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Dietary interventions designed to protect the perinatal brain from hypoxic-ischemic encephalopathy – Creatine prophylaxis and the need for multi-organ protection

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ABSTRACT

Birth asphyxia or hypoxia arises from impaired placental gas exchange during labor and remains one of the leading causes of neonatal morbidity and mortality worldwide. It is a condition that can strike in pregnancies that have been uneventful until these final moments, and leads to fundamental loss of cellular energy reserves in the newborn. The cascade of metabolic changes that occurs in the brain at birth as a result of hypoxia can lead to significant damage that evolves over several hours and days, the severity of which can be ameliorated with therapeutic cerebral hypothermia. However, this treatment is only applied to a subset of newborns that meet strict inclusion criteria and is usually administered only in facilities with a high level of medical surveillance. Hence, a number of neuropharmacological interventions have been suggested as adjunct therapies to improve the efficacy of hypothermia, which alone improves survival of the post-hypoxic infant but does not altogether prevent adverse neurological outcomes. In this review we discuss the prospect of using creatine as a dietary supplement during pregnancy and nutritional intervention that can significantly decrease the risk of brain damage in the event of severe oxygen deprivation at birth. Because brain damage can also arise secondarily to compromise of other fetal organs (e.g., heart, diaphragm, kidney), and that compromise of mitochondrial function under hypoxic conditions may be a common mechanism leading to damage of these tissues, we present data suggesting that dietary creatine supplementation during pregnancy may be an effective prophylaxis that can protect the fetus from the multi-organ consequences of severe hypoxia at birth.

1. Introduction

Birth asphyxia is a condition arising from complications during labour and delivery that lead to global oxygen deprivation for the fetus. The impaired placental gas exchange that leads to birth asphyxia results in fetal hypotension, hypoxemia and hypercapnia, with significant metabolic acidosis, to which the developing brain is particularly vulnerable (Low, 2004). The unpredictable nature of obstetric complications that contribute to birth asphyxia presents a unique set of challenges to clinical and research professionals devising strategies to reduce the incidence of perinatal asphyxia and the resulting morbidity and mortality. This is compounded by the multi-organ damage associated with intrapartum oxygen deprivation (Perlman et al., 1989).

Birth asphyxia and intrapartum complications account for 24% of deaths during the neonatal period (WHO, 2012). These figures equate to approximately 4 million neonatal deaths per annum being attributed to birth asphyxia (Lawn et al., 2005). Survivors often develop the clinical condition termed hypoxic-ischemic encephalopathy (HIE), with 20–70% of them having lifelong mental and physical disabilities, cerebral palsy and seizures (Graham et al., 2008). Characterization of brain injury following birth asphyxia has thus been the focus of many basic science and clinical

Abbreviations: AGAT, arginine:glycine aminotransferase; ADP, adenosine diphosphate; ANT, Adenine nucleotide translocator; ATP, adenosine triphosphate; CK, Creatine Kinase; Cr, Creatine; GAA, guanidinoacetate; GAMT, guanidinoacetate methyltransferase; MPTP, Mitochondrial Permeability Transition Pore; OXPHOS, Oxidative Phosphorylation; PCr, Phosphocreatine; ROS, Reactive Oxygen Species; SLC6A8, Creatine Transporter 1; VDAC, Voltage-dependent anion channel.

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investigations. However, in their clinical retrospective study, Hankins et al. (2002) observed that for 70% of cases, low oxygen was not the primary and direct cause of HIE, but rather HIE was a condition that developed secondary to renal, hepatic, and cardiac dysfunction following birth asphyxia (Hankins et al., 2002). These types of studies highlight the true nature of the global oxygen deprivation associated with birth asphyxia and the need to investigate all of the consequential injuries if the asphyxia-related neonatal morbidity and mortality is to be successfully reduced.

A disproportionate number of birth asphyxia-related deaths and morbidities occur in developing countries where poverty is a major socio-economic factor. Recent evaluations of the incidence of birth asphyxia in high income countries, where high quality obstetric care is available throughout the peripartum period, estimates that this occurs for 4.3%–8.5% of term live births (Graham et al., 2008). This is in stark contrast to an incidence rate of around 23% in developing countries where access to health care is low (Azra Haider and Bhutta, 2006). Despite recent advances in the understanding of the pathophysiology of brain damage following chronic fetal hypoxia or severe asphyxia at birth, little has been achieved in developing an effective treatment that is safe, easy to administer, cost effective, and therefore available to the majority of women and infants in cases of birth asphyxia worldwide (WHO, 2012). There is a need, therefore, to develop treatments appropriate for these settings. Nutritional interventions may provide the ideal platform for therapies that can be administered safely and prophylactically to reduce the number of babies suffering from severe neurological impairment following an asphyxic insult at birth.

Creatine (Cr) is an intracellular metabolite that is acquired through a diet rich in fish, meat and animal products, and is also synthesised by the body *de novo* (Walker, 1979; Brosnan and Brosnan, 2007). Creatine and its phosphorylated derivative phosphocreatine (PCr) have long been established as having a critical role in brain metabolism. The brain requires high ATP turnover to maintain function, and accounts for ~20% of the body's daily energy requirement (Shulman et al., 2004). Maintenance of this high and at times fluctuating level of ADP hydrolysis to produce ATP requires the co-ordination of mitochondrial oxidative phosphorylation and phosphate donation via phosphagens (Dzeja and Terzic, 2003). Phosphagen systems, consisting of a phosphate donor and a corresponding kinase enzyme to catalyse the reaction, provide support to cellular ATP turnover by mitigating temporal and spatial imbalances in ATP supply and demand (Ellington, 1989). The creatine/phosphocreatine/creatine kinase system is the sole phosphagen system of higher vertebrates, with creatine kinase isoforms expressed from early in development of the brain and spinal cord. This system plays an integral role in ATP turnover in the brain, particularly cellular subtypes with high and fluctuating energy demands, such as neurons (Andres et al., 2008). Thus, provision of creatine within the brain and maintenance of normal intracellular levels is critical for function.

Increased dietary consumption of creatine has been shown to increase the intracellular levels of creatine and phosphocreatine (Dechent et al., 1999), therefore potentially increasing the capacity to maintain ATP homeostasis under hypoxic conditions when oxidative phosphorylation may be compromised. Supplementary creatine has been shown to directly improve mitochondrial bioenergetics and protect structural integrity (Guidi et al., 2008), and it also acts as a mild antioxidant as the rephosphorylation of ADP via PCr consumes a proton (H^+). These properties give the creatine/phosphocreatine reaction the ability to prevent the cytosol from becoming acidic, particularly under hypoxic conditions (Sestili et al., 2011), thus protecting cells from damage associated with an acute hypoxic insult. Studies conducted in rodent models have shown that increasing the dietary availability of creatine during pregnancy leads to an increased amount of creatine in the fetal brain prior to birth, potentially giving offspring a greater capacity to cope with the episodes of oxygen deprivation and cellular energy failure that occur during birth asphyxia and which lead to neonatal HIE (Ireland et al., 2008).

In this review we discuss the pathophysiology of hypoxic ischemic encephalopathy following birth asphyxia and potential interventions that might lower the risk and severity of perinatal brain damage. We also present evidence on the importance of creatine for the brain, and that dietary supplementation with creatine during gestation in animal models prevents hypoxia-induced brain damage at birth.

1.1. Multifactorial scope of the problem of perinatal HIE

Magnetic Resonance Imaging (MRI) was important in identifying the patterns of brain injury in term infants with HIE (Azzopardi et al., 1989). *In vivo* spectroscopy (MRS) then identified a biphasic pattern of cerebral energy failure, giving rise to the concept that brain damage occurs in two phases - immediate cell death occurring largely by necrosis and driven by excitotoxicity; and delayed, apoptotic cell death largely driven by on-going inflammation and oxidative stress (Johnston et al., 2011). Given that the secondary phase develops slowly over hours and even days (although this is more rapid with more severe HIE), the time between the two phases has been considered as an opportunity ('window') in which to implement treatments to ameliorate the progress of apoptosis and cerebral inflammation. Hence, therapeutic hypothermia in neonates has been introduced as almost standard practice worldwide. Hypothermia treatment in the form of selective head or systemic (total body) cooling is a technique that involves lowering the infant's head (or body) temperature to between 32.0 °C and 33.5 °C for a period up to 72 h after the asphyxic insult (Shah et al., 2007). This technique aims to limit brain injury during the second wave of energy failure after oxygen deprivation by reducing cerebral metabolic demand and preventing cytotoxin accumulation and apoptotic cell death (Gunn et al., 1997). This is an approach that has a small therapeutic window, usually up to 6 h after birth, and is largely restricted to use in tertiary level medical facilities (Jacobs et al., 2008). Hypothermia is not more widely available because of certain technical demands, as well as the risks associated with decreased cardiac output, reduced cerebral blood flow, and activation of thermoregulatory processes (Erecinska et al., 2003). While adaptations of hypothermia procedures are under development for use in low-resource settings (Robertson et al., 2008), it remains an intervention that does not address the vast majority of birth asphyxia events that occur worldwide. And while it has been resoundingly successful in reducing the number of deaths in babies with access to tertiary level obstetric facilities, it is less effective in reducing some neurological disabilities that arise from birth asphyxia and HIE (Jacobs et al., 2013). Consequently, there has been intense focus on finding adjunct neuroprotective treatments using antagonists to excitotoxity ($MgSO_4$, topiramate, xenon gas), anti-oxidants (melatonin, allopurinol, N-acetylcysteine), and anti-inflammatory medications (indomethacin, minocycline, mast-cell inhibitors) (see review by (Bosch et al., 2009; Merchant et al., 2015). These treatments are under consideration for use in conjunction with neonatal hypothermia as strategies to either 'rescue' the already damaged brain of some infants, or prevent progression of damage from becoming a more serious, and perhaps irreversible condition. Another, perhaps more productive approach would be a treatment to decrease the likelihood of damage to the developing brain either in late gestation, or during birth.

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