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Research Paper

Reduced Cortical Excitability, Neuroplasticity, and Salivary Cortisol in 11–13-Year-Old Children Born to Women with Gestational Diabetes Mellitus

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ABSTRACT

Background: Children exposed to gestational diabetes mellitus (GDM) *in utero* are at increased risk of neurodevelopmental difficulties, including autism and impaired motor control. However, the underlying neurophysiology is unknown.
Methods: Using transcranial magnetic stimulation, we assessed cortical excitability, long-term depression (LTD)-like neuroplasticity in 45 GDM-exposed and 12 control children aged 11–13 years. Data were analysed against salivary cortisol and maternal diabetes severity and treatment (insulin [$N = 22$] or metformin [$N = 23$]) during pregnancy.
Findings: GDM-exposed children had reduced cortical excitability ($p = .003$), LTD-like neuroplasticity ($p = .005$), and salivary cortisol ($p < .001$) when compared with control children. Higher maternal insulin resistance (IR) before and during GDM treatment was associated with a blunted neuroplastic response in children ($p = .014$) and this was not accounted for by maternal BMI. Additional maternal and neonatal measures, including fasting plasma glucose and inflammatory markers, predicted neurophysiological outcomes. The metformin and insulin treatment groups had similar outcomes.
Interpretation: These results suggest that GDM can contribute to subtle differences in child neurophysiology, and possibly cortisol secretion, persisting into early adolescence. Importantly, these effects appear to occur during second trimester, before pharmacologic treatment typically commences, and can be predicted by maternal insulin resistance. Therefore, earlier detection and treatment of GDM may be warranted. Metformin appears to be safe for these aspects of neurodevelopment.

1. Introduction

Gestational diabetes mellitus (GDM) affects 5–10% of pregnancies, with a higher prevalence in obese women (WHO, 2014). Emerging evidence indicates that children exposed *in utero* to GDM are at higher risk of neurodevelopmental difficulties, including attention deficit hyperactivity disorder (Nomura et al., 2012), autism spectrum disorders (Xu et al., 2014), and impaired motor development (Ornoy et al., 1999). Additionally, maternal obesity has independently been associated with a range of neurodevelopmental and psychiatric disorders in the offspring (Edlow, 2016). Animal research underpins the hypothesis that oxidative stress and inflammation associated with maternal hyperglycemia are major drivers of altered neurodevelopment in GDM-affected fetuses (Sullivan et al., 2014), while obesity is associated with a chronic, low-grade, metabolically-induced inflammatory state (Pantham et al., 2015). Placental inflammation is observed in obesity- and GDM-affected pregnancies (Saloman et al., 2016), and intrauterine inflammation can evoke fetal brain injury (Elovitz et al., 2011). Further, maternal hyperglycemia can retard dendritic development in the fetal brain (Jing et al., 2014). Taken together, these findings suggest that GDM-exposed fetuses experience an adverse environment *in utero* that contributes to abnormal neurodevelopment. However, there are currently no neurophysiological data in children exposed to GDM (or maternal obesity), so the mechanisms underlying these neurodevelopmental disturbances are unknown.

Abbreviations: AMT, active motor threshold; cTBS, continuous theta burst stimulation; EMG, electromyogram; GA, gestational age; GDM, gestational diabetes mellitus; HOMA-IR, homeostatic model assessment for insulin resistance; HPA, hypothalamic-pituitary-adrenal; SI1mV, stimulus intensity to evoke 1 mV response; TMS, transcranial magnetic stimulation; IR, insulin resistance; LTD, long term depression; MEP, motor evoked potential; MiG, Metformin in Gestational diabetes trial; NMDAR, *N*-methyl-D-aspartate receptor; nmol/L, nano-moles per litre; RMT, resting motor threshold.

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Evidence for a link between inflammation and suboptimal neurodevelopment also comes from studies of children born preterm. While the aetiology of preterm birth is multifactorial, the only established pathological, causal factor is infection and/or inflammation (Romero et al., 2007), the exposure to which significantly increases the risk of alterations in cortical microstructure and functional connectivity (Counsell et al., 2008), and poor neurodevelopmental outcomes postnatally (Leviton et al., 2013). These subtle but significant changes are believed to underlie the high incidence of low severity neurodevelopmental impairments commonly reported in children born preterm, including cognitive, motor and behavioural impairments (Mwaniki et al., 2012) and altered neuroplasticity (*i.e.* the brain's ability to alter the strength of its synaptic connections) (Pitcher et al., 2012a). While GDM-exposed children born at term exhibit some of the same neurodevelopmental impairments as children born preterm, it is currently unknown if they exhibit similar abnormalities in neuroplasticity. Since neuroplasticity is widely accepted as a key mechanism underlying learning and memory, abnormalities in neuroplasticity may help to explain the physiological processes responsible for adverse neurodevelopmental outcomes in children exposed to GDM *in utero*.

Here we used transcranial magnetic stimulation (TMS) techniques to investigate cortical excitability and the capacity for long-term depression (LTD)-like neuroplasticity in children born to women enrolled in the Metformin in Gestational diabetes (MiG) randomised controlled trial (Rowan et al., 2008) to explore potential associations between maternal hyperglycemia and cortical function in the offspring. The MiG trial examined the safety and efficacy of the oral anti-hyperglycemic agent metformin *versus* insulin to treat GDM, and demonstrated that metformin is equally effective and safe as insulin for both mother and child (Barrett et al., 2013; Battin et al., 2015; Rowan et al., 2011; Wouldes et al., 2016). A secondary aim of the present study was to compare the neurophysiologic outcomes of metformin- *versus* insulin-exposed children, as there remains a lack of long-term and neurophysiological data assessing the potential impact of metformin. This is important because, unlike exogenous insulin, metformin crosses the placenta (Charles et al., 2006) and interacts with the fetus in largely unknown ways. Reassuringly, the available evidence suggests that the likely effects of metformin in the fetus are anti-inflammatory (Scheen et al., 2015) and neuroprotective (Chung et al., 2015). Thus, metformin may benefit the fetal brain in GDM-exposed pregnancies beyond its role in maternal glycemic control.

2. Methods

2.1. Ethical Approval

All procedures were approved by the Women's and Children's Health Network and University of Adelaide Human Research Ethics Committees, and conducted in accordance with the Declaration of Helsinki (2008 revision). Participants were pre-screened for contraindications to TMS (Rossi et al., 2009). Parents provided written, informed consent and accompanied their children to the experimental session.

2.2. Subjects

45 GDM-exposed (age: 11.8 ± 0.7 years [mean ± SD], 20 females) were recruited from the Adelaide arm of the MiG trial (Rowan et al., 2008). Their mothers had been treated for GDM not responding to lifestyle alteration, with a 1:1 random allocation at study entry to receive either insulin or metformin treatment (at 30 ± 2.6 weeks gestation). Eight of the metformin-treated women in the current study had required supplemental insulin to achieve euglycemia, but there was no difference in demographic or clinical characteristics of these women compared with the metformin-only treatment women, and these subjects are included in the metformin group. Twelve control children not exposed to GDM (age: 12.8 ± 0.8 years, 8 females) were recruited from labour ward records and matched as closely as possible for gestational age at birth (GA). Mothers in the GDM group had higher body mass indices (BMIs) (34.1 ± 6.8) than control group mothers (23.7 ± 4.6; $p < .001$).

2.3. Maternal Measures

Insulin resistance was measured in mothers in the MiG trial using the homeostatic model assessment of insulin resistance at trial entry (HOMA-IR-Trial) at 30 ± 2.6 weeks gestation (mean ± SD) and at 36 weeks gestation (HOMA-IR-36). Additional perinatal records were obtained.

2.4. Recording Procedures

Children were seated with their hands and forearms supported. Adhesive Ag/AgCl bipolar surface electrodes were applied over the right first dorsal interosseous (FDI) hand muscle to obtain surface electromyography (EMG) recordings. EMG signals were amplified (×1000; 1902 amplifier; CED), bandpass filtered (20 Hz–1 kHz), and digitized at 5 kHz (1401 interface; CED), and were stored offline for later analysis. Researchers were blinded to the treatment status of the participant's mother when collecting and analysing data.

2.5. Transcranial Magnetic Stimulation (TMS)

TMS is a non-invasive brain stimulation technique in which the motor cortex is electromagnetically stimulated to produce a motor evoked potential (MEP) recorded in a contralateral muscle using EMG (Di Lazzaro and Rothwell, 2014). Motor cortical excitability was assessed with single-pulse TMS applied to the left primary motor cortex (M1) representation of the right FDI muscle using a 70 mm figure-of-eight coil connected to a monophasic Magstim 200^2 stimulator (Magstim Co, Whitland, UK). The coil was oriented with the handle pointing postero-laterally at a 45° angle to the sagittal plane, producing a posterior-anterior current flow across M1. The optimal site for consistently evoking MEPs in the FDI was determined and marked on the scalp. Resting motor threshold (RMT) was determined as the lowest TMS intensity required to evoke MEPs of at least 50 μV peak-to-peak amplitude in the resting FDI in at least five of ten consecutive trials. Active motor threshold (AMT) was assessed while the subject maintained a voluntary contraction of approximately 10% of their maximum for FDI, and determined as the lowest TMS intensity required to evoke MEPs of at least 200 μV peak-to-peak amplitude in at least five of ten consecutive trials. The TMS intensity that evoked MEPs of ~1 mV peak-to-peak amplitude (SI_{1mV}) was also determined, and used throughout the experiment for evoking test MEPs (Pitcher et al., 2015).

2.6. LTD-like Neuroplasticity Induction With cTBS

Continuous theta burst stimulation (cTBS; a repetitive TMS protocol) over M1 was used to induce LTD-like suppression of MEP amplitudes. Pharmacological studies indicate cTBS-induced MEP suppression is NMDA-receptor-dependent and similar mechanistically to LTD (Huang et al., 2007). An air-cooled figure-of-eight coil connected to a Magstim Super Rapid stimulator (Magstim, Whitland, UK) was used to apply repetitive TMS to the optimal site for stimulating the right FDI. The cTBS protocol consists of 600 pulses applied in bursts of three pulses at 50 Hz, repeated at 5 Hz for a total of 40 s (Huang et al., 2005). Stimulation intensity was set to 80% of AMT. MEPs were recorded in blocks of 15 trials prior to cTBS (*i.e.* baseline) and at 0, 5, 10, 20 and 30 min following cTBS. The peak-to-peak amplitudes of the 15 MEPs in each block were measured and a mean amplitude calculated. Changes in MEP amplitude relative to baseline MEP amplitude were used as an index of LTD-like plasticity (Pitcher et al., 2012a). All MEPs were recorded at high gain and any with obvious EMG activity in the 100 ms before the TMS

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