
Fiber (g)	20.4	22.1 (6.4)	19.5	19.8 (5.7)	1.8 (0.006)
Protein (g)	82.0	81.8 (18.5)	79.5	83.2 (18.8)	-2.8 (0.198)
(% of energy)	16.7	16.2 (2.4)	16.5	16.8 (2.1)	-0.7 (0.016)
Carbohydrate (g)	250	259.7 (52.5)	241	252.0 (56.0)	2.0 (0.745)
(% of energy)	50.7	51.3 (5.0)	50.0	50.7 (5.5)	0.4 (0.585)
Fats , total, (g)	68.9	70.9 (20.3)	69.8	69.6 (20.8)	1.6 (0.528)
(% of energy)	31.2	31.0 (4.9)	32.1	31.0 (5.0)	0.4 (0.491)
Fatty acids					
SFA (g)	28.6	26.3 (8.2)	28.8	29.2 (9.8)	-2.9 (0.009)
(% of energy)	13.2	11.8 (2.4)	13.6	13.2 (2.7)	-1.2 (<0.001)
MUFA (g)	22.9	25.4 (8.3)	23.6	22.6 (7.2)	3.1 (0.002)
(% of energy)	10.5	11.4 (2.4)	11.2	10.3 (2.2)	1.3 (<0.001)
PUFA (g)	11.1	13.2 (4.5)	11.5	11.2 (4.3)	2.1 (<0.001)
(% of energy)	5.1	5.9 (1.3)	5.3	5.1 (1.4)	0.8 (<0.001)
Sodium † (g)	3.0	2.9 (0.7)	3.1	3.0 (0.7)	-0.02 (0.789)

SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids. * Dietary intakes during intervention (mean of 2nd and 3rd visits) were compared between groups (D vs C) using analysis of covariance, where the baseline intake (1st visit) was included as a continuous covariate. † NaCl = 2.54 x sodium intake

Table 6. Tertiles for intake of foods and energy-yielding nutrients in maternal diet during pregnancy and six months postpartum, and the proportion of infants with high (above the 85th percentile cut-off point) 32-33 split proinsulin concentration in each tertile of intake. (Modified from Study II)

Dietary Component	Study Visit	Tertile of intake			Infants with high split proinsulin	
			Mean	Range	Number (%)	P*
Grain Products, g †	1st trim.	T1	134	43-176	7 (10.8)	0.034
		T2	206	176-239	8 (11.4)	
		T3	**298**	**239-560**	15 (25.9)	
Fruits and Berries, g	2nd trim.	T1	147	17-232	6 (8.7)	0.006
		T2	**300**	**233-373**	17 (27.4)	
		T3	543	373-1443	7 (11.1)	
Fat, total, g	3rd trim.	T1	46	23-58	11 (18.6)	0.038
		T2	**67**	**58-76**	4 (6.1)	
		T3	93	76-175	14 (20.9)	
Cheese, g ‡	3rd trim.	**T1**	**19**	**0-30**	12 (18.8)	0.038
		T2	39	30-52	4 (6.1)	
		T3	77	52-177	13 (21.0)	
Butter, g	3rd trim.	T1	0.0	0.0-0.3	4 (6.5)	0.029
		T2	1.5	0.3-3.8	15 (23.4)	
		T3	11.5	3.8-53.3	10 (15.2)	
Vegetable-oil-based margarine and vegetable oil, g	3rd trim.	T1	15	1-22	15 (22.7)	0.045
		T2	28	22-34	4 (6.8)	
		T3	**47**	**34-76**	10 (14.9)	
Milk, g ‡	6 mo	T1	72	0-151	9 (13.6)	0.035
		T2	246	151-350	15 (25.4)	
		T3	**549**	**350-1016**	5 (8.5)	

Only dietary components significantly associated with infants' high 32-33 split proinsulin concentration are shown.
Tertiles closest reflecting the **recommended intake** are in bold.
* The association between tertiled intake of dietary components and infants' split proinsulin group were analyzed by Chi-squared test.
† All foods, including cereals (e.g. bread, porridge, pasta and rice) independent of fiber content.
‡ Independent of fat content

Table 7. Quartiles or tertiles for intake of energy-yielding nutrients in mothers and children.			
	Quartile or Tertile	**Intake** n	Range
Maternal intake during pregnancy			
Carbohydrate, grams	Q1	63	140; 215
	Q2	63	216; 254
	Q3	64	**255; 282**
	Q4	63	283; 447
Monounsaturated fatty acids, grams	Q1	63	10.4; 19.3
	Q2	63	19.4; 23.0
	Q3	64	**23.1; 27.8**
	Q4	63	27.9; 51.0
Fats total, % of energy intake	T1	36	23.1; 29.5
	T2	37	**29.6; 32.9**
	T3	36	33.0; 41.2
Carbohydrates total, grams	T1	36	157; 238
	T2	37	**239; 269**
	T3	36	270; 447
Child intake at 4 years of age			
Fats total, grams	T1	35	19.0; 39.1
	T2	34	**39.2; 50.4**
	T3	34	50.5; 69.0
Proteins, grams	**T1**	35	**33.3; 49.8**
	T2	34	49.9; 60.8
	T3	34	60.9; 86.3
Fiber, grams	T1	34	4.9; 99
	T2	35	10.0; 13.0
	T3	**34**	**13.1; 19.9**

Only components which were significantly associated with infants' blood pressure at six months or four years are given. The quartiles or tertiles closest to that recommended are in bold.

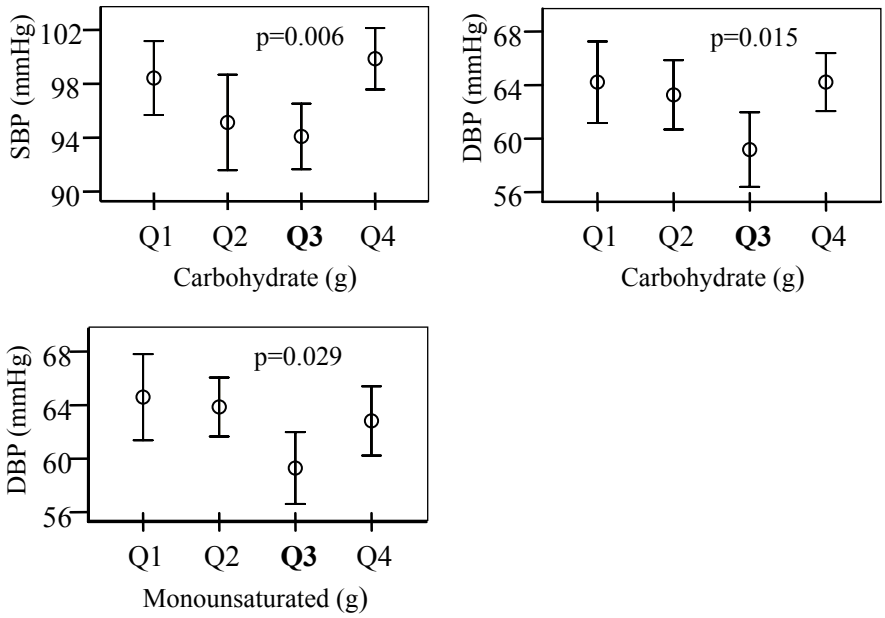


Figure 5. Association between maternal nutrient intake (in quartiles Q1 to Q4) during pregnancy and infant systolic (SBP) and diastolic (DBP) blood pressure (mean, 95%CI) at the age of six months. Results are based on analysis of variance. The quartiles closest to that recommended are in bold. Modified from study III.

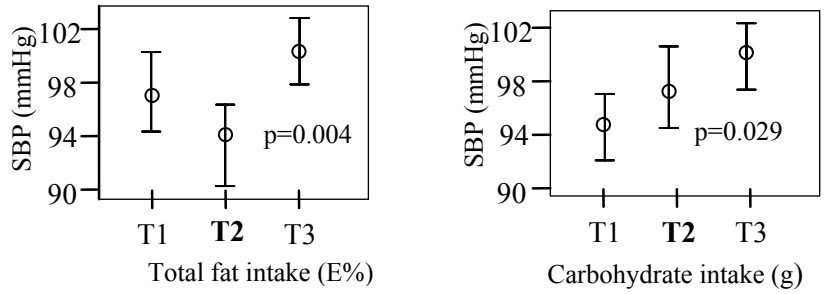


Figure 6. Association between maternal nutrient intake (in tertiles T1 to T3) during pregnancy and child systolic (SBP) blood pressure (adjusted mean 95%CI) at the age of four years according to final multivariate model. The tertiles closest to that recommended are in bold.

5.4. Child clinical characteristics and cardio-metabolic determinants

Fetal sympathovagal activation: The LF/HF ratio of fetal HR variability was positively associated with higher relative weight of the placenta (r=0.4, p=0.008), and decreased with advancing gestation (r=-0.3, p=0.04). The fetal HR was not associated with the maternal or infant characteristics presented in Table 2 (p>0.40). Cord blood leptin or

adiponectin separately were not correlated with the LF/HF ratio of fetal HR variability, and only a marginal correspondence was found between leptin/adiponectin ratio and the LF/HF ratio of fetal HR variability (r=0.293, p=0.098).

High 32-33 split proinsulin: Higher supra-iliac skinfold thickness (OR=1.24, 95%CI 1.00; 1.53, p=0.045), waist circumference (OR=1.14, 1.01; 1.29, p=0.035), leptin (OR=1.89, 1.01; 3.55, p=0.048) and leptin/adiponectin ratio (OR=1.90, 1.09; 3.44, p=0.029) at the age of six months, each included as continuous variables in logistic regression analysis, were associated with an increased likelihood of high 32-33 split proinsulin in infants. Lower (Q1: <3320g) birth weight infants carried an increased risk of subsequent high split proinsulin (OR=2.59, 1.11; 6.00, p=0.027), while other infants' anthropometrics or clinical characteristics at birth or at the age of six months were not statistically significantly related to high split proinsulin. Likewise other cardio-metabolic markers, the LF/HF ratio of fetal HR variability and blood pressures at six months or four years, were not associated with high split proinsulin concentration.

Blood pressure: The child's birth weight and length correlated positively with systolic blood pressure (r= 0.227, p=0.018 and r=0.248, p=0.009, respectively) measured at the age of four years but not at six months. The only infant anthropometric item correlating with blood pressure at six months was child length at the same age (systolic: r=0.256, p=0.001; diastolic: r=0.179, p=0.019). Fetal sympathetic activation was not associated with blood pressure measurements at six months or four years.

A multivariate model of anthropometrics (weight, length, body surface area (BSA), waist circumference, supra-iliac skinfold thickness) from birth to four years of age showed the increase in child BSA at four years (regression coefficient B=54.5, 95%CI 21.2; 87.7, p=0.002) to be associated with an increase in systolic blood pressure and the increase in supra-iliac skinfold at the age of four years showed a similar tendency (B=0.74, 95%CI -0.04; 1.52, p=0.064). The adjuster R^2 for the model was 0.17. According to the model for child diastolic blood pressure, child weight at the age of four was the only explaining factor (r=0.289, p=0.002).

Of the adipocyte-derived hormones measured from cord blood and at six months postpartum, leptin at both time-points tended to correlate positively with child's systolic (r=0.229, p=0.053 and r=0.188, p=0.062, respectively) and diastolic blood pressure (r=0.216, p=0.068 and r=0.193, p=0.055, respectively) at the age of four years. Leptin measured from cord blood but not at six months tended to be positively correlated with six months diastolic blood pressure (r=0.174, p=0.061), while no associations were found with systolic blood pressure at six months. Adiponectin did not correlate with blood pressure at six months or four years of age. As leptin is derived from adipose tissue, the partial correlations were analyzed by adjusting for BSA at birth or at six months. The partial correlation between cord blood leptin and

diastolic blood pressure at four years adjusted for BSA at birth was r=0.207 (p=0.083), while other observed correlations were attenuated.

5.5. Child postnatal dietary intake and cardio-metabolic determinants

32-33 split proinsulin: Those infants who were exclusively or partially breastfed for at least six months had a lower risk of high split proinsulin compared to infants with shorter duration of breastfeeding (OR=0.20, 95%CI 0.09; 0.46, p <0.001). Further, maternal milk consumption meeting the recommendations (T3) at six months postpartum resulted in contrast in a lower infant risk of high split proinsulin compared to tertiles T1 and T2 (Table 6).

Blood pressure: The diastolic blood pressure at the age of six months was lower in those infants who were being exclusively or partially breastfed at six months compared with those who were not. Further, a similar tendency was found for systolic blood pressure. The effect of breastfeeding was -2.6 mmHg (95%CI -5.6; 0.5, p=0.098) for systolic and -3.5mmHg (-6.6; -0.6, p=0.017) for diastolic blood pressure. Furthermore, children with longer total breastfeeding duration still tended to manifest lower systolic blood pressure at the age of four years (r=-0.166, p=0.085).

According to the multivariate model the children's own protein intake and total fat intake at the age of four years were the most important nutritional factors simultaneously explaining their systolic blood pressure at the same age. The child blood pressure was lowest in intake groups best meeting the recommendations. Further child fiber intake was shown to be linked to diastolic blood pressure. **Figure 7** shows the nonlinear patterns of associations between children's blood pressures and dietary intakes according to univariate analyses (ANOVA).

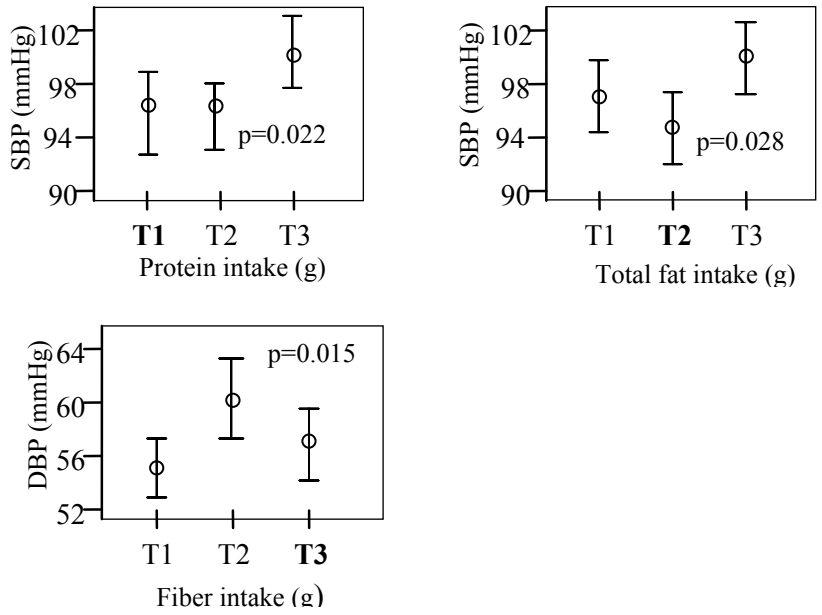


Figure 7. The association between child nutrient intake (in tertiles T1 to T3) and systolic (SBP) or diastolic (DBP) blood pressure (mean 95%CI) at four years. The tertiles closest to that recommended are in bold. Results are based on analysis of variance.

5.6. The applicability of maternal dietary intervention to modify the cardio-metabolic risk factors in infancy and childhood

As shown in Table 5, the maternal dietary counseling from the first trimester onwards increased maternal intakes of fiber and MUFA and PUFA during pregnancy, while the intake of SFA was reduced during pregnancy. These changes were explained by increased consumption of vegetable-oil-based margarine and vegetable oils and decreased consumption of butter and butter-based spreads. Further, the intake of proteins as a proportion of energy intake was reduced during pregnancy in the combined dietary intervention group compared to the controls.

In evaluating the applicability of dietary counseling to reduce the children's cardio-metabolic risk factors, we found that infants' risk of high 32-33 split proinsulin concentration was lower in the dietary counseling groups with probiotics or placebo compared to the control group (**Figure 8**); diet/probiotics OR=0.29 (95%CI 0.11; 0.80, p=0.016) and diet/placebo OR=0.31 (0.12; 0.80, p=0.015). No differences were found between the study groups in fetal sympathetic activation at birth. Also infants' blood pressure at the age of six months and four years were comparable between the study groups.

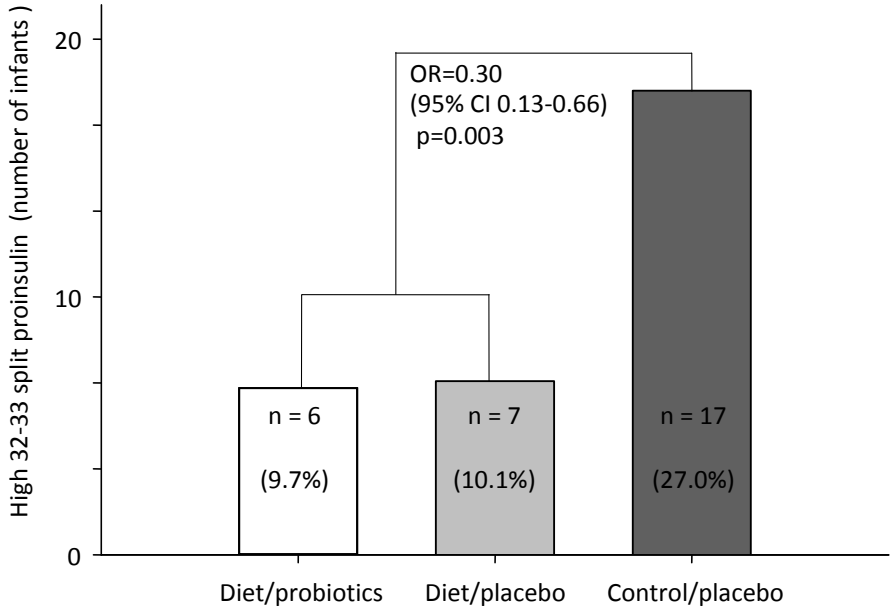


Figure 8. The impact of dietary intervention on the infants' high (above the 85th percentile cut-off point) 32-33 split proinsulin concentration.

6. DISCUSSION

The data implying early nutritional influences on cardio-metabolic risks in humans derive mainly from epidemiological studies of extreme prenatal circumstances such as exposure to famine (Ravelli et al. 1998, Painter et al. 2006). In these demonstrations low birth weight has been used as a marker of an adverse intrauterine environment as well as a predictor of a heightened risk of subsequent chronic diseases (Barker et al. 1989 and 1993, Hales et al. 1991). However, even a slight nutritional disturbance in utero producing no change in birth weight may nevertheless program the fetal cardio-metabolic system to function abnormally (Lucas 1991, Barker 1998). Further previous demonstrations suggest that this programming constitute a continuum where both intrauterine and postnatal life are of significance (Waterland and Garza 1999, Jones and Ozanne 2009). This notwithstanding, studies investigating the impacts of specific dietary components on offspring health in the case of well-nourished pregnant women are lacking, and most previous studies have concentrated on the effects of either intrauterine or postnatal nutritional environment and are not able to dissect the impacts of these periods on the offspring's later health.

6.1. Material and methodological aspects

The present prospective, randomized and controlled mother–infant nutrition and probiotic trial (NCT00167000; section 3, http://www.clinicaltrials.gov) in a healthy cohort was ideal for the purpose of evaluating the nutritional determinants influencing early cardio-metabolic programming. Firstly, the continuous food recordings of maternal diet during pregnancy and the evaluation of the children's dietary consumption at the age of four years allowed a study of the dietary intakes in detail and an evaluation of the direct impacts of specific dietary components on infant and child cardio-metabolic status. Indeed, food recording for three consecutive days by household measures as used here is the gold standard in measuring the intake of energy and macronutrients (Willet 1998). Three days, especially when at least one weekend day is included, is at group level a period long enough to allow characterization of the usual individual intake of energy and macronutrients representatively. Self-reporting against outside observer increases the vulnerability to biased reporting as well as dietary recording in the context of a nutritional intervention (Byers 2003, Livingstone et al. 2004). Subjects participating voluntarily in such intervention studies are, however, for the most part highly motivated and concerned to keep accurate records, although a modest under-reporting of total dietary intake is possible amongst some of the most overweight or obese women. The accuracy of recording was furthered by provision of written and verbal instructions for record-keeping beforehand. Further, the daily dietary intakes of energy, foods and nutrient were calculated by computerized program Micro-Nutrica nutrient analysis software. The procedure is based on Finnish

analysis of food compositions and on reliable foreign food composition databases used if some preparate is not available or is not relevant in Finnish databases. This program was one of the best available during the study years.

The second strength of the study protocol is that the prospective follow-up continued from the first trimester of pregnancy to early childhood enabled dissection of the effects of intrauterine and postnatal nutritional environments on the child. Further, the clinical follow-up was appropriately timed to cover critical window of programming (Field 2009). Thirdly, the intervention protocol included dietary counseling. All pregnant women attended municipal well-women clinics, and women in the dietary intervention groups (Diet/probiotics and Diet/placebo) received additional intensive dietary counseling at each study visit. The counseling was optimally targeted to modify the dietary elements associated with important developmental components; dietary fatty acid composition and fiber intake in conjunction with a balanced diet.

Finally, the comprehensive randomization scheme was combined with probiotics, as the overall aims of the trial were to study the impact of early nutrition on immunological, metabolic and microbiological programming, with long-term accurate follow-up, and thereby to reduce the risk of diseases in the child. Although the present study was not primarily focused on the impact of probiotic intervention, the inclusion of the Diet/probiotics group in the approach was justified in view of the demonstrations of a link between microbes and immune responses as well as fat metabolism and cardiovascular diseases. Indeed, inflammatory and immune mechanisms might be particularly important in the development of cardio-metabolic disorders (Pradhan 2007), and probiotics possess conspicuous immunoregulatory and particularly anti-inflammatory properties (Isolauri et al. 2001). Further, probiotics can correct an aberrant gut microbiota composition linked to the immunological and metabolic development of the child (Guarner and Malagelada 2003, Kalliomäki et al. 2008). Offsetting the abundant benefits of prospective study protocols, the loss of subjects is a challenge and a limitation which can be compounded by a bias due to intervention. Importantly, no such a bias was seen in the present study, as the reasons and the numbers of discontinuing women and children were comparable amongst the study groups.

Elevated blood pressure runs on from childhood (Fuentes et al. 2002) into adult ages (Chen and Wang 2008) and may predict hypertension and metabolic syndrome risks later in life (Sun et al. 2007). It is one of the most important risk factors for subsequent cardiovascular diseases (National High Blood Pressure Education Program Working Group 2004). However, it is not possible to measure blood pressure during fetal life in clinical studies. Sympathetic over-activation has been linked to the programming of hypertension and metabolic disorders (Reaven et al. 1996), and fetal sympathovagal activation – an important blood pressure regulator – can be evaluated during delivery by fetal ECG (Metsälä et al. 1995, Siira et al. 2007). With this procedure the evaluation of cardio-metabolic programming could be initiated as early as at intrapartum.

Fetal sympathovagal activation was evaluated by the LF/HF ratio of HR variability using well-established means, power spectral analysis. Some limitations to this procedure and the relevant data collection need to be acknowledged in that these may have affected subject loss. Continuous fetal ECG recording was possible only by fetal scalp electrode, and thus the membranes had to be ruptured. Further, spectral analysis requires a two-minute continuous signal segment at a stable HR and at least one hour's recording to obtain an optimal sampling rate (500Hz) in digitalizing the R-R intervals. The use of two-minute signal segments is also a strength in that interruptions arising from uterine activity do not disturb the analysis (Pello et al. 1991). Further, the holistic prospective study protocol made it possible to avoid the effects of external confounding factors such as analgesic procedures.

Direct intra-arterial measurement of arterial pressure with a catheter is the gold standard for blood pressure measurement but is not practical or appropriate for public health screening and thus an indirect procedure is generally used. Ambulatory measurement for 24 hours most reliably reveals the actual blood pressure level but cannot be performed before preschool age. These notwithstanding a clear methodological strength in this challenging situation are the validated blood pressure measurements made in serial manner. Measurement of blood pressure as early as at six months and four years of age has pros and cons. Although there is evidence of blood pressure tracking from six months onwards (Fuentes et al. 2002) it is possibly less precise than after the age of seven to eight years (Chen and Wang 2008).

Infant and child growth, including adiposity measures, waist circumference and skinfold thickness, were evaluated at study visits by the same research nurses. Only measures at birth were made by midwives at the hospital maternity ward. As about 97% of the children studied were born in Turku University Hospital and the remaining 3% in central or regional hospitals nearby, the small variation in measurements should be acceptable.

Non-fasting blood sampling in six-months-old infants was a limitation in this study, but fasting was not possible for ethical reasons. We did however standardize the collection so as to be carried out before noon and selected metabolic markers not particularly sensitive to the non-fasting state, unlike e.g. blood glucose and insulin concentrations (Glauber et al. 1986, Karlsson et al. 2004, Gil-Campos et al. 2010). A pre-feed blood sample, as in a very previous study (Ibanez et al. 2010), could have minimized some of the variation between infants' serum 32-33 split proinsulin, intact proinsulin, leptin and adiponectin concentrations. However, as six-month-old infants eat very often and are in general in an almost non-stop post-feed state, the error here should be acceptable. Further, the infants' blood samples were analyzed by accurate methods in experienced hands at the NIHR Cambridge Biomedical Research Centre, Core Biochemical Assay Laboratory (Cambridge, UK).

The infants' cardio-metabolic status was evaluated by various measures. High (above 85th percentile of the concentration) 32-33 split proinsulin at the age of six months was associated with simultaneously determined clinical measures of adiposity as well as with leptin to adiponectin. High split proinsulin at the age of six months very likely indicates susceptibility to adverse metabolic programming in these infants, and can be useful in its assessment.

6.2. Dietary intervention and cardio-metabolic programming

In previous intervention studies prenatal dietary interventions have been seen to influence offspring birth size (Olsen and Secher 2002, Merialdi et al. 2003, Moses et al. 2006), while postnatal interventions produced an effective impact for example on postnatal weight gain (Hakanen et al. 2006), blood pressure (Singhal et al. 2001, Forsyth et al. 2003, Niinikoski et al. 2009) and glucose-insulin metabolism (Singhal et al. 2003a). The results here would indicate that very early dietary counseling of pregnant women can benefit their offspring's metabolic development, as shown here by a decreased incidence of high split proinsulin in six-month-old infants in the dietary counseling groups compared to the control group. Since the maternal and fetal nutritional environments are closely related, an explanation for the beneficial effect of dietary counseling on the child could be extrapolated from the recent observation that a higher intake of unsaturated fatty acids can improve insulin sensitivity (Riserus et al. 2009), whereas a high saturated fat content in the diet promotes the secretion of pro-inflammatory cytokines (Cani et al. 2007), causally linked to adverse metabolic status by insulin resistance (Shoelson et al. 2006). Further, lower maternal dietary protein intake during pregnancy in the dietary intervention groups compared to the controls would mediate the benefit seen in infants. This notion is supported by a comparable finding in adults carrying favorable glucose-insulin metabolism after a lower maternal protein intake during pregnancy (Shiell et al. 2000).

Although maternal dietary counseling reduced the infants' risk of high split proinsulin, it showed neither a direct impact on fetal cardiac sympathovagal activation nor on blood pressure in infancy or childhood. There are several reasons for this. One is statistical power, which was insufficient here to study the effect on fetal cardiac sympathovagal activation. Another could be the limited follow-up period. In previous postnatally initiated studies similar dietary elements have benefited cardiac sympathovagal activation in adults and blood pressure in adolescents (Niinikoski et al. 2009, Sjöberg et al. 2010). Accordingly, the effectiveness of dietary counseling may emerge only after a longer follow-up. Further reasons explaining the result in offspring blood pressure can be: (1) the postnatal dietary counseling was not sufficiently efficacious to modify child dietary intake at the age of four years, (2) the relationships were non-linear, as seen between maternal dietary intake during pregnancy and infant blood pressure at six months, and (3) nutrients other than fats also proved to affect the connection. Indeed, the diet is always a mixture of a number of nutrients with possible complex interactions.

6.3. Maternal metabolic status and child cardio-metabolic programming

Maternal nutrition and energy metabolism determine the nutrient supply to the fetus and trigger its subsequent cardiovascular status (Roseboom et al. 1999 and 2001, Boney et al. 2005). Previous observations have shown that high maternal pre-pregnancy BMI may predispose the offspring to obesity-related risks in childhood (Thomas et al. 2007, Catalano et al. 2009, Fraser et al. 2010). Here higher maternal pre-pregnancy BMI was linked to fetal cardiac sympathovagal activation as measured by heart rate variability. Accordingly, heart rate variability has been shown to be related to BMI in obese adults (Molfino et al. 2009). The present finding is important, as the sympathetic over-activity, metabolic disorders and cardiovascular diseases frequently coexist (Reaven et al. 1996, Mancia et al. 2007).

Also excessive maternal weight gain during pregnancy has been associated with adverse pregnancy outcomes in both mother and child (Nohr et al. 2008, Wrotniak et al. 2008). The present findings support this in showing that mothers' inappropriate gestational weight gain was associated with a heightened risk of high split proinsulin in their infants. Further, as in previous studies, the results here evidence higher childhood systolic blood pressure in offspring whose mothers' weight gain during pregnancy was in the lowest or in the highest tertiles (Godfrey et al. 1994, Mamun et al. 2009) or whose mothers had elevated blood pressure (Gillman et al. 2004) compared to the offspring of other mothers.

One probable mediator between these maternal cardio-metabolic determinants and infant cardiovascular development is the placenta. This can be explained by the previous observations showing that the placenta mobilizes maternal adipose tissue fat stores and channels important fatty acids to the fetus (Duttaroy 2009), high maternal pre-pregnancy BMI may accelerate placental growth (Swanson and Bewtra 2008), and the adipose tissue in humans reflects the dietary fatty acid content during the last one to two years (Baylin and Campos 2006). On the other hand, a less health-conscious dietary pattern consisted of foods with high saturated fat and low fiber content has been associated with intima-media thickness (Nettleton et al. 2007, Mikkilä et al. 2009), and high saturated fat intake is known to impair endothelial function (Miller et al. 2009). The long-term dietary fat content of mother may thus impact her vascular structure and endothelial function, which are further reflected in placental development and function (Savvidou et al. 2003), and on the fetal development.

6.4. Intrauterine versus postnatal growth and cardio-metabolic programming

Small body size at birth and during infancy have been seen as markers of poor fetal nutrition. This may lead to fetal adaptations which program a future propensity to adult diseases such as disturbed glucose-insulin metabolism and elevated blood pressure (Barker 1994). The present results on infants' adverse metabolic status, i.e. high split

proinsulin concentration, support these epidemiological findings, as the lowest birth weight quartile (T1: <3320g) was a risk factor compared to higher quartiles.

In the context of the current obesity epidemic, the contribution of birth weight to subsequent blood pressure and glucose-insulin metabolism has been suggested to be non-linear (Launer et al. 1993, Huxley et al. 2000, Manderson et al. 2002, Wei et al. 2003). In accordance, birth weight correlated positively with childhood blood pressure in the present study, whereas no correlation was found between birth weight and blood pressure in infancy. This discrepancy could be explained by the fact that the effect of birth weight on blood pressure may change with age (Davies et al. 2006).

The birth weight-associated risks of subsequent cardio-metabolic diseases can be modified by later growth, and especially accelerated growth can be detrimental both during infancy and thereafter (Eriksson et al. 1999, Forsen et al. 2000, Ekelund et al. 2006). The results here suggest that particularly an early postnatal increase in adiposity may play an important role in cardio-metabolic programming. Indeed, the infant's current adiposity measures, supra-iliac skinfold thickness, waist circumference and the ratio of adipocyte-derived cytokines at six months were positively associated with high split proinsulin concentration. Further, at four years of age increased adiposity measures were associated with higher blood pressure in the child. The gender-specific effects of the growth pattern on the risks of cardio-metabolic diseases are also known (Flanagan et al 2000, Adair and Cole 2003) but were not studied here.

The mechanisms underlying the associations between different growth patterns and subsequent cardio-metabolic outcomes are particularly complex and not in fact fully understood (Gluckman and Hanson 2004b, Symonds et al. 2009a). For this reason only two heavily simplified outlines of possible mechanisms are presented here and illustrated in **Figure 9.** The adverse programming effect of low birth weight can be explained by its effect in provoking postnatal catch-up growth. This promotes the growth of adipose tissue, especially viscerally (Rogers 2003, Rolfe et al. 2010), and may thus lead to increased abdominal fat mass later in life (Singhal et al. 2003b, Ylihärsilä et al. 2007) and to adverse metabolic consequences. In the progression of hypertension the reduced number of nephrons in low-birth-weight babies plays a critical role (Luyckx and Brenner 2010, Vehaskari 2010). The nephrogenesis is completed at approximately the 36^{th} week of gestation and will not increase concomitant with postnatal growth. Although the kidneys' glomerular filtration rate, i.e. function, is normal in early life, their reserve to adapt to challenging "mismatch" situations such as obesity may be reduced.

High birth weight or macrosomia may result in obesity-related comorbidities even in childhood due to tracking of weight status and/or due to disturbed hormonal functions. Raised birth weight is linked to exposure to an abundant nutritional environment in utero, i.e. maternal obesity and hyperglycemia together with subsequent

hyperleptinemia and hyperinsulinemia, which are intricately involved in mechanisms of the developmental programming of cardio-metabolic dysfunction.

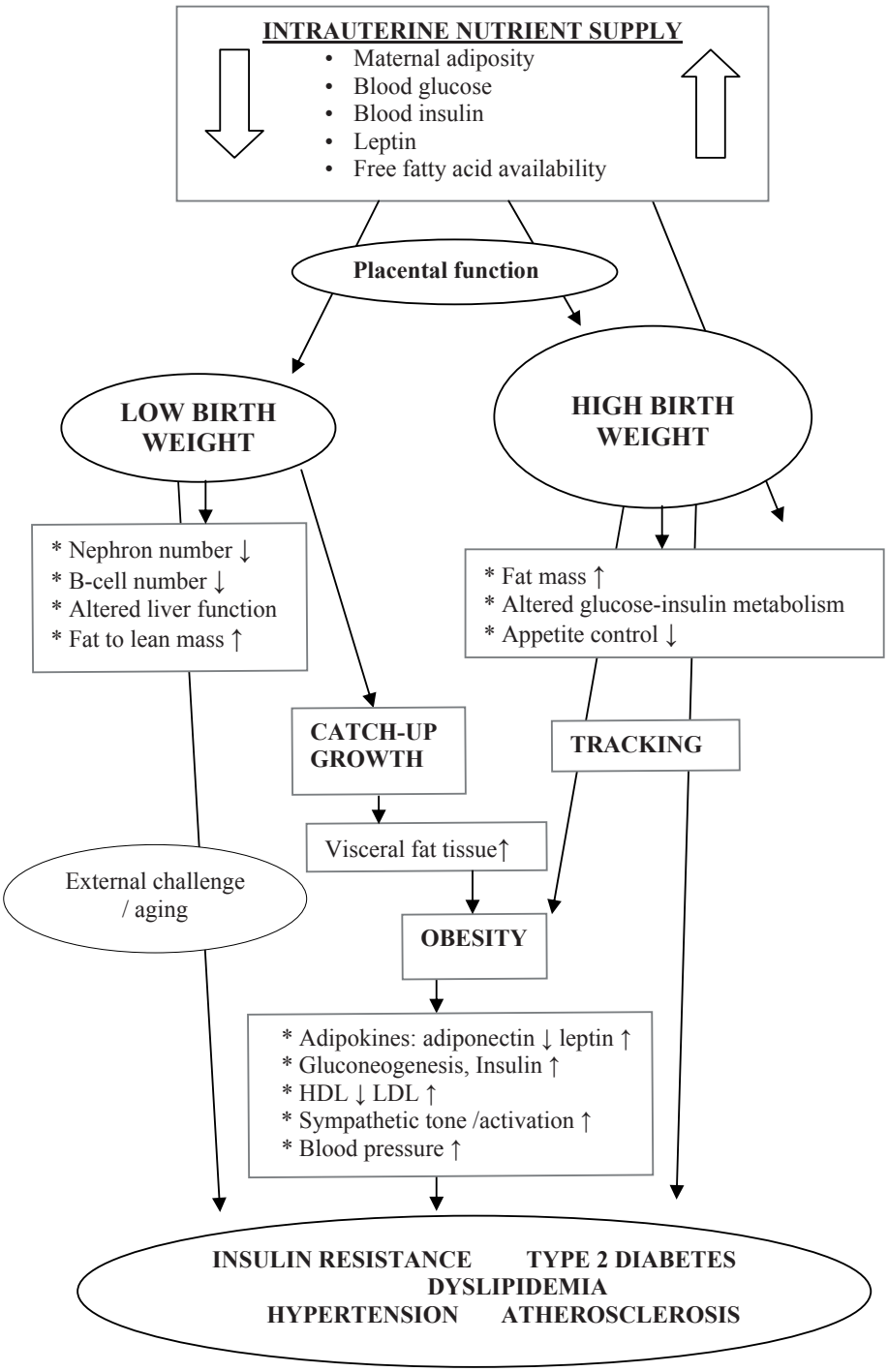


Figure 9. Mechanisms underlying child growth patterns and cardio-metabolic outcomes

6.5. Specific dietary components and cardio-metabolic programming

Thus far, conceptions of the importance and direct influences of maternal dietary fat and protein intake during pregnancy on the offspring's glucose metabolisms and blood pressure have remained inconsistent (Campbell et al. 1996, Godfrey et al. 1996 b, Shiell et al. 2000, Adair et al. 2001, Roseboom et al. 2001, Leary et al. 2005). The present results suggest a more important role for dietary fats than proteins during pregnancy in the offspring's cardio-metabolic programming, as the maternal intake of fat and specific fat-containing food products *i.e.* butter and fatty milk affected the offspring's risk of high split proinsulin and blood pressure in infancy and childhood, while protein intake had no independent effects. Further, the findings on maternal total fat and MUFA intake and butter and fatty milk consumption suggest that the recommended use of these food items and intake of these nutrients during pregnancy may benefit both the infant's glucose-insulin metabolism as well as blood pressure programming.

Interestingly, the intrauterine carbohydrate supply explained infant blood pressure at six months better than fat exposure to MUFAs, while maternal total fat intake as percentage of energy intake was more important for the offspring's blood pressure in childhood. This faster but briefer impact of total carbohydrates versus fats on the child's blood pressure could be induced by the anabolic properties of insulin. Higher insulin concentrations may be consequent on temporary minor hyperglycemic peaks (Simmons 1997) after increased consumption of refined carbohydrates. This adds significant knowledge to a previously controversial item, and suggests that maternal dietary carbohydrates during pregnancy influence child blood pressure programming mostly via effects on growth, while the fatty acid composition of the maternal diet has implications as early as during the intrauterine period, this possibly activating a long-lasting process in the child's vascular system.

A study made among adults has shown that increasing doses of fish oil supplementation (2g/day) reduced the LF/HF ratio of heart rate variability when compared to sunola oil (high oleic acid sunflower oil) supplementation (6g/day) (Sjöberg et al. 2010). This suggests that the maternal diet during pregnancy may influence fetal heart rate variability. Here, in contrast, maternal dietary intake during pregnancy was not linked to fetal cardiac sympathovagal activation. This may be a consequence of the very early age at measurement of outcome. Indeed, recent human and animal studies have pointed to the relevance of intrauterine as well as early postnatal life in cardio-metabolic development (Jones and Ozanne 2009). The other reasons could be the limited sample size, or on that the maternal use of fish oil supplements was not studied here, although it was evaluated during pregnancy and will be reported elsewhere. Findings of Sjöberg and associates (2010), but not our, are supported by the previous observations showing the importance of fish oil consumption in the prevention and management of cardio-metabolic diseases (Lee et al. 2009) and the impact of sympathetic over-activity on the risk of cardiovascular

diseases in the context of metabolic disorders (Reaven et al. 1996, Mancia et al. 2007). However, due to many physiological effects of the fish oil opposite results could also be possible in future studies. Indeed, the fish oil supplements have been shown to raise fasting plasma glucose in subjects with type 2 diabetes (Friedberg et al. 1998, Martin de Santa Olalla et al. 2009). Especially the high doses of n-3 fatty acids (~3-8g/day) showed to be deleterious (Martin de Santa Olalla et al. 2009) and may thus contribute to the development of impaired glucose metabolism. This controversy could be explained by the effect of fish oil to reduce insulin response to oral glucose without altering the glycemic response.

The results on child postnatal dietary intakes combined earlier findings of dietary fat and protein content as blood pressure determinants (Niinikoski et al. 2009, Singhal et al. 2007) and suggest that high protein intake during childhood may compound the adverse effect of high dietary fat consumption on child systolic blood pressure. Here systolic blood pressure was highest in children whose protein and/or fat intakes were in the highest tertiles and lowest in those whose intakes were as recommended. Although this was not found in our analyses, the reason could be that the high amount of protein or fat in the diet is associated with lower intake of vegetables, fruits and berries, and higher intake of salt inducing blood pressure elevation (Appel et al. 2006).

6.6. Breastfeeding and cardio-metabolic programming

The nutrient composition of breast milk together with its bioactive components are important links between breastfeeding and beneficial cardio-metabolic programming (Singhal et al. 2001 and 2003a). Here, as in previous studies (Martin et al. 2005), the longer duration of breastfeeding was seen to benefit blood pressure programming. In fact, the infants who were still being exclusively or partially breastfed at the age of six months manifested lower current blood pressure, and further, the longer duration of breastfeeding tended to lower blood pressure even at the age of four years. Furthermore, breastfed infants had a reduced risk of high split proinsulin at six months. This metabolic advantage of breastfeeding may be related to protection against obesity (Dewey 2003) and to more controlled and physiological growth pattern in breastfed versus formula-fed infants (Agostoni et al. 2009, Griffiths et al. 2009). It is also possible that the collective composition of the gut microbiota plays a role in metabolic development. Indeed, bifidobacteria, which typify the gut microbiota of the healthy breastfed infant (Fanaro et al. 2003), may dampen the systemic endotoxemia induced by bacterial lipopolysaccarides (Griffiths 2004), and thereby improve metabolic status (Shoelson et al. 2006, Cani et al. 2007).

6.7. Novel findings related to programming theory

This well controlled prospective intervention study allows analysis of the intrauterine and postnatal nutritional environments and evaluation of the impact of child growth

from birth to four years of age. The present findings add important information showing that the maternal metabolic status even before pregnancy may initiate the offspring's cardio-metabolic programming, and that postnatal factors related to nutrition, metabolism and growth can modulate this cascade originally initiated during fetal life.

The present results suggest that maternal dietary total carbohydrate intake, regardless of the source, during pregnancy influence child blood pressure programming mostly via the effects on growth, while the amount and quality of dietary fats as early as the intrauterine period may activate a long-lasting process probably by modifying the child's vascular system. Further, an attainment of recommended intake of total fat and MUFA, and use of fatty/low-fat milk as well as minor use of butter in the mother's diet during pregnancy may benefit the offspring's cardio-metabolic programming. The favorable programming could be strengthened by child's postnatal intake of proteins and total fats meeting the recommendations.

From the methodological point of view, the associations observed between adipokines and outcome measures suggest that leptin and adiponectin may play a role in the cardio-metabolic programming of the child. As the adipokine levels are attributable to adiposity and can possibly regulate placental growth (Nelson 2008), these may also mediate the effects of maternal pre-pregnancy BMI and pregnancy weight gain on the offspring's cardio-metabolic development.

6.8. Future prospects

The maternal metabolic status from the pre-pregnancy period onwards would appear to be an important denominator for the offspring's future health. To reverse the increasing rates of cardio-metabolic risk factors in subsequent generations interventions should be initiated at risk women already before pregnancy, in fact before they plan to conceive. Further, in coming studies the intervention should be carried out during pregnancy, as here, and be continued postnatally with a more frequent follow-up until childhood or even into adolescence. It has in fact been hypothesized that the mother's own nutrition, health and development in childhood and before pregnancy is the origin of disease susceptibility in the next generation. This calls for long-term follow-up especially in the present study population and also for research focusing on the clinical interactions between maternal cardio-vascular status, placental development and fetal long-term cardio-metabolic health.

7. SUMMARY AND CONCLUSION

Industrialized countries worldwide have been faced with a progressive increase in obesity-related conditions such as disturbed glucose metabolism and elevated blood pressure, and the velocity of propagation is particularly prominent in the pediatric population. Reversing this trend would represent an important breakthrough for public health care and well-being.

High maternal body mass index from the pre-pregnancy period onwards carries long-lasting consequences for the offspring's health in being associated with fetal cardiac sympathovagal activation, a denominator of cardio-metabolic diseases. Here appropriate maternal weight gain during pregnancy promoted beneficial metabolic programming in the infant and lower blood pressure in four-year-old children. Also maternal blood pressure in early pregnancy determined the offspring's blood pressure in childhood. Furthermore, the amount and type of carbohydrates and fats in the maternal diet during pregnancy proved to be especially important both for the infants' metabolic status and blood pressure in infancy and childhood. Indeed, the maternal intake of total fat and specific saturated fat- and carbohydrate-containing food products, *i.e.* butter, fatty milk and grain products, affected the infant's risk of high split proinsulin concentration. Further, the recommended intake of total fat and MUFA, and use of fatty milk as well as minor use of butter by the mother during pregnancy may benefit both the infant's glucose-insulin metabolism as well as blood pressure programming. The maternal carbohydrate intake during pregnancy explained better than fat intake the infants' blood pressure at six months, while maternal total fat intake during pregnancy was the most important intrauterine dietary factor for the offspring blood pressure in childhood. This suggests that the fatty acid composition of the maternal diet as early as the intrauterine period activates a long-lasting process affecting the child's cardiovascular and metabolic development.

Child birth weight was shown to be linked to early cardio-metabolic markers in infancy and childhood. However, postnatal growth, especially when linked to adiposity, proved to be more important for long-term programming. Indeed, the current adiposity status associated with the infants' risk of high split proinsulin at six months of age. The high concentration in this marker was proved to evince susceptibility to adverse metabolic programming, but it was not associated with fetal sympathovagal activation or blood pressure in infancy and childhood. Postnatal increase in body adiposity measures further played an important role in blood pressure in childhood. Furthermore, the present study points to the relevance of postnatal diet in cardio-metabolic programming. Particularly breastfeeding induced many subsequent health benefits and the recommended dietary total fat and protein amounts in the child's diet were associated with lowest blood pressure at the age of four years.

The intensive dietary counseling provided to the women during pregnancy and breastfeeding and targeting excessive saturated fat and low fiber content in the diet had the potential to improve the offspring's metabolic programming, determined here by a lower risk of high split proinsulin in six-month-old infants. To induce favorable blood pressure programming in the child, more frequent postnatal counseling to mother and child and long-term follow-up until adolescence may be warranted.

To conclude, the present results suggest that a healthy maternal metabolic status from fetal pre-implantation development onwards has long-lasting consequences for the offspring's well-being. This can be further reinforced by healthy maternal nutrition and appropriate weight gain during pregnancy as well as by controlling the child's inordinate postnatal adiposital growth and by following dietary recommendations. A modification of mother and child diet would be a safe, cost-effective and potent means to contribute to life-long health. Early and long-lasting dietary interventions could thus achieve health benefits over one generation.

8. ACKNOWLEDGEMENTS

Work for this thesis was carried out at the Department of Pediatrics, University of Turku and Turku University Hospital and the Functional Foods Forum, University of Turku, during the years 2003- 2011. I wish to express my gratitude to:

- My supervisors Doctor Tiina Ojala, MD, and Professor Erika Isolauri, MD, for all their guidance, support and continuous enthusiasm.

- Professor Olli Simell, MD, Department of Pediatrics, Marja-Riitta Ståhlberg, MD, retired Head of the Department of Pediatrics and Professor Jussi Mertsola, MD, Head of the Department of Pediatrics, and Professor Seppo Salminen, PhD, Head of the Functional Foods Forum for providing the facilities for this study.

- The reviewers of the thesis, Professor Johan Eriksson, MD, and Adjunct Professor Ursula Schwab, PhD, for their insightful and constructive criticism and excellent and valuable comments regarding the thesis.

- My other co-authors Adjunct Professor Kirsi Laitinen, PhD, Tuija Poussa, MSc, Sue Ozanne, PhD, and Johanna Ilmonen, MSc, for their major efforts and enjoyable collaboration. The contribution of Tuija Poussa in statistical analyses and consultation is also acknowledged.

- Research nurses Ulla-Maija Eriksson, Johanna Hvitfelt-Koskelainen and Sari Laksio for their support, practical assistance and invaluable efforts in conducting the study, and Ms Satu Leinonen for assistance in blood sample procedures.

- Dr Anu Huurre, MD, Dr Raakel Luoto, MD, Adjunct Professor Merja Nermes, MD, Katri Niinivirta, MD and Dr Tiina Ojala, MD, for clinical evaluation of the infants. I will also thank you for the enjoyable moments we have shared.

- Mr Robert MacGilleon for revision of the English language. I owe also my thanks to Eeva Uusitalo for her valuable comments related to Finnish abstract.

- All the other members of the NAMI research group and personnel at the Functional Foods Forum for pleasant moments and supportive working environment.

- The NIHR Biomedical Research Centre in Cambridge and the Core Biochemistry Assay Laboratory for laboratory analysis.

- The mothers and children for participating in the study.

- All friends and especially the “KS” group for their support and encouragement.

- My parents, Pirjetta and Raimo and my sister Tia for their love, care and support. Their contribution is further acknowledged for the invaluable effort to realize my most important hobby throughout my life and especially along with this work.

- My love, Niklas, for his love, support, patience and sense of humor without which this work could never have come true.

The study was financially supported by the Sigrid Juselius Foundation, the Foundation for Pediatric Research, an EVO grant from Turku University Hospital, the Turku University Foundation, the Emil and Blinda Miettinen Fund from the Faculty of Medicine / Turku Graduate School of Clinical Sciences in University of Turku, the Juho Vainio Foundation, the Varsinais-Suomi Regional Fund of the Finnish Cultural Foundation, and the Emil Aaltonen Foundation. Food products were donated by Raisio plc (Raisio, Finland), *L. rhamnosus GG* by Valio Ltd (Helsinki, Finland) and *B. lactis* by Chr. Hansen (Hoersholm, Denmark).

Turku, January 2011

Jonna Aaltonen

9. REFERENCES

Adair LS, Kuzawa CW, Borja, J. Maternal energy stores and diet composition during pregnancy program adolescent blood pressure. *Circulation* 2001;104:1034-9.

Adair LS and Cole TJ. Rapid child growth raises blood pressure in adolescent boys who were thin at birth. *Hypertension* 2003;41: 451-6.

Agostoni C, Braegger C, Decsi T, Kolacek S, Koletzko B, Michaelsen KF, et al. Breast-feeding: A commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2009;49:112-25.

Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* 1981;213:220-2.

American Diabetes Association. Gestational diabetes mellitus. *Diabetes Care* 2004;27:S88-90

Appel LJ, Brands MW, Daniels SR, Karanja N, Elmer PJ, Sacks FM. Dietary approaches to prevent and treat hypertension: a scientific statement from the American Heart Association. *Hypertension* 2006;47: 296-308.

Armitage JA, Taylor PD, Poston L. Experimental models of developmental programming: consequences of exposure to an energy rich diet during development. *J Physiol* 2005;565:3-8.

Bao W, Srinivasan SR, Berenson GS. Persistent elevation of plasma insulin levels is associated with increased cardiovascular risk in children and young adults. The Bogalusa Heart Study. *Circulation* 1996;93:54-59

Barker DJ and Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* 1986;1:1077-81.

Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ* 1989;298:564-7.

Barker DJ. Fetal and infant origins of adult disease. *BMJ* 1990;301:1111.

Barker DJ, Osmond C, Simmonds SJ, Wield GA. The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. *BMJ* 1993;306:422-6.

Barker DJ. Mothers, Babies, and Disease in Later Life. *BMJ Publishing Group*. London, UK. 1994

Barker DJ. In utero programming of chronic disease. *Clin Sci* 1998;95:115-28.

Barker DJ. The developmental origins of chronic adult disease. *Acta Paediatr Suppl* 2004;93:26-33.

Barker DJ, Osmond C, Forsen TJ, Kajantie E, Eriksson JG. Trajectories of growth among children who have coronary events as adults. *N Engl J Med* 2005;353:1802-9.

Barker DJ. The origins of the developmental origins theory. *J Intern Med* 2007;261:412-7.

Baylin A and Campos H. The use of fatty acid biomarkers to reflect dietary intake. *Curr Opin Lipidol* 2006;17:22-7.

Becker W, Lyhne N, Pedersen AN, Aro A, Fogelholm M, Phorsdottir P, et al. Nordic Nutrition Recommendations 2004 – intergrating nutrition and physical activity. *Scan J Nutr* 2004;48:178-187.

Ben-Dov IZ and Bursztyn M. Ambulatory blood pressure monitoring in childhood and adult obesity. *Curr Hypertens Rep* 2009;11:133-42.

Bodnar, L. M., A. M. Siega-Riz, et al. Severe obesity, gestational weight gain, and adverse birth outcomes. *Am J Clin Nutr* 2010:91:1642-8.

Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics* 2005;115:e290-6.

Brawley L, Itoh S, Torrens C, Barker A, Bertram C, Poston L, Hanson M. Dietary protein restriction in pregnancy induces hypertension and vascular defects in rat male offspring. *Pediatr Res* 2003;54:83-90.

Byers T. On the hazards of seeing the world through intervention-colored glasses. *Am J Clin Nutr* 2003;78:904-5.

Bäckhed FH, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004;101:15718-23.

Campbell DM, Hall MH, Barker DJ, Cross J, Shiell AW, Godfrey KM. Diet in pregnancy and the offspring's blood pressure 40 years later. Br *J Obstet Gynaecol* 1996;103:273-80.

Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, et al. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia* 2007;50:2374-83.

Catalano PM, Farrell K, Thomas A, Huston-Presley L, Mencin P, de Mouzon SH, et al. Perinatal risk factors for childhood obesity and metabolic dysregulation. *Am J Clin Nutr* 2009;90:1303-13.

Chen X and Wang Y. Tracking of blood pressure from childhood to adulthood: a systematic review

and meta-regression analysis. *Circulation* 2008;117:3171-3180.

Collins S, Kuhn CM, Petro AE, Swick AG, Chrunyk BA, Surwit RS. Role of leptin in fat regulation. *Nature* 1996;380:677.

Corvalan C, Uauy R, Kain J, Martorell R. Obesity indicators and cardiometabolic status in 4-y-old children. *Am J Clin Nutr* 2010;91:166-174.

Crowther NJ, Cameron N, Trusler J, Toman M, Norris SA, Gray IP. Influence of catch-up growth on glucose tolerance and beta-cell function in 7-year-old children: results from the birth to twenty study. *Pediatrics* 2008;121: e1715-22.

Damm P. Future risk of diabetes in mother and child after gestational diabetes mellitus. *Int J Gynaecol Obstet* 2009;104:S25-6.

Darendeliler F, Poyrazoglu S, Sancakli O, Bas F, Gokcay G, Aki S, et al. Adiponectin is an indicator of insulin resistance in non-obese prepubertal children born large for gestational age (LGA) and is affected by birth weight. *Clin Endocrinol* 2009;70:710-6.

Davies AA, Smith GD, May MT, Ben-Shlomo Y. Association between birth weight and blood pressure is robust, amplifies with age, and may be underestimated. *Hypertension* 2006;48:431-6.

de Rooij SR, Painter RC, Phillips DI, Osmond C, Michels RP, Godsland IF, et al. Impaired insulin secretion after prenatal exposure to the Dutch famine. *Diabetes Care* 2006;29:1897-901.

Dewey KG. Is breastfeeding protective against child obesity? *J Hum Lact* 2003;19:9-18.

Dietz WH. Critical periods in childhood for the development of obesity. *Am J Clin Nutr* 1994;59:955-9.

Drouillet PA, Forhan A, De Lauzon-Guillain B, Thiebaugeorges O, Goua V, Magnin G, et al. Maternal fatty acid intake and fetal growth: evidence for an association in overweight women. The 'EDEN mother-child' cohort (study of pre- and early postnatal determinants of the child's development and health). *Br J Nutr* 2009a;101:583-91.

Drouillet P, Kaminski M, De Lauzon-Guillain B, Forhan A, Ducimetiere P, Schweitzer M, et al. Association between maternal seafood consumption before pregnancy and fetal growth: evidence for an association in overweight women. The EDEN mother-child cohort. *Paediatr Perinat Epidemiol* 2009b;23:76-86.

Duttaroy AK. Transport of fatty acids across the human placenta: a review. *Prog Lipid Res* 2009;48:52-61.

Ehnholm C, Huttunen JK, Pietinen P, Leino U, Mutanen M, Kostiainen E, et al. Effect of diet on serum lipoproteins in a population with a high risk of coronary heart disease. *N Engl J Med* 1982;307: 850-5.

Ekelund UK, Ong K, Linne Y, Neovius M, Brage S, Dunger DB, et al. Upward weight percentile crossing in infancy and early childhood independently predicts fat mass in young adults: the Stockholm Weight Development Study (SWEDES). *Am J Clin Nutr* 2006;83:324-30.

Eriksson JG, Forsen T, Tuomilehto J, Winter PD, Osmond C, Barker DJ. Catch-up growth in childhood and death from coronary heart disease: longitudinal study. *BMJ* 1999;318:427-31.

Eriksson JG, Forsen TJ, Kajantie E, Osmond C, Barker DJ. Childhood growth and hypertension in later life. *Hypertension* 2007;49:1415-21.

Erkkola M, Salmenhaara M, Kronberg-Kippilä C, Ahonen S, Arkkola T, Uusitalo L et al. Determinants of breast-feeding in a Finnish birth cohort. *Public Health Nutr* 2010;13:504-13.

Fanaro S, Chierici R, Guerrini P, Vigi V. Intestinal microflora in early infancy: composition and development. *Acta Paediatr Suppl* 2003;91:48-55.

Fantuzzi G. Adiponectin and inflammation: consensus and controversy. *J Allergy Clin Immunol* 2008;121:326-30.

Fernandez-Twinn DS, Ozanne SE, Ekizoglou S, Doherty C, James L, Gusterson B, et al. The maternal endocrine environment in the low-protein model of intra-uterine growth restriction. *Br J Nutr* 2003;90:815-22.

Festa A, D'Agostino R Jr, Howard G, Mykkänen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation* 2000;102:42-7.

Field CJ. Early risk determinants and later health outcomes: implications for research prioritization and the food supply. Summary of the workshop. *Am J Clin Nutr* 2009;89:1533S-1539S.

Flanagan DE, Moore VM, Godsland IF, Cockington RA, Robinson JS, Phillips DI. Fetal growth and the physiological control of glucose tolerance in adults: a minimal model analysis. *Am J Physiol Endocrinol Metab* 2000;278:E700-6.

Forsen T, Eriksson J, Tuomilehto J, Reunanen A, Osmond C, Barker D. The fetal and childhood growth of persons who develop type 2 diabetes. *Ann Intern Med* 2000;133:176-82.

Forsyth JS, Willatts P, Agostoni C, Bissenden J, Casaer P, Boehm G. Long chain polyunsaturated fatty acid supplementation in infant formula and blood pressure in later childhood: follow up of a randomised controlled trial. *BMJ* 2003;326:953.

Fraser A, Tilling K, Macdonald-Wallis C, Sattar N, Brion MJ, Benfield L, et al. Association of maternal weight gain in pregnancy with offspring

obesity and metabolic and vascular traits in childhood. *Circulation* 2010:121:2557-64.

Friedberg CE, Janssen MJ, Heine RJ, Grobbee DE. Fish oil and glycemic control in diabetes. A meta-analysis. *Diabetes Care* 1998;21:494-500.

Fuentes RM, Notkola IL, Shemeikka S, Tuomilehto J, Nissinen A. Tracking of systolic blood pressure during childhood: a 15-year follow-up population-based family study in eastern Finland. *J Hypertens* 2002;20:195-202.

Gamborg M, Byberg L, Rasmussen F, Andersen PK, Baker JL, Bengtsson C, et al. Birth weight and systolic blood pressure in adolescence and adulthood: meta-regression analysis of sex- and age-specific results from 20 Nordic studies. *Am J Epidemiol* 2007;166:634-45.

Gardner DS, Hosking J, Metcalf BS, Jeffery AN, Voss LD, Wilkin TJ. Contribution of early weight gain to childhood overweight and metabolic health: a longitudinal study (EarlyBird 36). *Pediatrics* 2009;123:e67-73.

Gareau MG, Sherman PM, Walker WA. Probiotics and the gut microbiota in intestinal health and disease. *Nat Rev Gastroenterol Hepatol* 2010;7:503-14.

Gibson GR and Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* 1995;125(6): 1401-12.

Gil-Campos M, Aguilera CM, Ramirez-Tortosa MC, Canete R, Gil A. Fasting and postprandial relationships among plasma leptin, ghrelin, and insulin in prepubertal obese children. *Clin Nutr* 2010;29:54-9.

Gillman MW, Rich-Edwards JW, Rifas-Shiman SL, Lieberman ES, Kleinman KP, Lipshultz SE. Maternal age and other predictors of newborn blood pressure. *J Pediatr* 2004;144: 240-5.

Glauber HS, Revers RR, Henry R, Schmeiser L, Wallace P, Kolterman OG, et al. In vivo deactivation of proinsulin action on glucose disposal and hepatic glucose production in normal man. *Diabetes* 1986;35:311-7.

Gluckman PD and Hanson MA. Maternal constraint of fetal growth and its consequences. *Semin Fetal Neonatal Med* 2004a;9:419-25.

Gluckman PD and Hanson MA. Developmental origins of disease paradigm: a mechanistic and evolutionary perspective. *Pediatr Res* 2004b;56:311-7.

Godfrey KM, Forrester T, Barker DJ, Jackson AA, Landman JP, Hall JS, et al. Maternal nutritional status in pregnancy and blood pressure in childhood. Br J Obstet Gynaecol 1994;101:398-403.

Godfrey K, Robinson S, Barker DJ, Osmond C, Cox V. Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. *BMJ 1996a*;312:410-4.

Godfrey KM, Robinson S, Hales CN, Barker DJ, Osmond C, Taylor KP. Nutrition in pregnancy and the concentrations of proinsulin, 32-33 split proinsulin, insulin, and C-peptide in cord plasma. *Diabet Med* 1996b;13:868-73.

Goldstein BJ and Scalia R. Adiponectin: A novel adipokine linking adipocytes and vascular function. *J Clin Endocrinol Metab* 2004;89:2563-8.

Griffiths EA, Duffy LC, Schanbacher FL, Qiao H, Dryja D, Leavens A, et al. In vivo effects of bifidobacteria and lactoferrin on gut endotoxin concentration and mucosal immunity in Balb/c mice. *Dig Dis Sci* 2004;49:579-89.

Griffiths LJ, Smeeth L, Hawkins SS, Cole TJ, Dezateux C. Effects of infant feeding practice on weight gain from birth to 3 years. *Arch Dis Child* 2009;94:577-82.

Guarner F and Schaafsma GJ. Probiotics. *Int J Food Microbiol* 1998;39:237-8.

Guarner F and Malagelada JR. Gut flora in health and disease. *Lancet* 2003;361:512-9.

Guerre-Millo M. Adiponectin: an update. *Diabetes Metab* 2008;34:12-8.

Haapa ET, Toponen T, Pietinen P, Räsänen L. Annoskuvakirja. National Public Health Insitute and Department of Nutrition, University of Helsinki, Helsinki, Finland.1985

Hakanen M, Langström H, Kaitosaari T, Niinikoski H, Näntö-Salonen K, Jokinen E, et al. Development of overweight in atherosclerosis prevention trial starting in early childhood. The STRIP study. *Int J Obes* 2006;30:618-626.

Hakanen M, Lagström H, Pahkala K, Sillanmäki L, Saarinen M, Niinikoski H, et al. Dietary and lifestyle counselling reduces the clustering of overweight-related cardiometabolic risk factors in adolescents. *Acta Paediatr* 2010;99:888-95

Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, Winter PD. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ* 1991;303:1019-22.

Hales CN and Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* 1992;35:595-601.

Hales CN and Barker DJ. The thrifty phenotype hypothesis. Br Med Bull 2001;60:5-20.

Hasunen K, Kalavainen M, Keinonen H, Lagström H, Lyytikäinen A, Nurttila A, et al. Lapsi, perhe ja ruoka. Imeväis- ja leikki-ikäisten lasten, odottavien ja imettävien äitien ravitsemussuositus. *Sosiaali- ja terveysministeriön julkaisuja* 2004:11.

Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight

and obesity among US children, adolescents, and adults, 1999-2002. *JAMA* 2004;291:2847-50.

Higgins L, Greenwood SL, Wareing M, Sibley CP, Mills TA. Obesity and the placenta: A consideration of nutrient exchange mechanisms in relation to aberrant fetal growth. *Placenta* Epub 2010 Oct 26. doi:10.1016/j.placenta.2010.09.019

Huxley RR, Shiell AW, Law CM. The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. *J Hypertens* 2000;18:815-31.

Huxley R, Owen CG, Whincup PH, Cook DG, Rich-Edwards J, Smith GD, et al. Is birth weight a risk factor for ischemic heart disease in later life? *Am J Clin Nutr* 2007;85:1244-50.

Ibanez L, Ong K, Dunger DB, de Zegher F. Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. *J Clin Endocrinol Metab* 2006;91:2153-8.

Ibanez L, Sebastiani G, Diaz M, Gomez-Roig MD, Lopez-Bermejo A, de Zegher F. Low body adiposity and high leptinemia in breast-fed infants born small-for-gestational-age. *J Pediatr* 2010;156:145-7.

Ilmonen J, Isolauri E, Poussa T, Laitinen K. Impact of dietary counselling and probiotic intervention on maternal anthropometric measurement during and after pregnancy: a randomized placebo-controlled trial. *Clin Nutr* Epub 2010 Oct 21. doi:10.1016/j.clnu.2010.09.009

Institute of Medicine. Institute of Medicine, Subcommittee on Nutritional Status and Weight Gain During Pregnancy. Nutrition During Pregnancy. Washington, DC: *National Academy Press*.1990

Isolauri E, Sutas Y, Kankaanpää P, Arvilommi H, Salminen S. Probiotics: effects on immunity. *Am J Clin Nutr* 2001;73:444S-450S.

Isolauri E, Kalliomäki M, Rautava S, Salminen S, Laitinen, K. Obesity - extending the hygiene hypothesis. Nestle Nutr Workshop Ser Pediatr Program. 2009;64:75-85; discussion 85-9, 251-7.

Jones RH and Ozanne SE Fetal programming of glucose-insulin metabolism. *Mol Cell Endocrinol* 2009:297:4-9.

Kalliomäki M, Collado MC, Salminen S, Isolauri E. Early differences in fecal microbiota composition in children may predict overweight. *Am J Clin Nutr* 2008;87:534-8.

Karlsson FA, Engstrom BE, Lind L, Ohrvall M. No postprandial increase of plasma adiponectin in obese subjects. *Obes Res* 2004;12:1031-2; author reply 1032-4.

Kaufman FR. Type 2 diabetes mellitus in children and youth: a new epidemic. *J Pediatr Endocrinol Metab* 2002;15:737-44.

Kramer MS and Kakuma R. Optimal duration of exclusive breastfeeding. *Cochrane Database Syst Rev* (1) 2002 (Edited 2009): CD003517.

Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, et al. Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem* 2002;277:25863-6.

Kyttälä P, Erkkola M, Kronberg-Kippilä C, Tapanainen H, Veijola R, Simell O, et al. Food consumption and nutrient intake in Finnish 1-6-year-old children. *Public Health Nutr* 2010;13:947-56.

Laitinen K, Poussa T, Isolauri E. Probiotics and dietary counselling contribute to glucose regulation during and after pregnancy: a randomised controlled trial. *Br J Nutr* 2009;101:1679-1687.

Laitinen K, Hoppu U, Hämäläinen M, Linderborg K, Moilanen E, Isolauri E. Breast milk fatty acids may link innate and adaptive immune regulation: analysis of soluble CD14, prostaglandin E2, and fatty acids. *Pediatr Res* 2006;59:723-727.

Launer LJ, Hofman A, Grobbee DE. Relation between birth weight and blood pressure: longitudinal study of infants and children. BMJ 1993;307:1451-4.

Law CM, Barker DJ, Osmond C, Fall CH, Simmonds SJ. Early growth and abdominal fatness in adult life. *J Epidemiol Community Health* 1992;46:184-6.

Lawlor DA, Owen CG, Davies AA, Whincup PH, Ebrahim S, Cook DG, et al. Sex differences in the association between birth weight and total cholesterol. A meta-analysis. *Ann Epidemiol* 2006;16:19-25.

Leary SD, Ness AR, Emmett PM, Davey Smith G, Headley JE. Maternal diet in pregnancy and offspring blood pressure. *Arch Dis Child* 2005;90:492-3.

Lee JH, O'Keefe JH, Lavie CJ, Harris WS. Omega-3 fatty acids: cardiovascular benefits, sources and sustainability. *Nat Rev Cardiol* 2009;6:753-758.

Livingstone MB, Robson PJ, Wallace JM. Issues in dietary intake assessment of children and adolescents. *Br J Nutr* 2004;92:S213-22.

Lucas A. Programming by early nutrition in man. The childhood environment and adult disease. Whiley, Chichester. *Ciba Found Symp* 1991;156:38-55.

Luoto R, Laitinen K, Nermes M, Isolauri E. Impact of maternal probiotic-supplemented dietary counselling on pregnancy outcome and prenatal and postnatal growth: double-blind, placebo-controlled study. *Br J Nutr* 2010;103:1792-9.

Luyckx VA and Brenner BM. The clinical importance of nephron mass. *J Am Soc Nephrol* 2010;21:898-910.

Mamun AA, O'Callaghan M, Callaway L, Williams G, Najman J, Lawlor DA Associations of

gestational weight gain with offspring body mass index and blood pressure at 21 years of age: evidence from a birth cohort study. *Circulation* 2009;119::1720-7.

Mancia G, Bousquet P, Elghozi JL, Esler M, Grassi G, Julius S, et al. The sympathetic nervous system and the metabolic syndrome. *J Hypertens* 2007;25:909-20.

Manderson JG, Mullan B, Patterson CC, Hadden DR, Traub AI, McCance DR. Cardiovascular and metabolic abnormalities in the offspring of diabetic pregnancy. *Diabetologia* 2002;45:991-6.

Mantzoros CS, Rifas-Shiman SL, Williams CJ, Fargnoli JL, Kelesidis T, Gillman MW. Cord blood leptin and adiponectin as predictors of adiposity in children at 3 years of age: a prospective cohort study. *Pediatrics* 2009;123:682-9.

Martin RM, Gunnell D, Smith GD. Breastfeeding in infancy and blood pressure in later life: systematic review and meta-analysis. *Am J Epidemiol* 2005;161:15-26.

Martin de Santa Olalla L, Sanchez Muniz FJ, Vaquero MP. N-3 fatty acids in glucose metabolism and insulin sensitivity. *Nutr Hosp* 2009;24:113-127.

Matarese G, Moschos S, Mantzoros CS. Leptin in immunology. *J Immunol* 2005;174:3137-42.

Mbah AK, Kornosky JL, Kristensen S, August EM, Alio AP, Marty PJ, et al. Super-obesity and risk for early and late pre-eclampsia. *BJOG* 2010;117:997-1004.

McCance DR, Pettitt DJ, Hanson RL, Jacobsson LT, Knowler WC, Bennett PH. Birth weight and non-insulin dependent diabetes: thrifty genotype, thrifty phenotype, or surviving small baby genotype? *BMJ* 1994;308:942-5.

McDonald SD. Han Z, Mulla S, Beyene J. Overweight and obesity in mothers and risk of preterm birth and low birth weight infants: systematic review and meta.analyses. *BMJ* 2010;341:c3428

Melani F, Rubenstein AH, Steiner DF. Human serum proinsulin. *J Clin Invest* 1970;49:497-507.

Merialdi M, Carroli G, Villar J, Abalos E, Gulmezoglu AM, Kulier R, et al. Nutritional interventions during pregnancy for the prevention or treatment of impaired fetal growth: an overview of randomized controlled trials. *J Nutr* 2003;133:1626S-1631S.

Metcalfe NB and Monaghan P. Compensation for a bad start: grow now, pay later? *Trends Ecol Evol* 2001;16:254-260.

Metsälä T, Siimes A, Välimäki I. The effect of change in sympatho-vagal balance on heart rate and blood pressure variability in the foetal lamb. *Acta Physiol Scand* 1995;154:85-92.

Mikkilä V, Räsänen L, Laaksonen MM, Juonala M, Viikari J, Pietinen P, et al. Long-term dietary patterns and carotid artery intima media thickness: the Cardiovascular Risk in Young Finns Study. *Br J Nutr* 2009;102:1507-12.

Miller M, Beach V, Sorkin JD, Mangano C, Dobmeier C, Novacic D, et al. Comparative effects of three popular diets on lipids, endothelial function, and C-reactive protein during weight maintenance. *J Am Diet Assoc* 2009;109:713-7.

Molfino A, Fiorentini A, Tubani L, Martuscelli M, Rossi Fanelli F, Laviano A. Body mass index is related to autonomic nervous system activity as measured by heart rate variability. *Eur J Clin Nutr* 2009;63:1263-5.

Moses RG, Luebcke M, Davis WS, Coleman KJ, Tapsell LC, Petocz P, et al. Effect of a low-glycemic-index diet during pregnancy on obstetric outcomes. *Am J Clin Nutr* 2006;84:807-12.

Muntner P, He J, Cutler JA, Wildman RP, Whelton PK. Trends in blood pressure among children and adolescents. *JAMA* 2004;291:2107-13.

Mykkänen L, Haffner SM, Hales CN, Rönnemaa T, Laakso M. The relation of proinsulin, insulin, and proinsulin-to-insulin ratio to insulin sensitivity and acute insulin response in normoglycemic subjects. *Diabetes* 1997;46:1990-5.

Napoli C, D'Armiento F, Mancini F, Postiglione A, Witztum JL, Palumbo G, et al. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest* 1997;100:2680-2690.

National High Blood Pressure Education Program Working Group on Hypertension Control in Children and Adolescents. Update on the 1987 Task Force Report on High Blood Pressure in Children and Adolescents: a working group report from the National High Blood Pressure Education Program. *Pediatrics* 1996;98:649-658.

National High Blood Pressure Education Program Working Group. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics* 2004;114:555-576.

Nelson SM, Freeman DJ, Sattar N, Lindsay RS. Role of adiponectin in matching of fetal and placental weight in mothers with type 1 diabetes. *Diabetes Care* 2008;31:1123-5.

Nettleton JA, Steffen LM, Schulze MB, Jenny NS, Barr RG, Bertoni AG, et al. Associations between markers of subclinical atherosclerosis and dietary patterns derived by principal components analysis and reduced rank regression in the Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Clin Nutr* 2007;85:1615-1625.

Newburg DS, Woo JG, Morrow AL. Characteristics and potential functions of human milk adiponectin. *J Pediatr* 2010;156:S41-6.

Niinikoski H, Jula A, Viikari J, Ronnemaa T, Heino P, Lagstrom H, et al. Blood pressure is lower in children and adolescents with a low-saturated-fat diet since infancy: the special Turku coronary risk factor intervention project. *Hypertension* 2009;53:918-24.

Nohr EA, Vaeth M, Baker JL, Sorensen T, Olsen J, Rasmussen KM. Combined associations of prepregnancy body mass index and gestational weight gain with the outcome of pregnancy. *Am J Clin Nutr* 2008;87:1750-9.

Nordic Working Group on Diet and Nutrition. Nordic nutritional recommendations. *Scand J Nutr* 1996;40:161-165.

Olsen SF and Secher NJ. Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study. *BMJ* 2002;324:447.

Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ* 2000;320:967-71.

Ozanne SE and Hales CN. The long-term consequences of intra-uterine protein malnutrition for glucose metabolism. *Proc Nutr Soc* 1999;58:615-9.

Painter RC, de Rooij SR, Bossuyt PM, Simmers TA, Osmond C, Barker DJ, et al. Early onset of coronary artery disease after prenatal exposure to the Dutch famine. *Am J Clin Nutr* 2006;84;322-7.

Paturi M, Nieminen R, Reinivuo H, Ovaskainen ML. Ruokien annoskuvakirja, *Publications of the National Public Health Institute*. Helsinki, Finland. 2006

Pello LC, Rosevear SK, Dawes GS, Moulden M, Redman CW. Computerized fetal heart rate analysis in labor. *Obstet Gynecol* 1991;78:602-10.

Perloff D, Grim C, Flack J, Frohlich ED, Hill M, McDonald M, et al. Human blood pressure determination by sphygmomanometry. *Circulation* 1993;88:2460-70.

Pietinen P, Paturi M, Reinivuo H, Tapanainen H, Valsta LM. FINDIET 2007 Survey: energy and nutrient intakes. *Public Health Nutr* 2010;13:920-4.

Piirainen T, Isolauri E, Lagström H, Laitinen K. Impact of dietary counselling on nutrient intake during pregnancy: a prospective cohort study. *Br J Nutr* 2006;96:1095-104.

Pradhan A. Obesity, metabolic syndrome, and type 2 diabetes: inflammatory basis of glucose metabolic disorders. *Nutr Rev* 2007;65:S152-6.

Prasad M, Lumia M, Erkkola M, Tapanainen H, Kronberg-Kippilä C, Tuokkola J, et al. Diet composition of pregnant Finnish women: changes over time and across seasons. *Public Health Nutr* 2010;13:939-46.

Primatesta P, Falaschetti E, Poulter NR. Birth weight and blood pressure in childhood: results from the Health Survey for England. *Hypertension* 2005;45:75-9.

Puska P, Iacono JM, Nissinen A, Korhonen HJ, Vartiainen E, Pietinen P, et al. Controlled, randomised trial of the effect of dietary fat on blood pressure. *Lancet* 1983;1:1-5.

Rautava S and Isolauri E. The development of gut immune responses and gut microbiota: effects of probiotics in prevention and treatment of allergic disease. *Curr Issues Intest Microbiol* 2002;3:15-22.

Ravelli ACJ, van der Meulen JHP, Michaels RPJ, Osmond C, Barker DJP, Hales CN, et al. Glucose tolerance in adults after prenatal exposure to famine. *Lancet* 1998;351:173-77.

Ravelli AC, van Der Meulen JH, Osmond C, Barker DJ, Bleker OP. Obesity at the age of 50 y in men and women exposed to famine prenatally. *Am J Clin Nutr* 1999;70:811-6.

Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities ─ the role of insulin resistance and the sympathoadrenal system. *N Engl J Med* 1996;334:374-81.

Riserus U, Willett WC, Hu FB. Dietary fats and prevention of type 2 diabetes. *Prog Lipid Res* 2009;48:44-51.

Rogers I. The influence of birthweight and intrauterine environment on adiposity and fat distribution in later life. *Int J Obes Relat Metab Disord* 2003;27:755-77.

Rolfe EDL, Loos RJ, Druet C, Stolk RP, Ekelund U, Griffin SJ et al. Association between birth weight and visceral fat in adults. *Am J Clin Nutr* 2010:92:347-52.

Rolland-Cachera MF. Rate of growth in early life: a predictor of later health? *Adv Exp Med Biol* 2005;569:35-9.

Ronti T, Lupattelli G, Mannarino E. The endocrine function of adipose tissue: an update. *Clin Endocrinol* 2006;64:355-65.

Rose G. Familial patterns in ischaemic heart disease. *Br J Prev Soc Med* 1964;18:75-80.

Roseboom TJ, van der Meulen JH, Ravelli AC, van Montfrans GA, Osmond C, Barker DJ, et al. Blood pressure in adults after prenatal exposure to famine. *J Hypertens* 1999;17:325-30.

Roseboom TJ, van der Meulen JH, van Montfrans GA, Ravelli AC, Osmond C, Barker DJ, et al. Maternal nutrition during gestation and blood pressure in later life. *J Hypertens* 2001;19:29-34.

Rosenfeld E, Beyerlein A, Hadders-Algra M, Kennedy K, Singhal A, Fewtrell M, et al. IPD

meta-analysis shows no effect of LC-PUFA supplementation on infant growth at 18 months. *Acta Paediatr* 2009;98:91-7.

Rosner B, Cook N, Portman R, Daniels S, Falkner B. Determination of blood pressure percentiles in normal-weight children: some methodological issues. *Am J Epidemiol* 2008;167:653-66.

Salminen S, Gueimonde M, Isolauri E. Probiotics that modify disease risk. *J Nutr* 2005;135:1294-8.

Savino F, Costamagna M, Prino A, Oggero R, Silvestro L. Leptin levels in breast-fed and formula-fed infants. *Acta Paediatr* 2002;91:897-902.

Savino F, Liguori SA, Fissore MF, Oggero R. Breast milk hormones and their protective effect on obesity. *Int J Pediatr Endocrinol* 2009;2009:327505.

Savvidou MD, Hingorani AD, Tsikas D, Frolich JC, Vallance P, Nicolaides KH. Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. *Lancet* 2003;361:1511-7.

Schubring C, Kiess W, Englaro P, Rascher W, Dotsch J, Hanitsch S, et al. Levels of leptin in maternal serum, amniotic fluid, and arterial and venous cord blood: relation to neonatal and placental weight. *J Clin Endocrinol Metab* 1997;82:1480-3.

Semple RK, Soos MA, Luan J, Mitchell CS, Wilson JC, Gurnell M, et al. Elevated plasma adiponectin in humans with genetically defective insulin receptors. *J Clin Endocrinol Metab* 2006;91:3219-3223.

Shea S, Aymong E, Zybert P, Shamoon H, Tracy RP, Deckelbaum RJ, et al. Obesity, fasting plasma insulin, and C-reactive protein levels in healthy children. *Obes Res* 2003;11:95-103.

Shiell AW, Campbell DM, Hall MH, Barker DJ. Diet in late pregnancy and glucose-insulin metabolism of the offspring 40 years later. *BJOG* 2000;107:890-5.

Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* 2006;116:1793-801.

Siira S, Ojala T, Vahlberg T, Jalonen J, Välimäki I, Rosen KG. Marked fetal acidosis and specific changes in power spectrum analysis of fetal heart rate variability recorded during the last hour of labour. *BJOG* 2005;112:418-23.

Siira S, Ojala T, Ekholm E, Vahlberg T, Blad S, Rosen KG. Change in heart rate variability in relation to a significant ST-event associates with newborn metabolic acidosis. *BJOG* 2007;114:819-23.

Simmons D. Association between neonatal blood pressure and umbilical cord insulin concentration. *Diabet Med* 1997;14:196-9.

Singhal A, Cole TJ, Lucas A. Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials. *Lancet* 2001;357:413-9.

Singhal A, Farooqi IS, Cole TJ, O'Rahilly S, Fewtrell M, Kattenhorn M, et al. Influence of leptin on arterial distensibility: a novel link between obesity and cardiovascular disease? *Circulation* 2002;106:1919-24.

Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. *Lancet* 2003a;361:1089-97.

Singhal A, Wells J, Cole TJ, Fewtrell M, Lucas A. Programming of lean body mass: a link between birth weight, obesity, and cardiovascular disease? *Am J Clin Nutr* 2003b;77:726-30.

Singhal A, Cole TJ, Fewtrell M, Lucas A. Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. *Lancet* 2004;363:1571-8.

Singhal A, Cole TJ, Fewtrell M, Kennedy K, Stephenson T, Elias-Jones A, et al. Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? *Circulation* 2007;15:213-20.

Sjöberg NJ, Milte CM, Buckley JD, Howe PR, Coates AM, Saint DA. Dose-dependent increases in heart rate variability and arterial compliance in overweight and obese adults with DHA-rich fish oil supplementation. *Br J Nutr* 2010;103:243-8.

Smith-Kirwin SM, O'Connor DM, De Johnston J, Lancey ED, Hassink SG, Funanage VL. Leptin expression in human mammary epithelial cells and breast milk. *J Clin Endocrinol Metab* 1998;83:1810-3.

Steinberger J, Steffen L, Jacobs DR, Jr., Moran A, Hong CP, Sinaiko AR. Relation of leptin to insulin resistance syndrome in children. *Obes Res* 2003;11:1124-30.

Stocker CJ and Cawthorne MA. The influence of leptin on early life programming of obesity. *Trends Biotechnol* 2008; 26:545-51

Sun SS, Grave GD, Siervogel RM, Pickoff AA, Arslanian SS, Daniels SR. Systolic blood pressure in childhood predicts hypertension and metabolic syndrome later in life. *Pediatrics* 2007;119:237-46.

Surkan PJ, Hsieh CC, Johansson AL, Dickman PW, Cnattingius S. Reasons for increasing trends in large for gestational age births. *Obstet Gynecol* 2004;104:720-6.

Swanson LD and Bewtra C. Increase in normal placental weights related to increase in maternal body mass index. *J Matern Fetal Neonatal Med* 2008;21:111-3.

Symonds ME, Sebert SP, Hyatt MA, Budge H. Nutritional programming of the metabolic syndrome. *Nat Rev Endocrinol* 2009a;5:604-10.

Symonds ME, Stephenson T, Budge H. Early determinants of cardiovascular disease: the role of early diet in later blood pressure control. *Am J Clin Nutr* 2009b;89:1518S-1522S.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation* 1996;93:1043-1065.

Taveras EM, Rifas-Shiman SL, Belfort MB, Kleinman KP, Oken E, Gillman MW. Weight status in the first 6 months of life and obesity at 3 years of age. *Pediatrics* 2009;123:1177-83.

Temple RC, Carrington CA, Luzio SD, Owens DR, Schneider AE, Sobey WJ, et al. Insulin deficiency in non-insulin-dependent diabetes. *Lancet* 1989;1:293-5.

Thomas C, Hypponen E, Power C. Prenatal exposures and glucose metabolism in adulthood: are effects mediated through birth weight and adiposity? *Diabetes Care* 2007;30:918-24.

Tsai PJ, Yu CH, Hsu SP, Lee YH, Chiou CH, Hsu YW, et al. Cord plasma concentrations of adiponectin and leptin in healthy term neonates: positive correlation with birthweight and neonatal adiposity. *Clin Endocrinol* 2004;61:88-93.

Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006;444:1027-31.

Ulbak J, Lauritzen L, Hansen HS, Michaelsen KF. Diet and blood pressure in 2.5-y-old Danish children. *Am J Clin Nutr* 2004;79:1095-102.

Vaag A. Low birth weight and early weight gain in the metabolic syndrome: consequences for infant nutrition. *Int J Gynaecol Obstet* 2009;104:S32-4.

Valle M, Gascon F, Martos R, Bermudo F, Ceballos P, Suanes A Relationship between high plasma leptin concentrations and metabolic syndrome in obese pre-pubertal children. *Int J Obes Relat Metab Disord* 2003;27:13-8.

van Ravenswaaij-Arts CM, Kollee LA, Hopman JC, Stoelinga GB, van Geijn HP. Heart rate variability. *Ann Intern Med* 1993;118:436-47.

Vasudevan C, Renfrew M, McGuire W. Fetal and perinatal consequences of maternal obesity. *Arch Dis Child Fetal Neonatal* Epub 2010 Jun 7. doi:10.1136/adc.2009.170928

Vehaskari VM. Prenatal programming of kidney disease. *Curr Opin Pediatr* 2010;22:176-82.

Viikari JS, Niinikoski H, Juonala M, Raitakari OT, Lagstrom H, Kaitosaari T, et al. Risk factors for coronary heart disease in children and young adults. *Acta Paediatr Suppl* 2004;93:34-42.

Vogels N, Posthumus DL, Mariman EC, Bouwman F, Kester AD, Rump P, et al. Determinants of overweight in a cohort of Dutch children. *Am J Clin Nutr* 2006;84:717-724.

Vuori E, Kiuru K, Mäkinen SM, Väyrynen P, Kara R, Kuitunen P. Maternal diet and fatty acid pattern of breast milk. *Acta Paediatr Scand* 1982;71:959-63.

Välimäki I and Rantonen T. Spectral analysis of heart rate and blood pressure variability. *Clin Perinatol* 1999;26:967-80.

Walker A. Breast milk as the gold standard for protective nutrients. *J Pediatr* 2010;156:S3-7.

Waterland RA and Garza C. Potential mechanisms of metabolic imprinting that lead to chronic disease. *Am J Clin Nutr* 1999;69:179-97.

Wei JN, Sung FC, Li CY, Chang CH, Lin RS, Lin CC, et al. Low birth weight and high birth weight infants are both at an increased risk to have type 2 diabetes among schoolchildren in Taiwan. *Diabetes Care* 2003;26: 343-8.

Whincup PH, Kaye SJ, Owen CG, Huxley R, Cook DG, Anazawa S, et al. Birth weight and risk of type 2 diabetes: a systematic review. *JAMA* 2008;300:2886-97.

Widdowson EM and McCance RA. A review: new thoughts on growth. *Pediatr Res* 1975;9:154-6.

Willett W. Nutritional epidemiology. 24-Hour Dietary Recall and Food Record Methods. *Oxford University Press* 2nd ed. 1998;(Chapter 4):50-71.

WHO. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. *World Health Organ Tech Rep Ser* 1995;854:1-452.

WHO. The optimal duration of exclusive breastfeeding. Report of an Expert Consultation. Geneva, Switzerland. 2001.

Wrotniak BH, Shults J, Butts S, Stettler N. Gestational weight gain and risk of overweight in the offspring at age 7 y in a multicenter, multiethnic cohort study. *Am J Clin Nutr* 2008;87:1818-24.

Yannakoulia M, Yiannakouris N, Bluher S, Matalas AL, Klimis-Zacas D, Mantzoros CS. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. *J Clin Endocrinol Metab* 2003;88:1730-6.

Ylihärsilä H, Kajantie E, Osmond C, Forsen T, Barker DJ, Eriksson JG. Birth size, adult body composition and muscle strength in later life. *Int J Obes* 2007;31:1392-9.