

# **Nutritional Interventions in Infancy and Childhood for Prevention of Atherosclerosis and the Metabolic Syndrome**

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Atherosclerotic cardiovascular disease (CVD) is the leading cause of death and disability, and the most important public health priority in the West [1]. Yet, despite great progress in its clinical management, the prevalence of CVD continues to increase [1]. In the UK alone an estimated 2.7 million people are now living with coronary heart disease – a number that has risen sharply [1]. Consequently, the role of prevention has become a major priority for public health policy and future scientific research [1].

Atherosclerosis is now known to have a long preclinical phase with the development of pathological changes in the arteries of children and young adults well before the clinical manifestations of disease later in adulthood [2]. Nutritional factors have been shown to be particularly influential [2, 3] and affect a lifetime risk of CVD [3]. For instance, both observational and, more recently, experimental studies suggest that nutrition in fetal life ('the fetal origins hypothesis' [4]) and in the early postnatal period [5] influences, or programs, the long-term development of atherosclerosis and its complications. This effect occurs via programming of classical cardiovascular risk factors (e.g. obesity) and also by effects on the vascular biology of early atherosclerosis [6].

Other than via programming, nutrition also has an important direct impact on conventional cardiovascular risk factors in childhood. These risk factors show tracking into adult life [7], affect the earliest stages of atherosclerosis, and have a strong, independent influence on the long-term risk of CVD [8]. Obesity in childhood, for instance, adversely affects adult CVD risk independent of adult weight [8].

Based on the strong evidence for the early origins of atherosclerosis, the American Heart Association has recently published guidelines for the primary

prevention of CVD beginning in children and adolescents [9]. The present review focuses on the evidence and rationale for such interventions, emphasizing the role of nutrition in infancy and childhood.

### **The Childhood Origins of Atherosclerosis**

Autopsy studies of the atherosclerotic changes in the coronary arteries of young men killed in the Korean and Vietnamese wars first stimulated research into the early development of atherosclerosis [10]. Similar studies in children, particularly from the Bogalusa Heart Study, demonstrated a high prevalence of coronary atherosclerosis (up to 90%) by the third decade of life [10]. Strikingly, the presence and extent of atherosclerotic lesions in these reports correlated positively with established risk factors and, in accordance with the Framingham risk score for predicting cardiovascular mortality, their severity was associated with an increase in the number of risk factors [11]. Importantly, these autopsy findings have been confirmed in other populations (e.g. the Pathobiologic Determinants of Atherosclerosis in Youth Study) and extended to atherosclerosis at different vascular sites [12].

Intravascular ultrasound studies have confirmed and extended earlier findings from pathological studies [13]. One such study, performed in heart transplant recipients days after transplantation, found that up to 17% of asymptomatic teenagers had coronary atherosclerosis [13]. Similar observations were made using noninvasive measures of subclinical atherosclerosis, which, unlike coronary arteriography and intravascular coronary ultrasound, can be used in asymptomatic individuals and large population studies. For example, in the Bogalusa Heart Study, carotid intima-media thickness (especially in the carotid bulb) was increased in young adults aged 20–38 years [10]. This marker of atherosclerosis was associated with conventional cardiovascular risk factors and predicted by such risk factors in childhood [10]. Similarly, in 10- to 17-year-olds, arterial distensibility, a marker of arterial wall elasticity and the early atherosclerotic process, was related to lipid profile, blood pressure and parental history of myocardial infarction [10]. In fact, conventional cardiovascular risk factors have been shown to affect vascular health from early in childhood, and Leeson et al. [6] showed an inverse correlation between brachial arterial distensibility and cholesterol concentration from as early as the first decade of life.

Thus, in summary, there is now strong evidence for the presence of sub-clinical atherosclerosis in children from both pathological studies and ultrasound measures of vascular function. The latter are particularly important because they help elucidate risk factors for atherosclerosis in children, act as markers of the intensity of the burden of CVD, and could identify those at particular risk for future cardiovascular events. Ultimately, these noninvasive

techniques could help both in the investigation of mechanisms and the development of rational approaches to prevention.

### **The Metabolic Syndrome in the Young**

Postmortem studies and noninvasive measures of atherosclerosis both show that the same risk factors contribute to early atherosclerosis in the young as in adults [10]. As in adults, these risk factors correlate with each other and tend to occur in clusters. This clustering, first described in 1988 by Reaven [14] as the 'metabolic syndrome', links obesity, insulin resistance, dyslipidemia and hypertension with an increased risk of atherosclerosis, CVD and type-2 diabetes mellitus. In recent years, a number of new phenotypes have been added including microalbuminuria, endothelial dysfunction, and elevation in C-reactive protein and fibrinogen concentration [15]. However, obesity remains central to the development of the metabolic syndrome and subsequent cardiovascular risk [15].

#### *Role of Obesity*

As found in adults, obesity plays a major role in the development of the metabolic syndrome in the young. Obesity often precedes the development of other features such as insulin resistance, and each component of the metabolic syndrome worsens with increasing adiposity [16]. Recent evidence suggests that the syndrome is more common than previously suspected, with up to 50% of obese children and adolescents being affected [16].

That obesity (and particularly central obesity) is a major independent risk factor for CVD is well known. For instance individuals with a body mass index (BMI) of  $>30\text{ kg/m}^2$  are four times more likely to suffer from CVD than those with a BMI of  $<25\text{ kg/m}^2$  [17]. Obesity in children may be particularly detrimental [10]. Childhood obesity predicts the risk of developing a constellation of metabolic, hemodynamic and inflammatory disorders associated with CVD and, importantly, increases cardiovascular risk independent of adult weight [8]. Given that more than 30% of children in the USA are obese (BMI  $>95$ th percentile) [18], the current epidemic of childhood obesity has major implications for the prevalence of adult CVD.

The effect of childhood obesity on CVD is more complex than simply its association with conventional risk factors that make up the metabolic syndrome. The observation that insulin resistance cannot explain many newer features of the metabolic syndrome (e.g. features of inflammation) have led to suggestions that insulin resistance and atherosclerosis share common antecedents such as the adverse effects of inflammatory mediators secreted by adipocytes [15]. Adipose tissue could, therefore, have a direct detrimental effect on vascular health and particularly on endothelial cell dysfunction, a critical early event in the atherosclerotic process.

This concept, that adipose tissue is not just a passive energy store but is highly physiologically active, has become increasingly important in understanding the role of obesity in vascular disease. Adipose tissue secretes several biologically active cytokine-like molecules (e.g. C-reactive protein, interleukin-6, and leptin) collectively termed adipokines, which could affect vascular function by their local and distant actions. Leptin in particular, although predominantly involved in the regulation of appetite, has been shown to act via receptors on vascular cells to increase angiogenic activity and oxidative stress, and to promote vascular calcification and smooth muscle cell proliferation [19]. Consistent with this atherogenic action, we showed recently that leptin concentration was associated with lower arterial distensibility, independent of fat mass, inflammatory markers and insulin resistance [19]. These observations suggest that leptin is a key link between obesity and vascular disease. The fact that these findings were in healthy non-obese adolescents further suggests that this link is important early in the atherosclerotic process [19].

## **Mechanisms**

If we accept that atherosclerosis and the metabolic syndrome have their origins in childhood, then understanding the mechanisms becomes a high priority. Although a detailed discussion of mechanisms is beyond the scope of this chapter, such understanding will be critical in identifying the best interventions.

At its simplest level, the early origins of CVD may represent a genetic predisposition. Clearly, the development of atherosclerosis involves a complex interaction between the vascular endothelium, serum lipids, platelets, and inflammatory and vascular smooth muscle cells; a process in which poorly characterized genes must interact with a changing environment. Obesity, for instance, has been shown to have a 40–70% inheritance in twin studies [18]. Therefore, the same genes that predispose to obesity may be associated with other components of the metabolic syndrome and have a similar effect on atherosclerosis risk in the young as in older individuals.

Along these lines, the thrifty genotype hypothesis was first proposed over 40 years ago to explain the modern emergence of obesity and type-2 diabetes [20]. This hypothesis postulated that genetic selection of individuals, whose energy storage capacity allowed survival during time of famine, predisposed them to obesity and its complications during times of calorie excess (as in the modern world). However, while the development of atherosclerosis must involve complex gene-environmental interactions, family studies suggest only a moderate heritability for endothelial dysfunction (14%), a key early stage in this process [21]. The relative contribution of nature versus nurture for the development of atherosclerosis therefore remains largely unknown.

A second mechanism is the role of nutrition in the development of CVD. Nutrition has a major impact on most cardiovascular risk factors, which collectively account for more than 90% of the population-attributable risk of myocardial infarction (abnormal lipids, smoking, presence of diabetes, hypertension, abdominal obesity, psychological factors, consumption of fruits and vegetables, and alcohol, and regular physical activity) [22]. The same risk factors are important in both sexes, at all ages, and in all regions [22]. Furthermore, nutrition has the same effects on these factors in children as in adults [11] and, importantly, long-term follow-up studies have shown clear evidence for their tracking (particularly obesity, dyslipidemia, and blood pressure) from childhood into adult life [7, 10]. For instance, 2/3 of children obese at age 10 years are at risk of being obese as adults [18]. Not surprisingly, therefore, many features of the metabolic syndrome persist and extend from childhood into adult life [10]. Of particular concern in children is the long length of exposure to risk factors for vascular disease. For example, a prolonged exposure of arteries to the toxic metabolic milieu associated with obesity could explain the greater risk of CVD in those who are obese as children.

The third key mechanism for the early origins of CVD is the environmental equivalent to the thrifty genotype hypothesis, the thrifty phenotype hypothesis [23]. This concept has been recently proposed to explain the epidemiological evidence that factors in utero and in early life program the long-term risk of CVD. The thrifty phenotype hypothesis suggests that resetting of metabolism by early environmental factors affects the long-term phenotype, so that individuals exposed to under-nutrition in early life develop a thrifty phenotype which predisposes them to obesity and its risk factors if exposed to nutrient excess later in life [23]. The hypothesis is part of the broader concept of 'developmental plasticity' [24], shown by many plants and animals, which proposes that organisms are capable of developing in a variety of ways so that they are well adapted to the environment in which they are likely to live. Paradoxically, therefore, the modern epidemic of obesity and CVD may be related to the change in environment that has increased nutrient availability to individuals whose parents and grandparents lived in impoverished conditions.

Overall, it is clear that nutrition is key to most mechanisms for the early development of CVD. Nutrition is the major environmental factor that interacts with genetic predisposition to affect the metabolic syndrome and hence CVD risk. This applies equally to children as it does to adults. Nutrition in fetal life or in infancy is the major factor that programs the later propensity to CVD and, importantly, nutrition may interact with adverse prior programming to affect CVD risk [5]. For instance, the adverse effect of low birth weight on cardiovascular risk is greatest in individuals who become obese as adults [5]. Finally, obesity, itself strongly affected by nutrition, plays a central role in the early development of CVD. Prevention of obesity, along with key

nutrition interventions in infancy and childhood are therefore a logical and important focus for the prevention of CVD.

## **Nutrition Interventions**

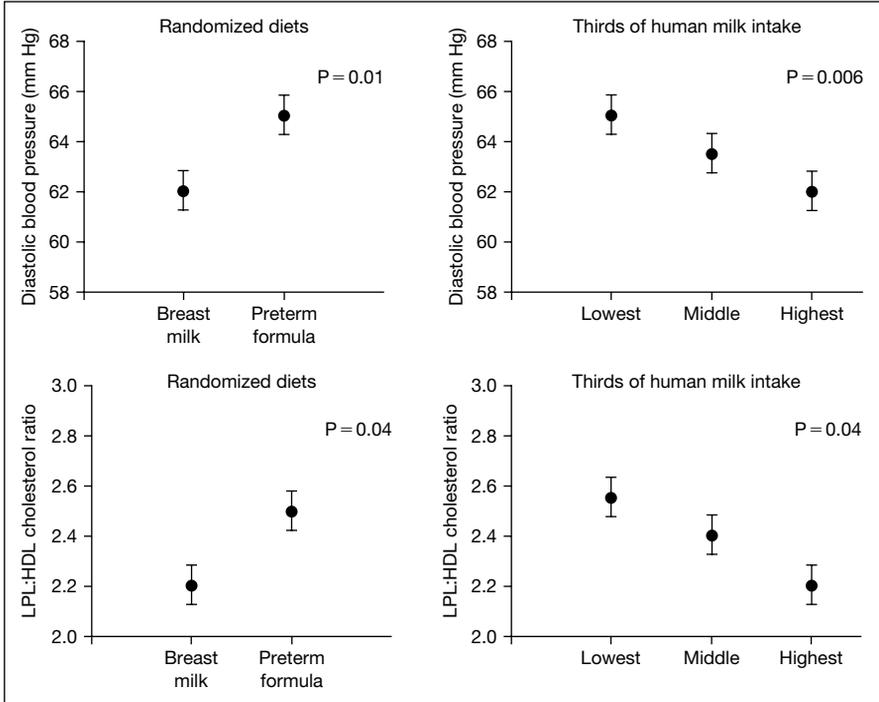
Critical to the argument that the primary prevention of CVD should begin in childhood is the observation that in up to 50% of individuals, the first manifestation of CVD is sudden death or myocardial infarction. This makes primary prevention particularly important for population health. Preventative efforts may be especially justified in children because lifestyle risk factors are often adopted in childhood and so, arguably, it is easier to intervene before these habits become established. Dietary and physical activity patterns, for instance, develop in childhood and are known to track into adult life. Risk-taking behaviors that affect CVD, such as smoking, are also often first acquired in childhood or adolescence. Nevertheless, despite the increasing epidemiological evidence, it remains difficult to establish the effectiveness of interventions at a population level using formal randomized trials.

The two main periods for interventions are in infancy and in childhood. However, importantly, there is no evidence to suggest age-related cutoffs that restrict preventative strategies to particular periods.

### *Interventions in Infancy*

The concept that nutrition in infancy can influence long-term risk factors for CVD first emerged in the 1960s with the pioneering work of McCance. He showed that rats raised in small litters, and therefore overfed early in postnatal life, were programmed for greater body size as adults. Subsequently, rats overfed in the brief suckling period were shown to have permanently higher plasma insulin and cholesterol concentrations while early nutrition in baboons was found to have a major impact on later obesity and atherosclerosis. In baboons, the effects of over-feeding in infancy for obesity emerged only after adolescence, demonstrating the later manifestation of some programming effects [5].

In humans, the major focus of nutritional programming has been the impact of breastfeeding. Breastfed infants have been shown in observational studies to have a lower risk of CVD, obesity, hypercholesterolemia, type-2 diabetes and high blood pressure [5]. These data could be confounded by socioeconomic and demographic differences between breastfed and formula-fed groups. In preterm infants, however, a causal association between breastfeeding and CVD risk was testable using an experimental approach. Infants whose mothers decided not to breastfeed were randomized to breast milk donated by unrelated lactating mothers or to formula milk. Infants assigned randomly to human milk versus formula, for an average of 4 weeks, were found to have marked benefits up to 16 years later for the major components of the metabolic



**Fig. 1.** Breast milk consumption in infancy and later blood pressure and lipid profile in adolescents born preterm.

syndrome (blood pressure, leptin ‘resistance’ suggestive of future obesity, insulin resistance and lipid profile; fig. 1) [5]. As further evidence of causation there were clear dose-response associations between the volume of breast milk intake and later cardiovascular benefit (fig. 1) [5].

The effect size for breast milk feeding on later cardiovascular risk factors is substantial. For blood pressure, for instance, a 3-mm Hg lower diastolic blood pressure in infants given breast milk compared to formula has major public health implications and represents an effect greater than all other non-pharmacological means of reducing blood pressure (such as weight loss, salt restriction, or exercise) [5]. Lowering population-wide diastolic blood pressure by only 2mm Hg has been estimated to reduce the prevalence of hypertension by 17%, the risk of coronary heart disease by 6% and the risk of stroke/transient ischemic attacks by 15%. Such an intervention would be expected to prevent an estimated 67,000 coronary heart disease events and 34,000 stroke/transient ischemic events annually among those aged 35–64 years, in the USA alone. Similarly, the 10% lowering of the cholesterol concentration with breastfeeding compares favorably with the effects of dietary

interventions in adults, which lower cholesterol by only 3–6%. Such an effect on cholesterol concentration would be expected to reduce the incidence of CVD by approximately 25% and mortality by 13–14% [5].

While breastfeeding is advantageous overall for later CVD risk, the optimal duration of breastfeeding remains unknown. In baboons, breastfeeding for the whole of ‘infancy’ followed by a Western diet increased later dyslipidemia and atherosclerosis compared to those previously fed formula [25]. Similarly, in young adults, breastfeeding beyond 3–4 months was associated with a ‘dose-related’ decline in vascular distensibility [25]. This study suggested that breastfeeding of longer duration interacted adversely with a subsequent (‘unphysiological’) Western-style diet, a hypothesis supported by further studies in humans. However, although intriguing, these preliminary and observational data cannot be used to guide public health interventions.

Understanding the mechanisms by which breastfeeding benefits cardiovascular health is essential in the development of preventative strategies for formula-fed infants. The most common explanation, confounding by socio-biological factors that influence both the mothers’ decision to breastfeed and later cardiovascular risk, is unlikely in view of experimental evidence from preterm infants. Other potential explanations include the long-term health benefits of specific nutrients in breast milk, which are absent from some formulas – such as the effect of long-chain polyunsaturated fatty acids in lowering later blood pressure [26]. Most recently, we have suggested that the cardiovascular advantages of breastfeeding may be due to slower growth in breastfed versus formula-fed infants [5].

#### *The Growth Acceleration Hypothesis*

The postnatal growth acceleration hypothesis suggests that faster growth (upward percentile crossing) particularly in infancy adversely programs the metabolic syndrome [5]. Consistent with this, faster neonatal growth was shown to program insulin resistance and endothelial dysfunction in adolescence. The size of the effect was substantial. Adolescents born preterm with the greatest weight gain had 4% lower flow-mediated dilation of the brachial artery than those with the lowest weight gain, an effect similar to that of insulin-dependent diabetes mellitus (4%) and smoking (6%) in adults [5]. These findings were not confined to infants born prematurely. In an intervention study of infants born full-term but small for gestation, those randomly assigned to a standard formula for the first 9 months had lower blood pressure 6–8 years later than those fed a nutrient-enriched formula that promoted growth (Singhal, unpublished). Further analysis suggested that faster growth explained the adverse effects of a nutrient-enriched formula on later blood pressure.

Data from further studies in both man and animals strongly support this hypothesis. The adverse effects of faster growth are consistent with previous data in animals showing that a higher plane of postnatal nutrition programs

the metabolic syndrome. In fact the adverse long-term effects of faster early growth emerge as a fundamental biological phenomenon across animal species [5]. Because growth acceleration is greatest in early infancy, this period may be critical. Consistent with this, faster growth in infancy has been associated with a greater risk of later obesity [5], and, from as early as 2 weeks, with later insulin resistance and endothelial function [5]. Importantly, both infants born prematurely or at term show these effects and, for obesity and endothelial function at least, faster gain in weight and length have both been shown to have adverse long-term effects.

Overall there is now strong evidence to support breastfeeding for the primary prevention of CVD. Whilst, clearly, randomized trials with clinical endpoints to prove efficacy are not possible, and the optimum duration of breastfeeding remains unknown, the effect size is considerable and has major implications for public health.

### **Nutritional Interventions in Childhood**

#### *Prevention of Obesity*

Given the strong evidence for the effects of obesity on the early development of atherosclerosis and the metabolic syndrome, there are surprising little data from randomized control trials that support a preventative role for weight loss in children. However, as in adults, weight loss in children is associated with improvements in insulin sensitivity, lipid profile and ultrasound measures of vascular health [27].

Perhaps the most compelling evidence for a cardiovascular benefit of weight loss in children is the effect on endothelial function [28]. In a randomized study, weight loss over only 6 weeks reversed the vascular dysfunction associated with obesity [28]. The addition of exercise training enhanced the beneficial arterial effects, which were sustained when the exercise program was continued for 1 year. These data underscore the importance of diet and exercise in reducing the impact of obesity on vascular disease even from an early age [28].

#### *Intervention Trials*

Ultimately, to affect public health policy, the efficacy and safety of nutritional interventions have to be demonstrated in large-scale randomized trials based in populations. Such data are difficult and expensive to collect but are now emerging.

For instance, in the Special Turku Coronary Risk Factor intervention project for babies, individual dietary counseling during and after infancy was shown to reduce the fat content of the diet (particularly for saturated fat), and improve the lipid profile up to 10 years later [29]. Similarly, in the dietary intervention study in children with hypercholesterolemia, dietary behavioral

intervention reduced fat (and particularly saturated fat) intake, and improved lipid profile at the 1- and 3-year follow-up but not after [30]. Importantly, in both of these studies, there were no adverse effects and the benefits were obtained without affecting growth.

Thus, although further large-scale trials are needed, there is increasing evidence for the efficacy and safety of interventions to reduce cardiovascular risk factors in childhood. However, the effects of specific therapies (e.g. the use of statins), specific nutrients (e.g. long-chain polyunsaturated fatty acids), or preventing obesity on long-term CVD have been little researched.

## Conclusion

A rapidly increasing prevalence of CVD and its risk factors makes it the most important health issue of the 21st century. The dramatic rise in obesity alone is expected to decrease life expectancy and threatens to reverse the reduction in cardiovascular mortality achieved in the past decades through control of hypertension, hyperlipidemia and smoking. There is now little doubt that the problem has its origins early in life. This has led to a major shift in focus for primary prevention from adults to children. Indeed, it is now reasonable to suggest that lifestyle modification, weight control and specific nutritional interventions (e.g. breastfeeding) in children could help reduce the burden of CVD in populations worldwide.

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## Discussion

*Dr. Laron:* I have several questions. Is there any new histological or pathological evidence that children who were killed in accidents had developed atherosclerosis? The classical examples are the soldiers killed in Korea. My second question is do you have any data on fatty liver? There are reports that 15% of obese children develop fatty liver. My third question is concerned with increasing growth velocity. Do you

think that the new approach of pushing growth hormone in very young children is deleterious?

*Dr. Singhal:* The growth hormone story is very interesting and I think there is some evidence that using growth hormone in children increases the risk of diabetes or insulin resistance [1]. Whether this is reversible when growth hormone is stopped is being investigated. The second question is the fatty liver story. I am not an expert on body composition but fatty liver correlates closely with visceral fat mass. Clearly visceral fat mass is likely to be detrimental for the metabolic syndrome. Regarding post-mortem studies, I am not aware of data on atherosclerosis in children who died from accidents. There are certainly data in from heart transplant patients that atherosclerosis is present in adolescence [2].

*Dr. Pereira-da-Silva:* What is your recommendation for neonatologists: (1) to continue providing additional nutrients to premature babies using fortified human milk, preterm formulas and PDF formulas and increase the risk of future morbidity, or to provide a suboptimal diet with the risk of a negative impact on brain structure as shown by Dr. Lucas, and also compromising catch-up growth, and (2) to consider different nutritional interventions for different populations of premature babies, i.e., in the case of being small versus appropriate for gestational age [3, 4], or asymmetrically versus symmetrical intrauterine growth retarded [5]?

*Dr. Singhal:* That is a very interesting question. One of the things that we have talked about is long-term effects and I think it is always important to remember that short-term effects, especially survival in preterm infants, is also important. You need to feed the preterm infant to make the baby strong enough to be extubated and to survive in the first place. So in preterm infants you need good nutrition and growth to make the baby strong enough, and to preserve the brain. I would also recommend that you add as much human milk as possible because of all the benefits with regard to necrotizing enterocolitis and long-term health. I am not aware of data which separate preterm infants into SGA or non-SGA. Our effects on later health were independent of the size at birth, but logically you would expect SGA preterm infants to be at higher risk of malnutrition.

*Dr. Saavedra:* With regard to weight gain, and you nicely showed that there is some evidence for a dose effect from the point of view of exclusive breastfeeding or the amount of breastfeeding versus non-exclusively breast-fed babies, within breast-fed babies and rate of weight gain, or within non-breastfed babies and rate of weight gain. Do you think we still see the kind of effects that you have shown?

*Dr. Singhal:* We do. Everybody would agree that breastfeeding is best, but in Iceland they have shown that faster weight gain in infancy is associated with a greater risk of obesity in breastfed babies. So I think the mechanism is more fundamental. I think breastfeeding works by reducing the growth rate compared to those infants fed formula but I think the growth rate is the important factor.

*Dr. Hanson:* I would like to ask you about the possible role of infections. Firstly, premature babies would have more infections, and secondly some children have repeated infections. In other words would early immunodeficiency be an advantage or not? Also children in poor countries who have lots of infections, what are the long-term effects of that?

*Dr. Singhal:* There is a very interesting paper which showed that in the ALSPAC study children who had more infections, had lower endothelial function [6].

*Dr. Hanson:* I could imagine that there might be a further variation of that based on genetic differences. We are just looking at alleles of cytokine genes, for instance TNF2, the allele of that for TNF- $\alpha$  is presumed to relay to a higher production of TNF- $\alpha$ , and we have seen that the presence of TNF2 relates to poorer outcome, rejection of a transplanted kidney for instance. One could imagine that such genetic variations also may result in variations in the response to infections.

*Dr. Singhal:* I completely agree. The effects have got to be different by different genotype, and the TNF story particularly in intensive care is very interesting.

*Dr. Cohen:* I enjoyed your discussion of leptin and the role of hyperinsulinism, obesity and the metabolic syndrome. Is it scientifically plausible that some day either molecular antibodies that would block the leptin receptor or possibly an anti-leptin vaccine that would produce anti-leptin antibodies could be developed as a treatment for obesity?

*Dr. Singhal:* I am not an expert on the role of leptin in the treatment of obesity and I think the anti-leptin story did not work because it is leptin-resistance rather than low leptin levels that are important in obesity. It is interesting that when a paper came out showing that leptin may be a link between obesity and vascular function [7], there was no interest among the pharmaceutical companies to see whether blocking the leptin receptor on endothelial cells could specifically reduce the risk of cardiovascular disease. The effect of obesity on blood levels occurs over many years. So I don't think a simple pharmaceutical bullet to block the actions of leptin on endothelial functions will work.

*Dr. Wang:* In our country, mothers who formula feed their infants normally have some reason for not breastfeeding, such as some form of cardiovascular disease. Do you have any data about family disease history in your studies?

*Dr. Singhal:* I think family history will obviously interact with the effects of early nutrition on long-term cardiovascular health. The advantage of randomized studies is that you remove the effect of the family history because of the randomization procedure. But I do agree that if you are prone to heart disease and have faster growth, this could theoretically make the situation worse. The important point is that we must try to do randomized studies to remove genetic and other confounding effects.

*Dr. Kroke:* I would like to know whether you have calculated the positive predictive value of rapid growth in infancy because I do not think that every child that is growing fast is going to be obese. So what is the quantitative effect?

*Dr. Singhal:* The best data are from Stettler et al. [8] who worked on the effects of faster growth in infancy and long-term risk of obesity. They suggested that the attributable risk of developing obesity or faster growth in infancy is approximately 20%. So there is a huge effect of faster growth in early life on the long-term risk of obesity. We haven't calculated the risk partly because our data are from preterm infants and so there may be other factors involved.

*Dr. Greeff:* With rapid growth is it really in our control to intervene with that? Many of those children will be just as miserable unless we feed them all, and they will have rapid growth whether we like it or not.

*Dr. Singhal:* I think that is a very important point. There is a paper which looked at which factors affect growth acceleration immediately after birth [9]. The most important is genetics, as you would expect, because if the parents are tall the baby is going to try to catch up. But postnatal nutrition also has an effect and this is the one factor that you can manipulate. So in other words if you have a SGA baby you are going to show a catch-up growth immediately after birth, but you don't need to make the situation worse by adding a high nutrient intake.

*Dr. Hursting:* In the cancer story it appears that the obesity cancer link may be driven primarily by insulin resistance, in part independent of the adiposity. I am wondering if a similar story is emerging in cardiovascular disease?

*Dr. Singhal:* I think the story is very similar. The coupling mechanisms between early growth and long-term health are often hormonal. For example faster growth in early life increases the IGF-1 concentration later in life [10], which links the cancer story and the cardiovascular story. So I think that if you set up hormonal systems soon after birth they then go on to effect other systems later in life.

*Dr. Sorensen:* For many years we have considered salt ingestion as a main cause for persistent hypertension and cardiovascular risk. How would you place salt ingestion as a risk factor in the context of these new concepts about increased growth etc., because I think that salt is something that is a lot easier to manage than many of these other factors. I wonder what you think about that?

*Dr. Singhal:* I think you are absolutely right. Nutritional programming effects are not going to work by themselves and the most obvious example of this is that you don't see heart disease in poor communities, regardless of the growth rate in early life. Conventional cardiovascular risk factors such as salt in take and obesity interact with the programming effects much earlier in life.

*Dr. Laron:* After your talk and what Dr. Lucas has said that breastfeeding is so important for the future public health issues, what should the message be from here to the regulatory organizations? We know that postnatal leave differs greatly from country to country, from none to 1 year in Finland, thus I think it would be of interest that something be said by those promoting this important issue.

*Dr. Singhal:* I completely agree. There should be enough time to breastfeed a baby adequately because we now know there are huge short- and long-term effects.

*Dr. Lucas:* I would just add the point that what we still don't know is the optimal duration of breastfeeding to achieve these benefits. There is obviously some concern at the moment that a long period of breastfeeding might actually increase cardiovascular risk. I don't think that that should alter our breastfeeding practices. What it should do is make us to worry about our weaning diet. But nevertheless in the end the amount of maternity leave should logically be based on the optimal duration of breastfeeding to achieve long-term health, and that needs to be resolved.

*Dr. Saavedra:* May I get back to the research related to weight gain and the possible influences it might have. Do we have anything yet to show if it is just weight gain versus changes in body composition for example in relation to adipose tissue in children?

*Dr. Singhal:* This is an area of active research: whether differences in body composition in early life effect the long-term risk. I can point out two studies in which an increase in length gain is associated with long-term cardiovascular risk. One was in preterm infants which showed an effect on endothelial function [11] and another showed an effect on obesity [12].

*Dr. Lake:* In your experimental models, when you provide your extra calories during that programming interval, this is almost always done by increasing the caloric density of the product that is given. Does it matter if the increased calories are provided by allowing a greater volume of intake by the individual during that time?

*Dr. Singhal:* The major, approximately 30%, difference was in the protein content. I think the standard and nutrient-enriched formulas were 68 and 72 kg/cal per 100 ml, respectively, so there wasn't a huge difference in energy. The volume of intake in the preterm infants was determined by the neonatal units and we had no control over this.

*Dr. Hamburger:* Just a comment to emphasize or illustrate the point that Dr. Lucas made a moment ago. I have recently been informed that in later old age lowering cholesterol, which we have all been trying to do, is associated with increased morbidity and mortality. I might agree with the caution not to rush to change practice without good evidence and good data.

*Dr. Singhal:* Sure, I agree with that. At the moment our findings don't change practice because we are encouraging breastfeeding, which is current practice anyway.

*Dr. Klish:* Since we are talking about cholesterol, I also have a question related to one of your early slides where you were talking about carotid artery distensibility and relating it to the cholesterol levels. An obese population has a normal distribution for cholesterol but the normal curve is about 10–15% greater than for a normal weight population. Can your distensibility studies show differences when the cholesterol rises

by 10–15%? If so, this could be an important tool to monitor the impact of the increase in cholesterol in obese children.

*Dr. Singhal:* The data that I presented showed that cholesterol was associated with decreased artery distensibility independent of body mass index, not body composition, so I do accept the criticism that obesity may still play a role in association. So certainly there are lots of data now showing that cholesterol concentrations in children influence vascular function.

*Dr. Lucas:* Can I just go back briefly to a point that was made earlier about volume intake. If you increase energy intake in babies they downregulate the volume intake so they control intake and several people have shown that. But if you actually alter protein intake, we have demonstrated that there is no downregulation. So in the studies in which we have measured it, the volume intake is exactly the same in babies on a high- or low-protein diet, so you see the full effect of the intervention. This is an extremely important point if you are influencing growth, then the protein strategy is much more effective.

*Dr. Arvanitakis:* Regarding the endocrine factors of obesity, the leptin data are more or less known. Do we have any evidence on other hormones more recently described such as ghrelin?

*Dr. Singhal:* I am not really the expert on endocrine factors in obesity, I am using the endocrine system as a link between early growth and long-term health.

*Dr. Kroke:* I was wondering whether you have any information about the effects of a woman being obese or having the metabolic syndrome on the composition or the quality of breast milk? Do you think the metabolic profile of the mother is having any effect?

*Dr. Singhal:* Yes it does, and Plagemann et al. [13] published these data showing that the breast milk of gestational diabetic mothers is richer and their babies grow faster and are more prone to obesity and glucose intolerance.

*Dr. Mora:* I wonder if there are any data regarding the use of hydrolyzed formula or amino acid-based formula in premature babies and term babies with regard to cardiovascular disease?

*Dr. Singhal:* I am not aware of anything in that area.

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