Early growth and cardiovascular disease

Ten years ago studies in Britain showed for the first time that people who had low birth weight were at increased risk of coronary heart disease and the disorders related to it: stroke, non-insulin dependent diabetes, raised blood pressure, and the metabolic syndrome. In a study of 16,000 men and women born in Hertfordshire death rates from coronary heart disease fell twofold between those at the lower and upper ends of the birth weight distribution. In groups investigated clinically the prevalence of non-insulin dependent diabetes and impaired glucose tolerance fell threefold. Such findings led to the “fetal origins hypothesis,” which states that cardiovascular disease and non-insulin dependent diabetes originate through adaptations that the fetus makes when it is undernourished. These adaptations, which include slowing of growth, permanently change the structure and function of the body.

Recently, associations between low birth weight and later disease have been widely replicated in studies in Europe and the USA. These studies have also confirmed that the associations are not the result of confounding variables, such as low socioeconomic status and smoking, which act in postnatal life. Influences that act in postnatal life do, however, add to the effects of low birth weight. For example, the highest prevalence of non-insulin dependent diabetes and impaired glucose tolerance fell threefold. Such findings led to the “fetal origins hypothesis,” which states that cardiovascular disease and non-insulin dependent diabetes originate through adaptations that the fetus makes when it is undernourished. These adaptations, which include slowing of growth, permanently change the structure and function of the body.

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Body proportions at birth and adult disease

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SHORT

Babies that are short in relation to their head circumference, and have a reduced abdominal circumference, tend to have persisting abnormalities of liver function, including raised serum LDL cholesterol and plasma fibrinogen concentrations. Replication of these observations has, however, been limited because few datasets include abdominal circumference at birth. Babies that have a small abdominal circumference in relation to their head circumference can result from “brain sparing” circulatory adaptations by which cardiac output is diverted to the brain at the expense of the trunk.

SHORT AND FAT

Studies in southern India have shown that babies who are short and fat tend to become insulin deficient and have high rates of non-insulin dependent diabetes. This is consistent with findings in Pima Indians and with observations in Sheffield that showed a U-shaped association between abdominal circumference at birth and death from coronary heart disease. Babies that are short and fat are thought to be the result of maternal hyperglycaemia, with consequent imbalance in the supply of glucose and other nutrients to the fetus.

LARGE PLACENTA

Studies in Preston showed that babies whose placenta are disproportionately large in relation to their own weight tend to have raised blood pressure. While this has been replicated in other studies it has not been found consistently; in a study in Aberdeen, raised blood pressure was associated with small placental size. Animal studies offer a possible explanation. In sheep the placenta enlarges in response to moderate undernutrition in mid-pregnancy. This is thought to be an adaptive response to extract more nutrients from the mother. It is not, however, a consistent
response but occurs only in ewes that were well nourished before pregnancy.

The malnourished fetus

The weight of evidence from studies of the birth weights of relatives has led geneticists to conclude that, although the growth of a fetus is influenced by its genes, it is usually limited by the nutrient and oxygen supply it receives.\(^1\)\(^2\) Constraint of fetal growth by the mother has been shown in embryo transfer and cross breeding experiments; a fetus transferred to a larger uterus will achieve a larger birth size. A study of babies born after ovum donation showed that while their birth weights were strongly related to the weight of the recipient mother, they were unrelated to the weight of the woman who donated the egg.\(^3\)\(^4\) It has been suggested that the associations with low birth weight reflect genes that determine both reduced fetal growth and later disease. The known plasticity of fetal growth and its control by the mother makes this speculation improbable. Rather there is a clear need to study interactions between genes and nutrient supply in utero. This focus on the nutrient supply to the fetus is supported by numerous animal experiments showing that poor nutrition may permanently affect the structure and physiology of the fetus in ways that can be related to disease in humans, including life long elevations of blood pressure and altered glucose–insulin metabolism in the offspring.\(^5\)

Mother’s body composition and diet

The availability of nutrients to the fetus is determined by the mother’s body composition at conception and her diet in pregnancy. In early studies in Britain there were few data on mothers. Cohorts that include this information as well as having detailed measurements of body size at birth have now been identified in Europe as well as in the developing world, and include mothers who are chronically malnourished. Preliminary findings suggest that many babies today may be malnourished and at increased risk of coronary heart disease and non-insulin dependent diabetes either because their mothers are too thin, or too fat, or eat unbalanced diets. The offspring of women who are thin, as measured by skinfold thickness, tend to have raised blood pressure,\(^6\) while the offspring of mothers with a high body mass index have increased rates of coronary heart disease and non-insulin dependent diabetes.\(^7\)\(^8\) Imbalance in the mother’s protein and carbohydrate intake is associated with raised blood pressure in the offspring.\(^9\)

Childhood growth

Studies in Helsinki have shown that the path of growth through childhood modifies the risk of disease associated with size at birth.\(^10\) The highest death rates from coronary heart disease occurred in men who were thin at birth but had accelerated weight gain in childhood. We do not yet know whether this association is because of the pathologi- cal effects of a high fat mass persisting into adult life, deleterious effects of catch up growth, or the intrauterine resetting of endocrine axes that control growth. It suggests that while the primary prevention of coronary heart disease and non-insulin dependent diabetes may ultimately depend on changing the body composition and diets of young women, more immediate benefit may come from preventing imbalances between prenatal and postnatal growth among children.

Primary prevention

To reduce chronic disease we need to understand how the human fetus is nourished and how malnutrition changes its physiology and metabolism. Fetal development is governed by complex, non-linear systems. Components of these systems interact, and the systems will have generic properties that do not depend on the details of their components.\(^11\) We cannot solve our problems by reductionist science alone, but need to combine this with further clinical and epidemiological research.

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Evidence for using nebulised antibiotics in cystic fibrosis

Standards of care for patients with cystic fibrosis (CF) have been defined largely on the basis of “best practice”, an accolade awarded to treatment regimens showing low chronic pulmonary infection rates, greatest patient longevity, and least patient morbidity. While acknowledging the wisdom accrued through clinical experience, paediatricians caring for children with CF are fighting for limited resources and must convince purchasers, who stroll the fashionable catwalk of evidence based medicine, of the scientific basis of our demands. They ask, “Where’s the beef?” For the use of nebulised antibiotics in CF care we can reply only that we have a lot of “topside” but few “prime cuts”.

Clinical trials, the results of which affect all of our prescription practices, have been generally parochial in conception, bedevilled by small patient numbers and underpowered, partly reflecting patient recruitment problems for research into an illness that affects only a small minority of the population and, until recently, a lack of multicentre trials. In his meta-analysis of the benefits and risks of nebulised antibiotic treatment in CF, Mukhopadhyay et al suggested that a definitive study would need 500 patients, the minimum that would allow for imbalances in patient characteristics that are not amenable to adjustments in the analysis.1 In Ramsey’s study of TOBI, a preservative free preparation designed for nebulisation, over 80% of almost 500 patients completed the trial,2 but the most comprehensive published multicentre study at the time of writing has only 71 patients.3 The antibiotics studied (gentamicin, tobramycin, colistin, ceftazidime), the antibiotic dose (20 mg to 600 mg of aminoglycoside), the type of nebuliser used, and the length of treatment have varied, making comparison between trials difficult.

Rationale for nebulised antibiotics

CF is a multisystem disease but morbidity and mortality closely correlate with progressive pulmonary damage, most often caused by chronic Pseudomonas aeruginosa endobronchial infection. Persisting infection promotes chronic airway inflammation and progressive lung destruction. While intravenous antipseudomonal aminoglycoside antibiotics are fundamental to the treatment of this infection, decreasing the pulmonary bacterial load and improving lung function,4 they do not penetrate well into sputum, peak levels approximating to 12% of the serum concentration.5 Aminoglycoside activity is further compromised by biological antagonism in sputum. Half of a gentamicin dose added to CF sputum may be bound by extracellular neutrophil DNA and the altered ionic environment interferes with drug accumulation by the bacteria, significantly decreasing its bactericidal activity.6 In vitro, an aminoglycoside bactericidal effect can only be reliably produced with concentrations 25 times the minimum inhibitory concentration (MIC).7 To achieve such high drug concentrations in sputum in vivo by intravenous antibiotic delivery would increase unacceptably the potential for nephrotoxicity and ototoxicity. Aerosol delivery could provide a high concentration at the desired site with minimal absorption and therefore low risk of toxicity.8

The widespread use of nebulised antibiotics in the management of patients with CF has evolved from three landmark publications; Hodson et al in 1981, Littlewood et al in 1986,2 and Ramsey et al in 1991.9 10 11 These studies validated that twice daily inhalations improved respiratory function and decreased hospital admissions,9 decreased the frequency of positive P aeruginosa cultures in recently colonised patients,10 and increased the chances of eradicating this organism when used to treat early infection in combination with oral antipseudomonal antibiotics.11 12

Aerosol delivery of antipseudomonal antibiotics has been widely used in Europe for over a decade, and is becoming increasingly popular in the USA where significant results have been obtained in trials of preservative free tobramycin (TOBI). It is a good time to look at the available evidence for its efficacy in maintaining respiratory function, treating acute exacerbations, and eradicating early P aeruginosa colonisation.

Treatment of patients with chronic P aeruginosa infection

In 1981 Hodson et al compared nebulised gentamicin 80 mg and carbenicillin 1 g (both bid) with placebo in a double blind crossover trial in 20 adult patients over a one year period.9 During active treatment patients showed improved respiratory function, felt better, and were hospitalised less frequently. Subsequent studies emphasised these positive findings: enhanced lung function tests,10 11 12 slower decline in respiratory function,13 14 15 decreased hospital admission rates,16 17 improved clinical score,18 better weight profile,19 20 21 and decreased P aeruginosa density,22 23 24 25 26 27 28 29 30 Patient numbers in these studies were small (nine to 41) and treatment length varied from three to over 32 months. Antibiotics used were ceftazidime, gentamicin and carbenicillin, colistin and tobramycin. Study designs were crossover, placebo controlled, and open label.

Only Ramsey et al’s paper3 can stand alone as evidence based research. Otherwise we need to turn to Mukhopadhyay et al’s meta-analysis to distil the evidence that confirms the benefit of nebulised antibiotics in CF care.9 Nine of 14 clinical trials were rejected because they lacked appropriate randomisation or failed to describe adequately outcome measures. These were defined in the meta-analysis as the number of acute pulmonary exacerbations, alterations in lung function, the number of patients with an altered pseudomonas respiratory load, alterations in the number of patients with resistant P aeruginosa, and the incidence of auditory, renal or respiratory side effects. The analysis of the five qualifying papers concluded that nebulised antibiotics significantly reduced respiratory P aeruginosa load and the frequency of respiratory exacerbations requiring systemic antibiotic treatment, and significantly increased lung function. The only adverse finding was a possible increase in in vitro bacterial resistance.

Ramsey et al’s studies of aerosolised tobramycin allow us to make evidence based decisions about clinical and bacteriological efficacy.2 3 In a multicentre, double blind, placebo controlled trial, 71 patients received half strength physiological saline or 600 mg TOBI in 30 ml half strength saline delivered by ultrasonic nebuliser.3 The drug dose was chosen to attain a sputum concentration of at least 400 µg/g—that is, 10-fold the MIC of tobramycin susceptible P aeruginosa. This sputum concentration is necessary to prevent bacterial growth.3 Group 1 received tobramycin for 28 days and placebo for 56 days, and group 2 received tobramycin for 56 days followed by placebo for 28 days. Quinine was added to placebo and drug to mask differences in taste. Nephrotoxicity was monitored by serum creatinine concentrations and urine analysis, ototoxicity by auditory acuity and vestibular function tests, and adherence by urine quinine concentrations. Sixty six patients completed the trial.
After 28 days, pulmonary function, expressed as a percentage of the predicted values, showed a 6–13 percentage point improvement in respiratory function in the active compared to placebo treatment periods: p < 0.001 for forced expiratory volume in one second (FEV₁) and forced expiratory flow (FEF₂₅₋₇₅), and p = 0.014 for forced vital capacity (FVC). At the end of the three month trial the magnitude of the treatment effect had fallen to between 4–6 percentage points. The increase for FEV₁, and FEF₂₅₋₇₅, remained significant at p = 0.002 and p = 0.001, respectively, but the difference between placebo and tobramycin for FVC was not significant. The results at three months possibly reflect the greater use of oral and intravenous antibiotics in the placebo periods (p = 0.006). Tobramycin also decreased the sputum density of P. aeruginosa by a factor of 100 (p < 0.001) as well as the frequency of acute respiratory exacerbations (p = 0.06). Adjustments for age, sex, study centre, baseline lung function, and adherence did not affect the outcome data. There were no toxic effects but delivery of 600 mg tobramycin by ultrasonic nebuliser poses problems of cost, patient inconvenience, and difficulties with nebuliser maintenance, and achieves higher than needed sputum aminoglycoside concentrations.

Two subsequent phase III, multicentre, randomised, placebo controlled, double blind studies of TOBI compared 300 mg of drug to a quinine based placebo, each in 5 ml of quarter strength saline administered twice daily for 10–15 minutes via a Pari LC Jet Plus nebuliser and Pulmo Aide compressor. The 300 mg dose has > 90% probability of achieving a peak sputum concentration at least 10-fold greater than the maximal MIC of the patients’ P. aeruginosa isolates. Patients received three treatment cycles over 168 days, each cycle consisting of 28 days with nebulisation followed by 28 days without. The on–off protocol design was based on evidence showing some treatment effect persisting at 28 days after stopping nebulised tobramycin, animal toxicology studies that showed resolution of any histological changes by that time, and because of the possibility of such a regimen minimising the potential for encouraging bacterial tobramycin resistance and increasing patient adherence (Ramsey BW. Presented at the 22nd European Cystic Fibrosis Conference, Berlin, June 1998). Adaptive randomisation ensured a balance between treatment groups for age, disease severity, sputum production, centre, concomitant recombinant human deoxyribonuclease (Pulmozyme; Roche Products Ltd, Welwyn Garden City, Herts, UK) inhalation, and the MIC of tobramycin susceptible P. aeruginosa. The treatment effect was defined as the mean relative change with active treatment minus the mean relative change with placebo from baseline at the end of the third treatment cycle. Over 80% of 491 patients enrolled completed the trial.

Nebulised tobramycin significantly improved respiratory function. The treatment effect for FEV₁ was about 12% (p < 0.001) evident after the first cycle and maintained throughout the study. Colony forming units per gram of sputum fell significantly (p < 0.001). Hospital admissions and the need for intravenous antibiotic treatment fell compared to placebo (p = 0.014), a difference that started to show at four weeks. Lung function data are now available for up to 12 months of treatment in over 250 patients and show maintenance of improvement at about 10% above baseline (Ramsey BW, June 1998). Nebulised tobramycin exerts a positive effect even in patients established on Pulmozyme treatment.

Safety of long term nebulised aminoglycosides
The main safety concerns centre on the risk of ototoxicity from aminoglycosides, nephrotoxicity from aminoglyco-

ides and colistin, increased P. aeruginosa antibiotic resistance that may compromise choices for intravenous antibiotic courses, and superinfection with naturally resistant organisms (such as Burkholderia cepacia, Alcaligenes xylosoxidans, Stenotrophomonas maltophilia, and Aspergillus species). There was no detectable ototoxicity or nephrotoxicity with tobramycin doses up to 600 mg tid when auditory acuity, vestibular function tests, serum creatinine, and urine analysis were used as monitoring tools. Systemic drug absorption from nebuliser delivery is minimal, but aminoglycoside toxicity remains a risk with prolonged treatment. Urinary n-acetyl-b-D-glucosaminidase concentrations, a sensitive indicator of tubulointerstitial lesions, show a straight forward dose–response relation with inhaled gentamicin.

Most studies report no increased resistance to the nebulised antibiotic and no increased new infection with multiresistant organisms. MacLuskey et al documented resistance developing in four of 12 patients receiving nebulised tobramycin for a mean of 32 months but in none of 12 controls. Any observed resistance is mostly minimal and transitory.

Nebulised antibiotics in the treatment of acute respiratory exacerbations
A small study comparing inhaled (eight patients) with intravenous (eight patients) antibiotic treatment for acute pseudomonas related pulmonary deterioration showed improvement and similar responses in both groups. A retrospective study of nebulised tobramycin for two weeks for acute P. aeruginosa infections had encouraging results. These have not been followed up in adequately powered randomised controlled trials and no conclusions can be drawn.

No study has shown benefit from adding inhaled antibiotics to routine intravenous treatment for acute respiratory exacerbations.

Nebulised antibiotics in the treatment of new P. aeruginosa infection
Stephens and colleagues observed eradication of P. aeruginosa infection in studies of inhaled antibiotics in acute pulmonary deterioration. Littlewood and colleagues showed that inhaled colistin given soon after the first pseudomonas isolation can decrease the number of organisms isolated, the frequency of isolation, and in some cases eradicate the infection. The Copenhagen centre progressed this knowledge and in 1991 described how chronic pseudomonas infection can be largely prevented by combined oral ciprofloxacin and nebulised colistin each given for three weeks. Further experience suggests that three months of high dose combination treatment is more effective in preventing or at least delaying chronic infection. The argument for this use of nebulised antibiotic stands on the excellent results achieved, but criticism can be and is directed at the use of historic controls. The recommendation to follow the Copenhagen protocol is not evidence based as there are no randomised controlled trials, but the results are so formidable that such a study is unlikely to receive ethical approval, even if anyone wished to do it.

Conclusion
We cannot precisely know for any individual patient how much of the nebulised antibiotic is delivered to the lungs, nor exactly where it will be deposited. Sputum drug concentrations are only a rough guide. We do not know from where the sputum has been expectorated, nor how much of the antibiotic in the sputum is from the upper airways. Nonetheless, nebulised antibiotics can improve lung
function, slow the rate of respiratory decline, and decrease the need for intravenous treatment. In combination with oral ciprofloxacin, nebulised colistin can eradicate early P. aeruginosa infection. Aerosolised antibiotic treatment appears safe in short term trials but long term safety data are not available and patients should be monitored routinely.

It is with interest and anticipation that studies with the Halolite nebuliser (Medic-Aid Ltd, Bognor Regis, West Sussex, UK) are awaited. Timing of antibiotic delivery to the first part of the inspiratory cycle should improve drug deposition and efficacy, reduce waste, and possibly improve compliance. Patient adherence to nebulised antibiotics delivered with standard equipment is likely to be only about 60%. We still do not know the minimum effective dose for nebulisation. Individual practices vary between 80 mg and 300 mg bid for aminoglycosides. Colistin is empirically prescribed at doses between 0.5 MU and 2 MU according to the child’s weight. The Halolite, if fulfilling its promise, will necessitate revision of dosage schedules.

Nebulised antibiotic treatments in CF have progressed from a good idea derived from clinical experience to a more scientific approach to drug dosage and delivery. Only the preservative free tobramycin has been studied in an adequately powered trial, but many others document significant clinical and microbiological response to less expensive drug formulations—that is, the standard preparations for intravenous use delivered as an aerosol, to evaluate TOBI fully we need a direct comparison between them.

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Is prolonged rotavirus infection a common cause of protracted diarrhoea?

Before Ruth Bishop’s identification of human rotavirus in 1973, paediatricians could offer only platitudes by way of explanation when confronted with young children with acute diarrhoea. Since then it has become clear that rotavirus is the most common cause of gastroenteritis in children under 2 years of age living in either developed or developing countries. The aetiological mechanisms have been largely elucidated, mainly by studying an analogous infection in piglets caused by transmissible gastroenteritis agent. Rotavirus probably causes diarrhoea by increasing the turnover of enterocytes along the villus axis, leading to the population of blunted villi by immature cells that are incapable of normal absorption, and are more crypt-like and secretory in nature. Rotavirus can also reduce sucrase–isomaltase expression in human enterocytes by blocking sucrase–isomaltase transport to the apical membrane without apparent cell destruction. Reduction in activity is correlated to rotavirus induced alterations in the enterocyte cytoskeleton. There is also a single unconfirmed report of elaboration of a proinflammatory cytokine by rotavirus. Its neuronal (non-structural) glycoprotein of rotavirus) potentiates chloride secretion by a calcium dependent signalling pathway.

In some children a clinical episode indistinguishable from acute gastroenteritis is followed by protracted diarrhoea, so called post-gastroenteritis syndrome. This is more likely to occur in developing countries, where pre-existing malnutrition and enteropathies may lead to...
protracted diarrhoea in 8–20% of children initially presenting with acute gastroenteritis. In developed countries this happens in up to 5% of cases, and its causes are far from clear. Secondary lactase intolerance may occur, and many children exhibit an intolerance to cows’ milk protein and often several other proteins. Management is pragmatic, and comprises mainly nutritional support. Most children in developed countries survive, but in developing countries mortality is still high.

A recent report from the Melbourne group now suggests that persistent rotavirus infection may play a greater role in post-gastroenteritis syndrome and protracted diarrhoea than previously thought. Until now, rotavirus excretion was generally thought to stop within 10 days of the onset of symptoms in most children, and within 20 days of onset in all. Enzyme immunoassay (EIA) is the technique commonly used for detection of rotavirus in stool, but reverse transcriptase-polymerase chain reaction (RT-PCR) is up to 25 times more sensitive than EIA in detecting rotavirus in serially diluted faecal suspensions. It has both higher sensitivity and specificity. Using an RT-PCR technique, Bishop’s group studied the duration of rotavirus excretion in 37 children admitted to hospital with acute rotavirus infection. Excretion ceased within 10 days in 17 children and within 20 days in 26 of the 37 children. However, extended excretion between 25–57 days was seen in 11 children. Only one child seemed to have been re-infected with rotavirus. In contrast, rotavirus excretion estimated by EIA ranged from 4–29 days, and only 12 children had detectable excretion between 10 and 29 days. Extended excretion of rotavirus was significantly linked with intermittent antitrovirus IgA coproantibody boosts. Most of these boosts were associated with rotavirus excretion. Eight of the 11 children with extended rotavirus excretion between 25 and 57 days developed mild diarrhoea and vomiting associated with excretion or coproantibody boosts during the 100 days’ surveillance.

It is clear that prolonged rotavirus excretion is not only a feature of immunodeficiency but is also seen in about one third of immunocompetent young children. Intermittent mild diarrhoea seen in significantly increased numbers of children excreting rotavirus for one to two months could explain some cases of post-gastroenteritis syndrome. Moreover, children excreting rotavirus for prolonged periods could become reservoirs of human rotavirus and contribute to its survival between epidemics. As new diagnostic procedures indicate that rotavirus infection may be the cause of protracted diarrhoea in some children, who may have otherwise undetected infection, an effective rotavirus vaccine becomes even more important.

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