The Search for Biomarkers of Long-Term Outcome after Preterm Birth

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Abstract

Preterm birth and survival rates are rising globally, and consequently there is a growing necessity to safeguard life-long health. Epidemiological and other studies from around the world point to a higher risk of adverse adult health outcomes following preterm birth. These reports encompass morbidities in multiple domains, poorer reproductive health, and reduced longevity. The contributions of genetic inheritance, intrauterine exposures, and postnatal care practices to this altered adult phenotype are not known. Early detection is essential to implement preventive measures and to test protective antenatal and neonatal interventions to attenuate aberrant health trajectories. A satisfactory biomarker of outcome must be predictive of later functional health and ideally remain stable over the period from infancy to childhood and adult life. To date, blood pressure is the index that best fulfils these criteria. High throughput ‘omic’ technologies may identify biomarkers of later outcome and health risk. However, their potential can only be realized with initial investment in large, longitudinal cohort studies, which couple serial metabolomic profiling with functional health assessments across the life course.

Introduction

The number of preterm births is rising and is recognized to be a serious global health issue. The World Health Organization reports that eleven countries now have a preterm birth rate in excess of 15%; these include Pakistan and...
Indonesia, with the remaining nine in sub-Saharan Africa [1]. In high-income countries, around 6–12% of births are preterm. The third trimester of development experienced by preterm infants (born below 37 weeks of gestation) differs substantially from the conditions they would have experienced in utero, exposures that may plausibly affect multiple biological pathways and organ systems. In this review, we describe current knowledge of the health of adults born preterm (excluding neurodevelopmental, neurocognitive, and neuropsychiatric disorders), discuss the suitability of existing biomarkers to predict later metabolic and cardiovascular disease, and indicate suitable approaches to advance this field.

The Adult Phenotype following Preterm Birth

In comparison to their term-born counterparts, the life course following preterm birth is marked by greater health problems at every stage. Large national cohort studies have demonstrated independent associations between preterm birth and reduced longevity, poorer reproductive outcomes, and greater morbidity in multiple domains in relation to counterparts born at full term [2, 3]. Low gestational age at birth is independently associated with increased mortality in young adulthood, and, of note, there appears to be a greater impact with increasing immaturity [2]. Preterm women, but not men, are at increased risk of having preterm offspring, and reproductive rates of both men and women are reduced by about a third to a half [3]. Being born preterm, in addition to, and independent of, being small for gestational age, is also associated with a nearly twofold increased risk of later having pregnancy complications [4]. A large number of studies have identified preterm birth as a risk factor for features of the metabolic syndrome in adulthood, notably higher blood pressure (BP), insulin resistance, and markers of cardiovascular disease. It is important to recognize that epidemiological studies are highly susceptible to confounding, and the extent to which adverse health outcomes are reflective of remediable exposures, as opposed to genetic predisposition, is unknown.

Plausible Determinants

The multitude of intrauterine and neonatal factors that may contribute to the development of a preterm phenotype are summarized in figure 1, with current evidence of outcomes in preterm compared to term infants and adults summarized in table 1.
Potential Biomarkers

Body Mass Index
Overweight and obesity are linked to a number of chronic disease states, and the risk of overweight/obese children or adolescents becoming obese adults is at least twice as high compared to their normal-weight counterparts [5]. Body mass index (BMI) might, therefore, represent an easily obtainable, reliable marker for quantifying future risk. However, avoiding overweight in childhood does not protect against the development of the metabolic syndrome should they become obese in adult life. Furthermore, obese children who go on to be normal-weight adults are not at greater risk of developing cardiovascular disease [6]. The application of anthropometric variables such as BMI to predict the development of features of the metabolic syndrome is further complicated by the fact that adults born preterm remain shorter and lighter, and have a lower BMI compared to their term born peers throughout infancy and childhood, a reduction which is attenuated during adolescence. In a meta-analysis of published studies, we found no difference in BMI between preterm- and term-born adults (pooled mean difference –0.04; 95% CI –0.33, 0.24) [7]. Taken together, these data suggest that measures of height, weight, and BMI do not effectively predict the future risk.

Body Composition and Ectopic Lipid Deposition
Bioelectrical impedance, dual X-ray absorptiometry, and other techniques used to measure percentages of fat and fat-free mass represent a potentially better marker for the cardiovascular risk than anthropometric variables [8]. However,
information on how adipose tissue depots track from infancy into adulthood is sparse, while interactions with puberty and sex further limit their application [9]. An association between lower weight of children and adolescents born preterm and reduced fat mass rather than reduced fat-free mass has been reported in several studies [10]. These conclusions were not reflected in our meta-analysis of five available studies. We identified no differences in the percent fat mass between adults born preterm and full term [7]. We have, however, identified increased intra-abdominal (visceral) adiposity and intrahepatocellular lipid levels, independently, in preterm infants [11] and adults born preterm [12], and increased intramyocellular lipid levels in adults born preterm [12]. Intra-abdominal adiposity located centrally around the organs and ectopic lipid deposits are strongly associated with inflammation, insulin resistance, and

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<th>Table 1. Current knowledge: the phenotype of preterm compared to term-born infants and adults</th>
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<td><strong>Anthropometry</strong></td>
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74 Parkinson · Hyde · Modi
cardiovascular disease [13]. While these cross-sectional data suggest that alterations in body composition and ectopic lipid deposition persist into adult life, and thus represent potential biomarkers of outcome, larger, confirmatory longitudinal studies are required.

**Insulin Sensitivity**
A recent review and meta-analysis implicates preterm birth as a significant and independent risk factor for the development of both type-1 and type-2 diabetes [14]. Hyperinsulinemia and decreased skeletal muscle insulin sensitivity are key features of the metabolic syndrome and precursors to the development of type-2 diabetes. However, we identified no difference in fasting glucose or insulin between adults born preterm and full term, possibly a reflection of inconsistencies in the published literature [7]. In a systematic review examining the link between preterm birth and insulin sensitivity throughout the life course, an association was noted. However, the authors acknowledge published data are conflicting and that associations were likely to be affected by the heterogeneity of each study population and multiple confounding factors [15].

**Blood Lipids**
Hyperlipidemia is considered a cardinal driver of atherogenesis. Fatty streaks have been noted in the aortic wall in fetal life and peak in prevalence in infancy. Infants born to hypercholesterolemic mothers have significantly more aortic fatty streaks which persist into adolescence. Circulating levels of blood lipids track from childhood into adulthood in term-born populations, but the association is lost following adjustment for adult BMI [16]. In a meta-analysis, we noted evidence of increased LDL cholesterol in adults born preterm (pooled mean difference 0.14 mmol/l; 95% CI 0.05, 0.21 mmol/l; p = 0.01) but no statistically significant differences in HDL or total cholesterol [7]. A study involving the 1986 Northern Finland Birth Cohort showed that compared with term-born participants, boys born preterm had higher total cholesterol (mean 6.7%; 95% CI 0.2, 3.7%), LDL cholesterol (mean 11.7%; 95% CI 2.1, 22.3%), and apolipoprotein B (mean 12.3%; 95% CI 3.1, 22.4%); no differences were noted in girls. Differences were stronger when adjusted for maternal smoking, birth weight standard deviation score, parental education, pubertal stage, BMI, and lifestyle; similar associations were noted with gestation as a continuous variable [17]. It has been suggested that total cholesterol is a promising biomarker of long-term cardiovascular health, as maximal blood cholesterol recorded in the neonatal period has been shown to be strongly associated with greater aortic stiffness in young adults who were born preterm [18].
Cardiovascular Indices
Endothelial dysfunction is considered a precursor to the development of vascular disease. Conflicting data exist on arterial stiffness and endothelial function in children and adolescents born preterm, with a positive association found in some but not all studies [19, 20]. In a meta-analysis of the published literature, we found no significant difference between preterm and term adults in either flow-mediated dilation, intima-media thickness, or pulse wave velocity, though the number of studies was small [7]. However, sophisticated imaging techniques have recently shown that preterm birth impacts the long-term development of cardiac structure and function, with increases in myocardial mass and reductions in ventricular function [20].

Blood Pressure
Life course trajectories of BP are well established; data from a number of studies indicate that BP tracks from childhood into adulthood [21]. Higher BP in late adolescence is associated with early incidence of coronary heart disease and stroke [22]. A large number of studies have shown a clinically relevant increase of around 3–4 mm Hg in systolic BP in children and adults born preterm compared to those born at term [23]. Furthermore, a large cohort analysis has demonstrated a dose-response relationship between prematurity and BP [24]. In a meta-analysis of 13 studies, we showed that adults born preterm had clinically highly relevant increases in systolic BP (pooled mean difference 4.2 mm Hg; 95% CI 2.8, 5.7 mm Hg) and diastolic BP (pooled mean difference 2.6 mm Hg; 95% CI 1.2, 4.0 mm Hg) compared to term-born adults [7]. Ambulatory monitoring is considered a more reliable approach to assess BP as it is less affected by the anxiety response that accompanies one-off measurements. Increased BP reactivity to psychosocial stressors or ‘white coat hypertension’ has been observed in women born preterm, suggesting a heightened response to stress rather than a change in basal BP [25]. In the two studies that involved measurement of ambulatory BP to date, the results confirmed a difference in systolic BP in preterm compared with term-born women, but not men [7].

Metabonomics
Increasingly, the search for biomarkers has turned to the cutting-edge ‘omic’ technologies. These techniques generally have a high throughput, are noninvasive, and require a minimal sample volume, and thus allow screening of large numbers of potential biomarkers simultaneously. Metabonomics aims to characterize the global profile of the metabolites within a biological system, and hence provides a reflection of genomic, transcriptomic, and proteomic phenotypes in combination, i.e. an integrated profile of the biological status.
Metabonomic analysis of urine samples has identified putative biomarkers for the later development of type-1 and type-2 diabetes; for example, elevation in the glutamic acid concentration preceded the production of glutamic acid decarboxylase antibodies and overt type-1 diabetes [26], and elevation in 2-aminoadipic acid precedes the manifestation of type-2 diabetes [27]. Implementation of metabonomic technologies has revealed intriguing initial results regarding differences in urinary metabolites between preterm and term-born adults. Increased levels of metabolites were associated with inflammation and an altered microbiome, including an inverse association between urinary hippurate levels in adulthood and BP [12]. Hippurate is formed predominantly by hepatic glycine conjugation of intestinal microbial-derived benzoate produced from plant phenolics. Intestinal microbiota promote host energy recovery from dietary sources through catabolism of otherwise poorly digestible nutrients such as resistant starches, a phenomenon implicated in the development of human obesity, hypertension, and cardiovascular disease. Perturbations in the intestinal microbiome are a plausible effector pathway especially as the preterm neonate is heavily exposed to antimicrobials. Although the clinical implications of differences in the urinary metabolite profile remain uncertain, preliminary data such as these indicate a role for metabonomic technologies in the identification of biomarkers of risk in preterm populations and potential for insight into mechanisms mediating preterm birth and later health outcomes [12]. The use of metabonomics in neonatal studies has been reviewed recently [28].

Genomics and Epigenomics
It is hypothesized that high levels of oxidative stress in preterm infants reduces telomere length, explaining why they manifest signs commensurate with accelerated aging [29]. Reduced telomere length has been reported in low-birthweight infants compared to normal-birth-weight neonates [30]. Epigenomics, the profiling of chemical alterations to the DNA without base pair sequence changes, has also been used in the search for biomarkers of later life outcome. Correlations have been demonstrated between DNA methylation in samples obtained in early life and the obesity phenotype in later life [31]. Early catch-up growth in preterm infants has also been shown to alter DNA methylation, but it remains unclear whether this methylation is the cause or the outcome of the catch-up growth [32].

Sex Differences
Sex-specific differences in biological outcomes are well recognized. An increased susceptibility to adverse outcomes in preterm men compared to preterm women has been shown in several studies in relation to adiposity and markers of
inflammation [12]. Furthermore, we found the preterm-term difference in BP for women was significantly greater than the preterm-term difference in men (systolic BP, mean difference 2.9 mm Hg; 95% CI 1.1, 4.6 mm Hg; p = 0.004); diastolic BP (mean difference 1.6 mm Hg; 95% CI 0.3, 2.9 mm Hg; p = 0.02) [7]. In the 1986 Finnish Birth Cohort study, girls born preterm had a 6.7 mm Hg (95% CI 3.1, 10.2) higher systolic BP and a 3.5 mm Hg (95% CI 1.1, 5.8) higher diastolic BP than those born at term, whereas boys showed no BP differences [17]. These data suggest that certain aberrant trajectories associated with preterm birth are sex specific. This is an important consideration that should be factored in the design of clinical studies.

Conclusions

It appears clear that preterm birth is associated with adverse health outcomes across the life course. However, the extent to which adverse health outcomes reflect intrauterine or immediate postnatal experiences is unclear. This notwithstanding, it is plausible that the health care received by a preterm baby has the potential to aggravate or attenuate aberrant biological trajectories, regardless of whether these reflect genetic predisposition, the intrauterine environment, or postnatal exposures. Preterm birth may also be viewed as a natural experiment that provides the opportunity to understand the biology of human third-trimester development and to develop new approaches to slow the explosive rise in noncommunicable diseases witnessed worldwide.

A cardinal difficulty in testing new experimental approaches in preterm care, and resolving uncertainties in accepted but inadequately evidenced practices, is the need for long-term follow-up. The identification of reliable biomarkers of long-term health would immeasurably enhance the ability to test and evaluate new treatment strategies and those that are established but insufficiently evidence based. This, however, requires that long-term, longitudinal follow-up studies are conducted to identify and validate biomarkers of later health risks. The use of ‘omic’ technologies in the search for bio-markers of long-term outcomes of preterm birth is gathering pace. However, to date, no biomarkers with sufficiently high specificity and sensitivity for long-term outcomes of infants born preterm have been identified. This requires the comprehensive ‘omic’ characterization and parallel functional assessment of a large prospective cohort of preterm and term-born infants at multiple, longitudinal time points throughout their life course. The availability of large population databases holding detailed longitudinal clinical information offers the opportunity to facilitate the acquisition of information on functional outcomes. Given the increasing
prevalence of preterm survivors within the total population pool globally, implementa-
tion of such a research strategy would appear to be a sound and essential
investment.

**Disclosure Statement**

All authors declare that no financial or other conflicts of interest exist in relation to the
contents of the chapter.

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