

Integration of physiological and molecular mechanisms of the developmental origins of adult disease: new concepts and insights

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It is now well established that an imbalance or reduction in the maternal diet either through pregnancy and lactation or at defined time points therein can have long-term effects on cardiovascular and metabolic health in the resulting offspring; the exact outcome varying greatly with the period of development or growth targeted. The EARly Nutrition programming – long-term follow up of Efficacy and Safety Trials and integrated epidemiological, genetic, animal, consumer and economic research (EARNest), or metabolic programming, project aims to determine the primary physiological and molecular mechanisms that cause long-term changes in both cardiovascular function and metabolic homeostasis. Thereafter, it also aims to examine nutritional interventions that could be adopted in order to overcome such complications. The present review summarises some of the more recent findings from a range of nutritional interventions in both small and large animals that are beginning to uncover novel pathways by which long-term health can be determined. These interventions include nutritional manipulations that can increase or decrease blood pressure in the resulting offspring as well as indicating their dissociation from adaptations in the kidney. Particular emphasis will be placed on growth during lactation in conjunction with the extent to which central and peripheral tissue adaptations can act to amplify, or protect, the offspring from later disease when born to nutritionally-manipulated mothers.

Blood pressure: Growth: Lactation: Metabolism

To date, the most-widely-studied stage of development in relation to the early origins of later adult disease has been during pregnancy (McMillen & Robinson, 2005; Symonds *et al.* 2007). The reason is that birth weight (with or without placental weight) has been the main end point from the majority of historical and prospective human studies of later adverse health-outcome measures (Barker, 1994; Godfrey & Robinson, 1997; Barker, 1998). It is becoming clear, from more extensive investigations in which postnatal and/or early childhood growth measurements are available, that changes in growth over this period may be as, or even more, important in predicting future health problems (Barker *et al.* 2005; Singhal, 2005). Indeed, prevention of accelerated growth over the first 6 months of life could potentially reduce the incidence of later metabolic disturbances (Ekelund *et al.* 2007). The aim of the EU Early Nutrition Project is, thus, to integrate results from clinical, epidemiological and animal studies in

order to optimise nutrition during early development (Demmelmaier *et al.* 2006).

The concept that postnatal growth rather than prenatal growth is a more robust predictor of later adult disease is supported by findings from contemporary intervention studies (Singhal & Lucas, 2004) that have shown strong relationships between postnatal growth and components of the metabolic syndrome. This notion is perhaps not unexpected, given the increasing number of reports showing the close relationship between infant ‘catch-up’ growth and later obesity (Rogers & the EURO-BLCS Study Group, 2003). In addition, a combination of low birth weight and faster growth after birth impair vascular structure and function (Singhal & Lucas, 2004). The extent to which these adverse outcomes can have the same origin remains a matter of debate. To date, a majority of animal studies have focused on blood pressure control, because it is potentially the greatest health concern in adult populations

Abbreviations: LP:HC, low-protein high-carbohydrate.

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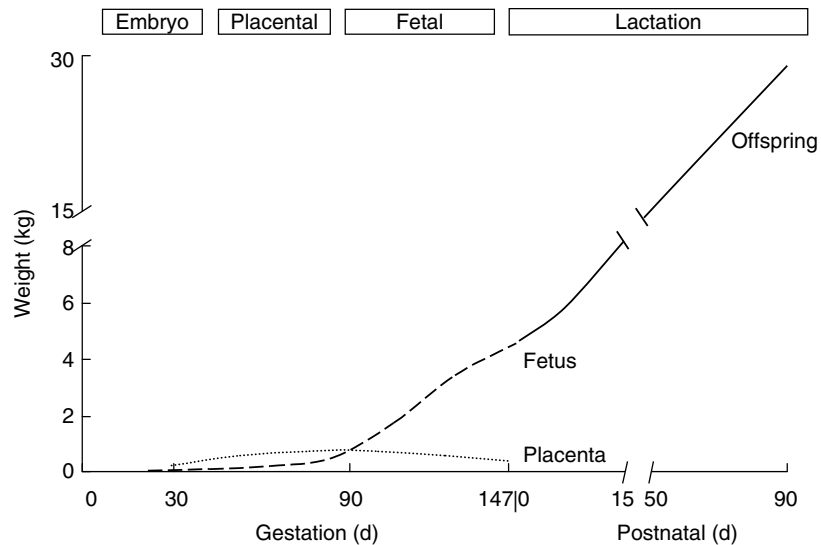


Fig. 1. Summary of the change in body weight, and hence growth rate, between conception and the end of lactation for the sheep.

(Armitage *et al.* 2004). In addition, in small-animal models long-term cardiovascular effects following changes in maternal dietary composition appear to be more readily programmed (McMillen & Robinson, 2005), whereas effects on obesity are only seen with much-more-severe changes in the maternal diet (Vickers *et al.* 2001).

It is the aim of the present review, therefore, to consider the extent to which the findings from animal studies may add support to the concept of ‘postnatal origins of adult disease’. Thus, it will be necessary to compare and contrast those investigations in which prenatal nutritional interventions specifically compromise weight at birth as opposed to how a switch in the maternal diet soon after birth may impact on maternal lactational performance and, thereby, represent the primary mediator of longer-term cardiovascular and metabolic maladaptation in the offspring. Currently, one study (Wyrwoll *et al.* 2006) has shown the potential for the fetal programming effects of maternal dexamethasone administration (for the development of the lungs of babies expected to be born prematurely) to be overcome when the offspring are cross-fostered onto dams fed a diet rich in *n*-3 fatty acids.

The impact of the timing and duration of changes in the maternal diet during development on later blood pressure

To date, a majority of animal studies have focused on the impact of manipulating the mother’s diet either throughout pregnancy or at specific periods of pregnancy (Symonds *et al.* 2006, 2007), with little attention given to the lactational or post-weaning period, which represents the period of most-rapid early postnatal growth (Fig. 1). As will be summarised, this period could be very important when assessing the relative impact of any nutritional manipulations on cardiovascular control in the offspring. At the same time, it must be remembered that maturation of a

number of key organ and endocrine pathways likely to mediate many of the cardiovascular and related adaptations in nutritionally-programmed offspring occur after birth rather than before birth in rodents, which is not the case in infants or large mammals (Symonds *et al.* 2007). These pathways include central pathways in the brain involved in appetite control (Bouret *et al.* 2004), as well as the establishment of nephron number in the kidney (Wintour & Moritz, 1997).

A further critical component in the interpretation of rodent experiments is how blood pressure is actually assessed, because it is now apparent in studies using nutritionally-manipulated adult rats that very different interpretations can be drawn depending on the technique used (D’Angelo *et al.* 2005). Blood pressure as measured by the tail-cuff method tends to be higher than intra-arterial blood pressure, and this disparity is likely to be a result of the considerable stress imposed during the handling, warming and restraint involved (Tonkiss *et al.* 1998). This outcome is not surprising given that the technique was only originally fully validated in adult hypertensive rats (Bunag, 1973). In this context adult rats fed on a high-fructose diet (i.e. 660 g fructose and 120 g lard/kg) for 8 weeks show no change in resting blood pressure when measured by telemetry, whereas the blood pressure in response to stress, as induced by subjecting the animals to the tail-cuff procedure, is raised (D’Angelo *et al.* 2005). These animals are also insulin resistant and hyperglycaemic. The differential outcomes on blood pressure under resting conditions as opposed to external stress is a fundamental concept that must be taken into account when considering this type of results obtained from nutritionally-programmed animals. As a result, specific mention will be made throughout the present review when blood pressure is only measured by the tail-cuff procedure.

The most-widely-adopted maternal nutritional interventions that result in higher blood-pressure responses in the offspring are feeding a diet that has a 50% reduction in

protein (in conjunction with a proportionate increase in carbohydrate content, i.e. low-protein high-carbohydrate; LP:HC) or reducing total food intake by 70% compared with *ad libitum* intake (Armitage *et al.* 2004; McMillen & Robinson, 2005). It should be noted that these types of diets are not typical of those consumed by contemporary women living in either the developing or developed world, in which excess rather than insufficient macronutrient intake is becoming increasingly prevalent.

Another important consideration is the potential gender-specific divergence in the postnatal outcome. Males appear to be far more susceptible to some of the detrimental effects of nutritional programming compared with females (Khan *et al.* 2003). One contributing factor for this gender difference is presumably the much higher body weight attained by male rats compared with female rats and, thus, their faster juvenile growth rates (Symonds & Gardner, 2006). Consequently, the metabolic and oxidative stress imposed on some (if not all) organs can be much greater in males than females, thereby reducing their lifespan considerably (Tarry-Adkins *et al.* 2006).

Maternal dietary manipulation during development and programming of lower blood pressure in the offspring

When considering the relative contributions of the prenatal and postnatal diets to later blood pressure control it is necessary to focus on more-recent studies that strongly indicate that there is not a simple relationship between maternal consumption of an LP:HC diet and raised blood pressure in the offspring (Fernandez-Twinn *et al.* 2006; Hoppe *et al.* 2007). Consequently, depending on how long this diet is fed to both the mother and the offspring, very different effects on blood pressure are seen, as summarised in Fig. 2.

When the mother continues to consume a LP:HC diet through lactation the offspring do not exhibit 'catch-up' growth and their blood pressure is the same as that of the controls, even when switched to the same standard diet at weaning (Fernandez-Twinn *et al.* 2006). In contrast, if the offspring are maintained on a LP:HC diet after weaning, then as adults they actually show a lower blood pressure than controls, an adaptation that is maintained even when they are fed a diet that is high in salt (Hoppe *et al.* 2007). Again, this adaptation is accompanied by the maintenance of a lower body weight through to adulthood.

The finding that maternal nutrient restriction programmes lower blood pressure in the offspring is not an isolated report and is confirmed by the findings of another study in which a 50% global reduction in maternal food intake was maintained through pregnancy (Brennan *et al.* 2006). After maternal diet was restored during lactation, the adult offspring were found to maintain a lower blood pressure throughout the 24 h period of assessment. This outcome was found despite the offspring of nutrient-restricted mothers being smaller from birth up to 2 d of age. A notable finding in this study was that nutrient-restricted mothers did not increase their body weight until the final 3 d of gestation. However, the weights of all their

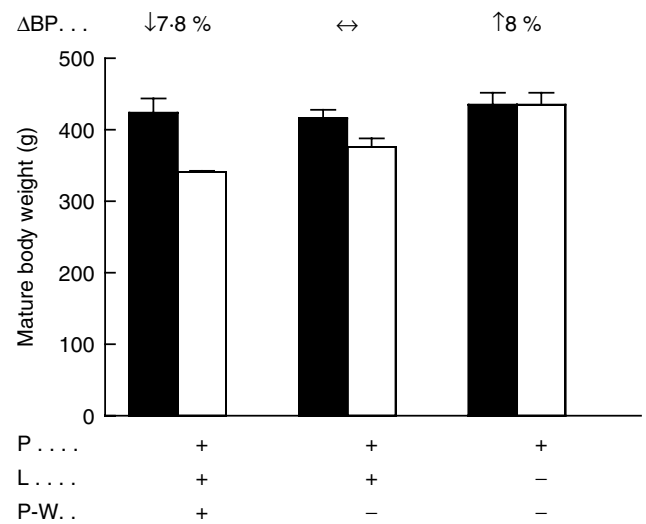


Fig. 2. Summary of the relationship between the duration of feeding a low-protein high-carbohydrate diet (□) and mature body weight in male rats and its relationship with the difference in blood pressure (Δ BP) between these offspring and those born to control dams (■). ↓, Decrease; ↔, no change; ↑, increase; P, pregnancy; L, lactation; P-W, post-weaning. Values are means with their standard errors represented by vertical bars. There was no difference in mature body weights between any of the control groups. (Based on Woods *et al.* 2001; Fernandez-Twinn *et al.* 2006; Hoppe *et al.* 2007.)

major organs were maintained, which suggests that their lower body weight was a consequence of fat and muscle tissue mobilisation; adaptations that may be important in ensuring lactational performance is not greatly impaired and, thus, postnatal growth rate is unaffected. This finding may be important because in earlier studies the offspring of mothers that were more-severely globally nutrient restricted (i.e. fed only 30% *ad libitum* intake) did not restore their body weight to that of controls even when they were cross-fostered onto normally-fed dams (Vickers *et al.* 2000). Cross-fostering could, however, result in a substantially greater increase in milk supply to these offspring such that even though they maintain a lower body weight their blood pressure (as measured by the tail-cuff technique) is elevated (Vickers *et al.* 2000). It is, therefore, necessary to consider both actual growth rate achieved as well as what would be optimal for longer-term health. In this context, it is remarkable that the adverse outcomes in offspring born to dams fed only 30% *ad libitum* intake are prevented when leptin is administered from day 3 to day 13 after birth, resulting in a dramatic loss in weight of these pups (Vickers *et al.* 2005).

An explanation for the impact of specific maternal nutritional interventions on later blood pressure, even when these interventions are terminated at birth, therefore needs to take into account differences in growth rates between groups. In this context, the apparent gender-specific effect of blocking maternal glucocorticoid synthesis on the offspring's blood pressure (as measured by the tail-cuff procedure) at 4 weeks of age may be explained by the fact that it is accompanied by a pronounced reduction in postnatal growth rate (McMullen & Langley-Evans, 2005). Given

Table 1. Summary of the differences in kidney structure and function between male and female adult rats

| Kidney measurement | Male | Female | Reference |
|--|----------|----------|----------------------------------|
| Weight (g/kg) | 0.70 | 0.75 | Woods & Weeks (2005) |
| Nephron no. | 300 000* | 250 000* | Woods <i>et al.</i> (2001, 2005) |
| NO synthase activity (pmol/min per mg protein) | 7.3 | 4.2 | Attia <i>et al.</i> (2003) |
| Glomerular filtration rate (ml/min) | 3.8 | 2.1 | Woods (2006) |
| Effective renal plasma flow (ml/min) | 10.5 | 6.4 | Woods (2006) |
| Urinary protein excretion (mg/d) | 16 | 2 | Woods & Weeks (2005) |

*Approximate nos.

the critical importance of glucocorticoids in ensuring normal mammary development and function (Quirk *et al.* 1986; Stelwagen *et al.* 1998), it is not unexpected that when maternal glucocorticoid synthesis is blocked her lactational capacity would be impaired.

Another recent study (Torrens *et al.* 2006) has shown that supplementation of the LP:HC diet with folic acid (5 mg/kg) can also reduce blood pressure in the resulting offspring when assessed by the tail-cuff procedure at 15 weeks of age. This reduction may be considered a surprising adaptation given that the LP:HC diet has adequate folic acid (i.e. 1 mg/kg). Furthermore, rats exhibit coprophagia, so there is a substantial contribution of intestinal fermentation to folic acid synthesis. Consequently, the rats must be given succinyl sulfathiazole to inhibit this process in order to induce folate deficiency (Devlin *et al.* 2005). Folic acid supplementation in women, at least from 20 weeks of gestation, has no placental effects (Klingler *et al.* 2006). There is currently no information as to whether postnatal growth is affected in offspring born to dams that consume excess folic acid (Torrens *et al.* 2006) or whether folate could affect mammary development or function.

It is conceivable that lactational performance could be affected because folic acid supplementation can impair folate secretion into milk (Mackey & Picciano, 1999). It also disrupts the normal inverse relationship between dietary folate and plasma homocysteine (Mackey & Picciano, 1999), which may be related to changes in maternal folic acid metabolism (Smith *et al.* 1983). Lactation is accompanied by a dramatic reduction in folate concentration (Houghton *et al.* 2006), so such adaptations would be potentially exacerbated when dams are fed a diet in which folic acid supplementation is stopped after birth. It would therefore appear imperative that any effects of nutritional manipulation during pregnancy on growth and/or body composition through lactation must be studied subsequently.

Further indirect evidence that when postnatal growth is unaffected then, irrespective of the prenatal environment, blood pressure is similarly unaffected, is provided from large-animal studies (Gardner *et al.* 2004; Gopalakrishnan *et al.* 2005). These studies indicate that resting blood pressure is initially lower in nutrient-restricted offspring (Gopalakrishnan *et al.* 2005), and when they are raised under standard field conditions blood pressure is only modestly increased before feeding (Gopalakrishnan *et al.* 2004). Any potentially adverse outcomes may, however, be

amplified when later growth is enhanced (Symonds *et al.* 2006).

Nutritional programming of the kidney and its relationship with blood pressure control

One organ that is particularly sensitive to nutritional programming is the kidney, in which the most consistent response is a reduction in nephron number (Zandi-Nejad *et al.* 2006). In rats there is an appreciable difference in kidney structure and function between males and females, which appears to be directly related to longevity (Tarry-Adkins *et al.* 2006). As summarised in Table 1, the kidneys of males possess more nephrons than those of females, and urinary protein excretion is substantially raised (approximately 6-fold) together with effective renal plasma flow and glomerular filtration rate (Woods, 2006). In addition, male rats have lower endothelial NO synthase activity (Attia *et al.* 2003). Ultimately, a combination of increased workload on the kidney, together with greater susceptibility to renal damage, could explain why male kidneys show increased cellular senescence, loss of function and, ultimately, male rats die earlier than females (Tarry-Adkins *et al.* 2006).

There is no evidence, however, that blood pressure is substantially different between male and female rats (Woods, 2006; Wyrwoll *et al.* 2006) and, indeed, there can be substantial functional deficits in the kidney of males that can actually be accompanied by a reduction in blood pressure (Hoppe *et al.* 2007). Male rats appear to be potentially more susceptible than females to the effects on later blood pressure after being born to mothers that consumed a LP:HC diet (Woods *et al.* 2001, 2005). This gender-specific outcome has been suggested to reside in the lack of any nutritional programming effect on the renin-angiotensinogen axis within the female kidney (Woods *et al.* 2005); gender has no influence on this axis in large mammals such as sheep (Whorwood *et al.* 2001). A comparison of postnatal growth rates between the two studies (Woods *et al.* 2001, 2005) conducted in the same laboratory indicates a striking difference (Fig. 3). Male offspring of mothers fed a LP:HC diet throughout pregnancy, but not the offspring of those fed a control diet, can be seen to have grown substantially faster during lactation. Furthermore, only those offspring that show a relative acceleration of postnatal growth go on to exhibit a higher blood pressure as adults (Fig. 3). There are a number

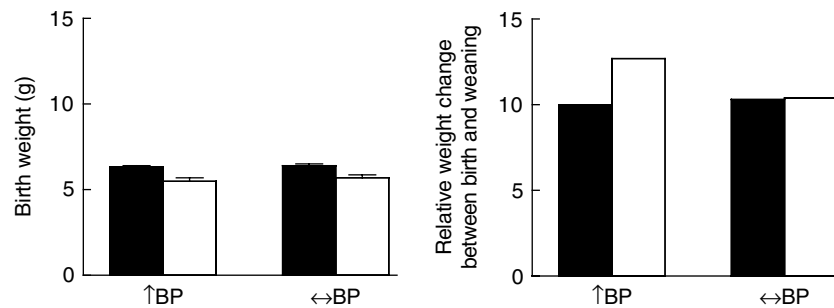


Fig. 3. Comparison of the difference in growth rates between birth and weaning and its relationship with changes in blood pressure in these offspring. (□), Low-protein high-carbohydrate diet; (■), controls; BP, blood pressure; ↑, increase; ↔, no change. Values are means with their standard errors represented by vertical bars. (From the work of Woods *et al.* 2001, 2005.)

of additional explanations as to why males selectively show this adaptive response, including the possibility that their higher growth rate is critical and may be related to the differential effects of lactation on postnatal development between males and females. It is therefore essential that similar lactational environments are maintained between studies in order to ensure valid comparisons are made.

Other factors determining the relationship between kidney function and blood pressure control

Given the increasing evidence that a deficit in nephron number does not directly impact on later blood pressure control in nutritionally-programmed offspring (Hoppe *et al.* 2007) and in the general population (Kett & Bertram, 2004), it is necessary to consider other explanations for how kidney function can be differentially affected such that hypertension may result. Observations from adult animals and human subjects indicate that excess weight gain is a major risk factor for CVD that impacts on kidney-mediated blood pressure regulation through impaired renal pressure natriuresis (Hall, 2003). This effect is primarily mediated through renal sympathetic activity, which is increased in obese individuals (Landsberg & Krieger, 1989; Esler, 2000; Hall *et al.* 2001). Increased food intake and a concomitant rise in body fat mass, plasma leptin concentrations and glucose intolerance could lead to enhanced sympathetic stimulation of the kidney, thereby causing hypertension. However, can these maladaptations be programmed *in utero* (Symonds *et al.* 2005)?

In relation to the relative importance of the prenatal and the postnatal diet in excess fat deposition, a majority of small and large mammals are born with minimal fat reserves in which the major component is brown adipose tissue rather than white adipose tissue (Cannon *et al.* 1988; Bispham *et al.* 2005). Critically, brown adipose tissue is located in very different regions in small and large mammals; it is found in the intrascapular region in rodents but around the central organs such as the kidney in young sheep and infants. In the latter species brown adipose tissue is rapidly activated at birth, as both sheep and human

subjects are precocial thermoregulators (Clarke *et al.* 1997b). For altricial thermoregulators, such as the rat, their brown fat is not fully activated until they start to leave their nest, which is coincident with maturation of their hypothalamic–pituitary axis (Obregon *et al.* 1996). Furthermore, in sheep, but not rats, perirenal adipose tissue is the most-rapidly-growing tissue after birth (Clarke *et al.* 1997a), although the extent to which this process can be preferentially promoted has yet to be shown.

Gender differences in fat deposition have been shown in rats. Females possess appreciably more intrascapular brown fat than males from 1 month of age (Pearce *et al.* 2003), although this disparity does not appear to be accompanied by any difference in their white fat stores or plasma leptin concentrations (Wyrwoll *et al.* 2006). In this context it is possible that rats may never deposit sufficient adipose tissue around their kidneys for it to have a deleterious effect on their function, except in extreme situations in which the rat has a genetic predisposition to become obese (Kasiske *et al.* 1988). Thus, it is necessary to give due consideration to these comparative aspects of animal development in order to interpret data from studies in terms of current models of nutritional programming of later disease.

In providing insights into the current epidemic of obesity, which is also occurring at a much earlier age (Budge *et al.* 2005), it must be noted that a range of other explanations are available outside those currently given most attention, i.e. increased consumption of processed food and/or reduced physical activity. These aspects have recently been reviewed (Keith *et al.* 2006), and they include a number of developmental explanations, e.g. maternal age and a combination of intrauterine and inter-generational effects. To these explanations can readily be added the recognition that formula-fed infants show an accelerated growth trajectory compared with those who are breast-fed (Ong *et al.* 2002), and that this outcome becomes predictive of both later weight gain and obesity (Ong *et al.* 2006). Nutritional advice that is offered to parents in the future will need to reflect the importance of avoiding excess growth (Ekelund *et al.* 2006). Whilst optimal growth rate may differ between individuals, avoiding excess growth is also an important consideration

in the nutritional support offered to previously-sick and/or premature infants in their recovery phase when it may have the potential to improve later cardiovascular health (Regan *et al.* 2006).

The impact of diet on postnatal growth and later risk of metabolic disease

With the increasing evidence that excess postnatal growth, particularly in those infants that are born premature or small at birth, can have deleterious longer-term consequences, it is perhaps surprising that the vital role that nutrition has after birth has not been more closely investigated within animal studies. It should be noted that milk produced by the rat has an approximately 10-fold higher protein content and protein:sugar than in human milk (Prentice & Prentice, 1995), which reflects the very different types of organ growth that occur after birth in these two species. An excessively-high carbohydrate intake in itself appears to be related to later insulin insensitivity in preterm infants (Regan *et al.* 2006), although whether this outcome is a direct effect of sugar or the accompanying lower protein intake is not known. It is suggested that this type of effect can be mediated by excess central fat deposition (Ibanez *et al.* 2006), which in offspring born to nutritionally-compromised mothers appears to be the primary factor that accompanies glucose intolerance in young adult sheep (Gardner *et al.* 2005). Indeed, the function of the pancreas appears to be largely unaffected in growth-restricted infants (Beringue *et al.* 2002).

Accelerated postnatal growth in small-for-gestational-age children is accompanied by a comparatively rapid rise in plasma insulin-like growth factor-I concentrations (Iniguez *et al.* 2006), which in young growing sheep closely follows the increase in perirenal adipose tissue (Clarke *et al.* 1997a). At the same time the accelerated adipose tissue growth in first-born offspring is accompanied by increased insulin-like growth factor sensitivity and hepatic secretory potential (Hyatt *et al.* 2007). Not only is adipose tissue the fastest growing organ in the postnatal period, but food intake is the primary factor determining its growth (Gate *et al.* 2000). Furthermore, the composition and endocrine sensitivity of adipose tissue soon after birth is very different compared with that in later life (Symonds *et al.* 2003; Dunger & Ong, 2004; Gnanalingham *et al.* 2005; Lomax *et al.* 2007). This factor, together with the high carbohydrate composition of milk (Prentice & Prentice, 1995), may contribute to its capacity to maintain such a high growth rate (compared with the fetus, Fig. 1) as well as having a greater impact on overall metabolic regulation (Budge & Symonds, 2006).

It is not unexpected that there is a substantial drive towards increased adiposity, particularly in formula-fed infants who appear to consume more in the first 2 months of life than in later months (Ong *et al.* 2006). Breast-feeding is therefore protective against promoting early fat deposition (Harvey *et al.* 2007) and later obesity (Arenz *et al.* 2004). In the past, any extra weight, especially fat, gained during infancy may well have been lost in childhood, but in the current environment, in which weight gain

rather than weight loss is the norm (Popkin *et al.* 2006a), the adverse long-term health (Kim & Popkin, 2006) and socio-economic consequences are substantial (Popkin *et al.* 2006b).

In conclusion, an increased understanding of the components of milk that act to differentially affect adipose tissue growth and endocrine action has the potential to enhance the long-term health outcomes of not only the large proportion of the population that are fed infant formula, but particularly those that are born prematurely or small-for-dates. In this context, the type of projects funded by the EU in which there is a close working relationship between academic, clinical and industrial partners has a distinct opportunity to make substantial progress in this complex arena (Demmelmair *et al.* 2006). A combination of small- and large-animal models of the relative impact of both prenatal and postnatal nutritional interventions and their differential impact on long-term outcomes is thus predicted to make substantial progress in this area.

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