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NUTRITIONAL INFLUENCES ON HUMAN NEUROCOGNITIVE FUNCTIONING

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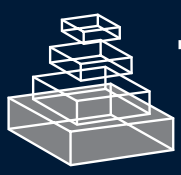
Michael Smith and Andrew Scholey



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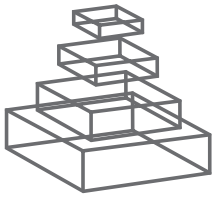

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NUTRITIONAL INFLUENCES ON HUMAN NEUROCOGNITIVE FUNCTIONING

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'You are what you eat'. It's a saying that we've all heard time and time again. The notion that good nutrition is essential for adequate growth and sound physical wellbeing is very well established. Further, in recent years, there has been an overwhelming increase in research dedicated to better understanding how nutritional factors influence cognition and behaviour. For example, several studies have suggested that higher foetal exposure to omega-3 fatty acids and B vitamins such as folate promotes neurodevelopment. B vitamins may also play a role in neurocognitive functioning in later life, with some suggestion that lower vitamin B levels are associated with increased risk of dementia (although randomised controlled trials investigating B vitamin supplementation as a cognitive enhancer in the elderly have provided inconclusive evidence as to the benefits of such therapy for dementia). In fact, the nutritional underpinnings of Alzheimer's disease and other disorders of cognitive ageing is becoming a much researched topic.

In addition, consumption of several other foods has been found to convey more acute cognitively enhancing effects. For example, ingestion of carbohydrates (e.g. glucose), caffeine, resveratrol and several 'nutraceutical' herbal extracts has been associated with short-term improvements in cognitive performance. Beyond specific micronutrients and macronutrients, the current literature seems to support anecdotal evidence that consumption of a balanced breakfast is crucial to various measures of school performance, including attention in the classroom.

What is clear from this emerging literature is that the relationship between nutritional status and neurocognitive functioning at various stages of the lifespan is complex. An aim of this Research Topic is to bring together some recent empirical findings, reviews and commentaries of the literature to date and opinion pieces relating to future directions for this burgeoning field.

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EDITORIAL
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Nutritional influences on human neurocognitive functioning

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The notion that good nutrition is essential for adequate growth and sound physical wellbeing is very well established. Further, in recent years, there has been an overwhelming increase in research dedicated to better understanding how nutritional factors influence cognition and behavior (Riby et al., 2012). An aim of this Research Topic was to bring together Review, Opinion and Original Research articles reflecting the current science in this discipline. These include the effects of a range of foods and nutritional substrates on acute and chronic human neurocognitive functioning. The 13 accepted papers which form this Research Topic cover a diverse range of topics relating nutritional factors to neurocognitive functioning and performance. The articles demonstrate that neurocognitive performance is influenced by nutritional factors ranging from the dietary level (e.g., whole diet and meal composition) through to effects of macronutrients (such as glucose and omega-3 fatty acids) and micronutrients (vitamins, iron) on neurocognitive performance.

An objective of this research topic was to consider how various nutritional factors impact upon neurocognitive functioning at different stages of the lifespan. A number of the submissions focused on effects of nutrition in childhood, during which time nutrition plays an important role in growth and development, including via influences on constituents of the human central nervous system. A review by Nyaradi et al. (2013) considered the role of nutrition from a very broad perspective on neurocognitive development from the prenatal period through to childhood. This suggested that while observational studies have supported an important role for several individual nutrients (such as omega-3 fatty acids, B vitamins, iron) in the neurocognitive development of children, intervention studies aimed at supplementing intake of these individual nutrients have demonstrated inconclusive benefits. The authors of this review also highlighted the beneficial neurocognitive effects of breastfeeding and regular breakfast consumption as well as the impairing neurocognitive effects of childhood malnutrition. Kitsao-Wekulo et al. (2013) aimed to extend current understanding of this link between childhood malnutrition and poor cognitive outcomes, by investigating nutritional status as a mediator of the relationship between several socio-demographic variables and cognitive function in a sample of predominantly rural-dwelling Kenyan children. Nutritional status was found to mediate the relationship between socio-demographic factors and (i) language, (ii) motor function, and (iii) executive functioning in this study. With respect to specific micronutrient deficiencies that translate to adverse neurocognitive outcomes, Radlowski and Johnson (2013) reviewed the literature relating to the most common global nutrient deficiency, namely iron deficiency. They report that maternal anemia during the perinatal period increases the risk of delayed neurocognitive development. A further nutrient for which intake is typically below recommended levels in Western individuals is the omega-3 docosahexaenoic acid (DHA). Low dietary levels of this essential fatty acid are potentially problematic given (i) the involvement of this nutrient in mediating several critical brain functions, and (ii) DHA is derived from the diet alone. Similarly to the review of Nyaradi et al. (2013), Heaton et al. (2013) review concludes that dietary and plasma DHA levels in infancy appear to be associated with enhanced cognitive development, but that RCTs investigating infant DHA supplementation have been inconclusive with respect to beneficial effects on cognitive development. However, these authors note substantial methodological issues with RCTs of infant DHA supplementation studies, which could in part explain the equivocal findings (see also Meldrum et al., 2011). In a further review by Whiteley et al. (2013), it was argued that several dietary interventions have been effective in attenuating the neurocognitive and other adverse psychological outcomes in developmental disorders. The authors focused specifically on an intervention involving dietary elimination of gluten (the major protein in wheat, barley and rye) and casein (found in mammalian dairy products), and reported that this gluten and casein free dietary intervention was effective in enhancing such functions as language, attention and motor control in individuals with autism spectrum disorders.

Caroline Edmonds has conducted several studies investigating the influence of hydration status on cognitive functioning, with previous studies observing that access to water improves cognitive performance in children (Edmonds and Jeffes, 2009). In the paper included in this Research Topic, Edmonds et al. (2013) observed that beneficial effects of water consumption may be limited to individuals with relatively higher levels of subjective thirst, with thirsty individuals who were not provided with water exhibiting slower simple reaction times compared with (i) those who were administered water and (ii) those who were not administered water but reported lower levels of subjective thirst. In a further empirical study, Gibson et al. (2013) found that younger women with a higher dietary intake of saturated fat showed deficits in learning and memory.

Three papers accepted into our Research Topic considered the role of breakfast, which has been argued by many nutritionists

to be the "most important meal of the day," in neurocognitive performance. A review by Adolphus et al. (2013) reported that (i) the quality and frequency of the habitual breakfast meal and (ii) engagement with school breakfast programmes in children and adolescents influences academic attainment. In addition Defeyter and Russo (2013) investigated the acute effect of breakfast consumption (compared to fasting) in adolescent non-habitual breakfast consumers, and observed that breakfast consumption enhanced verbal memory (under conditions of greater cognitive load) and backwards counting performance. However, no effects were observed in a range of other cognitive domains. Conversely, Zilberter and Zilberter (2013) highlight the equivocal findings of previous studies investigating the relationship between breakfast consumption and neurocognitive performance. These authors report that several different breakfast effects which have been investigated previously (e.g., glycemic load of the breakfast meal, nutritional composition, breakfast vs. no breakfast) have yielded positive, negative, and null effects on neurocognitive performance across a range of different populations under investigation. Thus it appears that more studies are needed to ascertain the specific benefits of breakfast on neurocognitive performance.

Finally, in recent years neuroimaging studies have made a substantial contribution to our understanding of the neurocognitive mechanisms underpinning nutritional influences on human cognitive performance. Three papers within this Research Topic specifically discuss the role of neuroimaging in investigating the link between nutrition and cognitive functioning. With respect to carbohydrate intake and neurocognitive performance, it is well established that glucose ingestion enhances memory performance, but no such beneficial memory effect of glucose is typically observed for emotionally laden stimuli (Smith et al., 2011). Schopf et al. (2013) report that following glucose ingestion, the hypothalamus becomes inactive in response to emotional material, providing a mechanistic explanation for the previously observed behavioral observations. Further, Jackson and Kennedy (2013) discuss the ways in which near-infrared spectroscopy has proven useful in detecting changes in cerebral blood flow following ingestion of dietary constituents including caffeine, polyphenols and omega-3 fatty acids. A paper which reviewed the literature relating to neuroimaging studies that have investigated the mechanisms underpinning the influence of early diet on cognitive and brain development by Isaacs (2013) provides a sound overview of the work which has been conducted on this topic.

In summary, it is clear that nutritional status, diet and the ingestion of a range of nutrients impacts upon neurocognitive development, function, and performance. The papers within this Research Topic consider a range of these effects. However, equivocal findings have emerged from many studies which have investigated the relationship between nutrition and cognition. Neuroimaging studies are informative with respect to the precise mechanisms which mediate these effects, and future studies in this area will contribute greatly to our understanding of the relationship between nutrition, diet and human neurocognitive functioning.

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REVIEW ARTICLE
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The role of nutrition in children's neurocognitive development, from pregnancy through childhood

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This review examines the current evidence for a possible connection between nutritional intake (including micronutrients and whole diet) and neurocognitive development in childhood. Earlier studies which have investigated the association between nutrition and cognitive development have focused on individual micronutrients, including omega-3 fatty acids, vitamin B12, folic acid, choline, iron, iodine, and zinc, and single aspects of diet. The research evidence from observational studies suggests that micronutrients may play an important role in the cognitive development of children. However, the results of intervention trials utilizing single micronutrients are inconclusive. More generally, there is evidence that malnutrition can impair cognitive development, whilst breastfeeding appears to be beneficial for cognition. Eating breakfast is also beneficial for cognition. In contrast, there is currently inconclusive evidence regarding the association between obesity and cognition. Since individuals consume combinations of foods, more recently researchers have become interested in the cognitive impact of diet as a composite measure. Only a few studies to date have investigated the associations between dietary patterns and cognitive development. In future research, more well designed intervention trials are needed, with special consideration given to the interactive effects of nutrients.

Keywords: nutrition, cognitive development, children, micronutrients, diet quality

INTRODUCTION

Cognition represents a complex set of higher mental functions subserved by the brain, and includes attention, memory, thinking, learning, and perception (Bhatnagar and Taneja, 2001). Cognitive development in pre-schoolers is predictive of later school achievement (Tramontana et al., 1988; Clark et al., 2010; Engle, 2010). As Ross and Mirowsky (1999) state: "Schooling builds human capital - skills, abilities, and resources—which ultimately shapes health and well-being." Indeed, more education has been linked to better jobs, higher income, higher socio-economic status, better health care access and housing, better lifestyle, nutrition, and physical activity (Florence et al., 2008), which are all well-known health determinants. Education increases an individual's sense of personal control and self-esteem; these factors have also been shown to influence better health behavior (Ross and Mirowsky, 1999; Logi Kristjánsson et al., 2010). Academic achievement is important for future personal health, and is therefore a significant concern for public health.

Cognitive development is influenced by many factors, including nutrition. There is an increasing body of literature that suggests a connection between improved nutrition and optimal brain function. Nutrients provide building blocks that play a critical role in cell proliferation, DNA synthesis, neurotransmitter and hormone metabolism, and are important constituents of enzyme systems in the brain (Bhatnagar and Taneja, 2001; Lozoff and Georgieff, 2006; Zeisel, 2009; De Souza et al., 2011; Zimmermann, 2011). Brain development is faster in the early years of life compared to the rest of the body (Benton, 2010a), which may make it more vulnerable to dietary deficiencies.

In this literature review, we assess the current research evidence for a link between nutritional intake in pregnancy and childhood and children's cognitive development. We first discuss individual micronutrients and single aspects of diet, which represents earlier research in this area. We next consider the more encompassing aspects of diet, which have emerged as researchers became more interested in diet as a comprehensive measurement. The most recent research trend in this area suggests a broader analysis of the role of nutrition in neurocognitive development, which we offer here in comparison to previous reviews (Black, 2003b; Bellisle, 2004; Stevenson, 2006; Georgieff, 2007; Benton, 2010a).

BRAIN DEVELOPMENT IN HUMANS

The understanding of the functional and structural development of the human brain has emerged from a range of methodologies (including clinical lesion and experimental animal studies) and lately as a result of greatly improved neuroimaging methods, in particular Positron Emission Tomography and Magnetic Resonance Imaging (MRI) (Levitt, 2003; Uddin et al., 2010).

Brain development is a temporally extended and complex process, with different parts and functions of the brain developing at different times (Grossman et al., 2003). By 5 weeks after conception in humans, the anterior-posterior and dorsal-ventral axes of the neural tube have already developed (Levitt, 2003). The cortical plate (which is the forerunner of the cerebral cortex) and some inter-neuronal connections form from 8 to 16 weeks of gestation (Kostović et al., 2002; Levitt, 2003). From 24 weeks of gestation until the perinatal period, the neurons in the cortical plate die and are replaced by more mature cortical neurons. During this time, significant refinement in neural connections take place (Levitt, 2003). From 34 weeks post-conception until 2 years of age, peak synapse development, and significant brain growth occurs (Huttenlocher and Dabholkar, 1997; Levitt, 2003). By preschool age, synaptic density has reached the adult level. The myelination of some parts of the brain (particularly those that control higher cognitive functions, such as the frontal lobes) continues well into adolescence, whilst myelination occurs earlier in other parts of the brain that coordinate more primary functions (Toga et al., 2006). Although the gray matter (which contains the bodies of nerve cells) reaches asymptote by the age of 7–11 in different regions of the brain, it is thought that the growth of the white matter (which represents axonal nerve tracts) continues beyond 20 years of age. Studies have shown that the maturation of specific brain areas during childhood is associated with development of specific cognitive functions such as language, reading, and memory (Nagy et al., 2004; Deutsch et al., 2005; Giedd et al., 2010). The development of the frontal lobes, which are believed to control higher cognitive functions (including planning, sequencing and self-regulation), appears to occur in growth spurts during the first 2 years of life, and then again between 7 and 9 years of age and also around 15 years of age (Thatcher, 1991; Bryan et al., 2004). The development of some subcortical structures including the basal ganglia, amygdala, and hippocampus (which are also centrally involved in some mediating higher cognitive functions, including memory, executive functions, and emotion) also continues until late adolescence. In addition, a meta-analysis has confirmed a connection between the size of the hippocampus and memory performance during brain development in children and young adults (Van Petten, 2004). Overall, the research evidence suggests that cognitive development is strongly connected with micro and macro-anatomical changes which take place throughout childhood (Levitt, 2003; Herlenius and Lagercrantz, 2004; Ghosh et al., 2010).

Individual brain development follows a genetic program which is influenced by environmental factors including nutrition (Bryan et al., 2004; Toga et al., 2006; Giedd et al., 2010). Environmental influences may modify gene expression through epigenetic mechanisms, whereby gene function is altered through the processes of DNA methylation, histone modification and the modulating effect of non-coding RNAs, without the alteration of the gene sequence *per se*. These epigenetic factors can cause long lasting or even heritable changes in biological programs (Levi and Sanderson, 2004; Rosales et al., 2009; Murgatroyd and Spengler, 2011; Lillycrop and Burdge, 2012). It has been shown in animal and more recently in human studies that nutrition is one of the most salient environmental factors, and that nutrition can have a direct effect on gene expression (Levi and Sanderson, 2004; Rosales et al., 2009; Attig et al., 2010; Lillycrop and Burdge, 2011; Jiménez-Chillarón et al., 2012). One of the first and best known human studies in the rapidly growing field of "Nutritional Epigenomics" relates to the Dutch Hunger Winter during the 1940's in which the offspring of mothers exposed to famine during pregnancy had an increased risk of cardiovascular, kidney, lung, and metabolic disorders and reduced cognitive functions (Roseboom et al., 2006; De Rooij et al., 2010). More specifically, evidence has been obtained of hypo- and hyper-methylated DNA segments from the blood cells of the affected individuals (Heijmans et al., 2008).

Evidence suggests that the timing of nutritional deficiencies can significantly affect brain development. For example, it is well known that folic acid deficiency between 21 and 28 days after conception (when the neural tube closes) predisposes the foetus to a congenital malformation, called a neural tube defect. Hence, this is a critical period, because during that time an irreversible change in the brain structure and function occurs if there is inadequate folic acid present (Blencowe et al., 2010). A critical period is a specific period within a sensitive timeframe (Knudsen, 2004). A sensitive period tends to reflect a broader timeframe; during such a developmental period the brain is more sensitive to specific interventions. However, skills and abilities can still be acquired outside this time period, albeit with less proficiency (Knudsen, 2004). An example is that deaf children who receive cochlear implants within a sensitive period for brain development (i.e., before the age of 3–5 years) show better language development than those who receive a cochlear implant after this period (Penhune, 2011).

Since rapid brain growth occurs during the first 2 years of life (and by the age of 2 the brain reaches 80% of its adult weight), this period of life may be particularly sensitive to deficiencies in diet (Bryan et al., 2004; Lenroot and Giedd, 2006). Adolescence is also a significant and sensitive developmental period, with research indicating that structural reorganization, brain and cognitive maturation and—in particular—major developments in the prefrontal cortex take place during puberty (Luna and Sweeney, 2001; Sisk, 2004; Peper et al., 2009; Asato et al., 2010; Blakemore et al., 2010).

DIETARY INFLUENCES ON COGNITIVE DEVELOPMENT

MICRONUTRIENTS AND COGNITIVE DEVELOPMENT

Omega-3 fatty acids

In recent years, there has been an increasing interest in the effect of essential fatty acids, particularly long chain polyunsaturated fatty acids (LCPUFA), on cognitive brain development. Of the human brain's dry weight 60% is comprised of lipids, of which 20% are docosahexaenoic acid (DHA; which is an omega-3 fatty acid) and arachidonic acid (AA; an omega-6 fatty acid). These represent the two core fatty acids found in gray matter (Benton, 2010b; De Souza et al., 2011). Furthermore, the supply of LCPUFAs from food, especially the omega-3 fatty acids, including DHA and eicosapentaenoic acid (EPA), is frequently inadequate for children as well as for adults (Schuchardt et al., 2010).

Essential fatty acids play a central functional role in brain tissue. They are not only the basic components of neuronal membranes, but they modulate membrane fluidity and volume and thereby influence receptor and enzyme activities in addition to affecting ion channels. Essential fatty acids are also precursors for active mediators that play a key role in inflammation and immune reaction. They promote neuronal and dendritic spine growth and synaptic membrane synthesis, and hence influence signal processing, and neural transmission. In addition, essential fatty acids regulate gene expression in the brain (McCann and Ames, 2005; Eilander et al., 2007; Innis, 2007; Cetina, 2008; Wurtman, 2008; Ramakrishnan et al., 2009; Ryan et al., 2010; Schuchardt et al., 2010; De Souza et al., 2011). Therefore, the existing literature strongly suggests that essential fatty acids are critical for brain development and function.

It has been suggested that the fast growth of the human cerebral cortex during the last two million years was strongly related to the balanced dietary intake of LCPUFAs (Broadhurst et al., 1998), specifically with an equal ratio of omega-6 and omega-3 fatty acids in the diet (Simopoulos, 1999). Evidence proposes that the modern Homo sapiens, whose brain developed significantly relative to its ancestors, lived near rivers and oceans, where seafood and fish were abundant (Crawford et al., 1999). The rise in intellectual and brain development in Homo Sapiens also coincided with tool making and language development (Crawford et al., 1999; Broadhurst et al., 2002). During the last 150 years, it is believed that the balance of omega-6 to omega-3 fatty acids has shifted in favor of omega-6 fatty acids in the diet, resulting in a ratio of 20–25:1 and a dietary deficiency in omega-3 fatty acids (Simopoulos, 1999). A diet that is deficient in omega-3 fatty acids may have health and developmental implications (Simopoulos, 2008).

A number of epidemiological studies have shown a positive association between maternal fish intake (which is a rich source of omega-3 fatty acids) during pregnancy and cognitive development in children (Daniels et al., 2004; Hibbeln et al., 2007; Jacobson et al., 2008; Oken et al., 2008a,b; Boucher et al., 2011). Data from the Avon Longitudinal Study of Parents and Children (ALSPAC) in the UK regarding fish consumption and child cognitive development were analyzed in two studies (Daniels et al., 2004; Hibbeln et al., 2007). The earlier study found evidence that higher maternal fish consumption was associated with higher language and social skills (after appropriate adjustments) in 7421 British children assessed at 15 months, using the MacArthur Communicative Development Inventory (MCDI), and at 18 months using the Denver Developmental Screening test (Daniels et al., 2004). The later ALSPAC study demonstrated that those children whose mothers consumed lower levels of seafood during pregnancy had lower IQ, measured by the Wechsler Intelligence Scale for Children III (WISC-III) at the age of 8 (after adjusting for a wide range of relevant covariates). Lower maternal seafood consumption was also linked to suboptimal behavior at age seven (measured using the Child Behavior Checklist) and to lower levels of social, fine motor and language development (measured using the Denver Developmental Screening test) at six, 18, 30, and 42 months of age in the same study (Hibbeln et al., 2007). Although higher fish intake may result in higher erythrocyte mercury concentration (which has been shown to alter neurodevelopment adversely), research in American schoolchildren (Project Viva, a prospective pre-birth cohort study) demonstrated that higher maternal fish intake was still positively associated with improved language scores on the Peabody Picture Vocabulary Test (PPVT), after adjustment for many potential confounders and covariates (Oken et al., 2008a). The Danish National Birth Cohort study investigated the developmental milestones of 25,446 six- and 18-month old children on a developmental scale created by the researchers, and found that higher maternal fish intake was beneficial for cognitive development even after adjusting for breastfeeding and many sociodemographic factors (Oken et al., 2008b). Two other studies of Inuit children in Arctic Quebec, Canada, showed that higher umbilical cord DHA concentration was associated with: (1) improved infant cognitive development, measured on the Fagan Test of Infant Intelligence at 6 months, (2) on the Bayley Scales of Infant Development test used at 11 months (Jacobson et al., 2008), and (3) better memory performance of school children on both the Digit Span Forward subtest of the WISC-IV and the California Verbal Learning Test-Children Version (Boucher et al., 2011). These results were independent of mercury contamination in seafood. Both studies adjusted for a wide range of socioeconomic and demographic factors. However, these investigations used smaller samples, specifically 109 infants (Jacobson et al., 2008) and 154 schoolchildren (Boucher et al., 2011), compared to the previously described studies. In conclusion, the positive association between maternal fish intake and cognitive development is supported by evidence from the studies cited above.

However, intervention studies of LCPUFA supplementation during pregnancy have produced conflicting results so far. Some studies have reported positive associations between DHA supplementation and cognitive developmental parameters (Helland et al., 2003, 2008; Colombo et al., 2004; Judge et al., 2007; Dunstan et al., 2008). A randomized placebo-controlled double- blind study undertaken by Helland et al. (2003) in Norway used a design which supplemented women from 18 weeks of pregnancy until 3 months postpartum with cod liver oil containing 1183 mg DHA. Children's cognitive status was assessed at 6 and 9 months on the Fagan Test of Infant Intelligence (Helland et al., 2001), and at 4 years of age by the Kaufman Assessment Battery for Children (K-ABC), specifically the Mental Processing Composite comprising the Sequential Processing, Simultaneous Processing, and Non-verbal Scales (which reflects children's problem solving and information processing skills; Helland et al., 2003). The results indicated that maternal DHA supplementation improved children's mental processing skills at 4 years of age (Helland et al., 2003), but not recognition memory at 6 and 9 months of age (Helland et al., 2001). However, in a follow-up study conducted at the age of 7, there was no difference in overall IQ between supplemented versus non-supplemented children, but a positive correlation was observed between the concentration of α-linolenic acid (ALA) and DHA in maternal plasma phospholipid and performance on the Sequential Processing scale (Helland et al., 2008). A possible explanation of these findings is that by 7 years of age cognitive development is influenced by many other intervening factors, and the test battery used in the

research may not have been sensitive enough to detect the association between diet and cognition at this later age (Helland et al., 2008). The results of a study reported by Judge et al. (2007) also supported the findings of the previous study, providing evidence that maternal DHA supplementation results in better problem solving ability (speed of processing) in 9 month old infants on the Infant Planning Test, but not on recognition memory evaluated using the Fagan Test of Infant Intelligence. These findings may indicate that DHA is more important in the development of problem solving and processing ability than other cognitive functions such as memory. Visual attention or look duration declines during the first year of life, giving place to more complex mental processing (Frick et al., 1999; Colombo et al., 2004; Judge et al., 2007). Colombo et al. (2004) reported an association between maternal DHA supplementation and faster decline in visual attention during infancy, and better resistance to distractibility during the second year of life. Researchers in Western Australia supplemented a small sample of women ($n = 98$) from 20 weeks of gestation until delivery with high dose (2200 mg) DHA or olive oil and showed significant improvements in hand and eye coordination in the supplemented group at 2½ years of age, after adjusting for maternal age, maternal education, and breastfeeding (Dunstan et al., 2008). These researchers also demonstrated better performance in other elements of cognitive development (locomotor, social, speech and hearing performance, and practical reasoning), evaluated using the Griffiths Mental Development Scales, and on language development, evaluated using the PPVT (Dunstan et al., 2008). However, these latter differences were not statistically significant, perhaps due to the relatively small sample size in the study.

Contrary to expectations, some studies did not find a relationship between LCPUFA supplementation during pregnancy and cognitive development in children (Tofail, 2006; Makrides et al., 2010; Campoy et al., 2011). One of these studies was conducted in a developing country, Bangladesh, where a high proportion of mothers suffer from undernutrition, and possibly from multiple micronutrient deficiencies important for brain development. The pregnant mothers in this study were randomized into fish-oil or soy-oil supplemented groups, and their infants' cognitive development was measured on the Bayley Scales of Infant Development at ten months of age. In this study, the mothers were only given supplements during the third trimester, which may not have represented a sufficient timeframe for supplement administration (Tofail, 2006). In a recent study conducted in Europe on 270 women from three countries, cognitive development was measured on the K-ABC at 6½ years of age after 500 mg DHA prenatal supplementation. In this study, the co-authors did not find significant differences in intelligence between the intervention and control groups (Campoy et al., 2011). The explanation offered was that the positive effect of prenatal supplementation may have been overshadowed by other important factors (not all of which it is possible to control for) including social stimulation, other nutrients taken, diet as a whole, illnesses, and drugs prescribed by the age of 6–7 years (Helland et al., 2008). Makrides et al. (2010) conducted a well-designed multicenter double-blind randomized controlled trial in Australia on 2399 women between 2005 and 2009 from 21 weeks of gestation until birth and did not find any difference on the Bayley Scales of Infant and Toddler Development at the age of 18 months between intervention (supplemented with 800 mg DHA) and control groups (supplemented with vegetable oil capsules), after adjustment for potential confounders. Given the size of this study, the Makrides et al. (2010) investigation was certainly adequately powered to detect a statistically significant difference; yet, no such difference was observed.

Some published studies have also considered supplementation in lactating mothers in order to examine the effect of increased omega fats in breast milk on the cognitive development of children. Reviews of these studies have concluded that there are indications that supplementing lactating mothers with fish oil may positively influence cognitive development in children (Eilander et al., 2007; Hoffman et al., 2009).

In conclusion, the current findings show inconsistencies in the efficacy of maternal supplementation with omega-3 fatty acid. In seeking to account for the contrasting findings, it seems that the following considerations may be relevant: the interventions were applied in different groups of women, using a wide range of DHA dosage, with different durations of supplementation, and the outcomes were measured on different cognitive instruments and at different ages. The more consistent results obtained in epidemiological studies (compared with supplementation trials using only omega-3 fatty acid) may be explained by the possibility that fish is a whole food, and it contains other nutrients important to cognitive development. Furthermore, by eating fish rather than taking fish supplements, other possibly unhealthy or potentially inflammatory foods may also be displaced—i.e., red meat and processed meats. Epidemiological studies may also be better powered but they may also potentially have less control for confounding.

Postnatal studies have considered the effect of omega-3 fatty acid supplementations on term and preterm infants. Epidemiological studies and supplementation trials have also been undertaken in older children in relation to cognitive development. The DHA component is believed to be one of the main reasons why breast milk may improve the cognitive performance of children. Humans are able endogenously to synthesize DHA from precursor α-linolenic acid. However, the conversion rate varies according to genetically determined polymorphisms in two genes, namely FADS1 and FADS2. Moreover, in infants the conversion to DHA seems to be very limited (Hoffman et al., 2009; Guesnet and Alessandri, 2011). Research consistently shows that the blood levels of DHA in formula-fed infants are lower than in breastfed infants, irrespective of the level of precursors in formulas (Hoffman et al., 2009). Therefore, this topic has generated a great deal of scientific interest, especially with respect to the results of clinical trials that have supplemented infant formulas with LCPUFA and investigated the relationship with cognitive development in either term or preterm infants.

A Cochrane review undertaken by Simmer et al. (2008) included 11 studies and concluded that there is currently not enough evidence to support the supplementation of infant formulas with LCPUFA to benefit cognitive development in children born at term. Another review by McCann and Ames (2005) considered animal as well as human supplementation studies. These authors found that, although animal studies provided convincing

evidence for DHA supplementation and improved cognitive performance, these studies were undertaken under extreme dietary conditions in which (in most cases) animals were severely deficient in essential fatty acids. By contrast, in formula and breastfed infants the differences in brain DHA concentrations are small, such that the positive effects of LCPUFA on cognitive development may be difficult to detect. Eilander et al. (2007) also concluded that there is no evidence that formula supplementation in term infants is beneficial for cognitive development. On the other hand, Hoffman et al. (2009) reviewed 20 studies and suggested that those trials which supplemented formula milk with a similar level of DHA to breast milk (i.e., to an average of 0.32%) found beneficial outcomes. A major intake of DHA in the brain happens in the last trimester of pregnancy; therefore, preterm infants are disadvantaged and have decreased brain concentration of this vital LCPUFA. Eilander et al. (2007) indicated that supplementing formula milk with LCPUFA may be beneficial for the cognitive development of preterm infants. However, a recent Cochrane review undertaken on preterm infants reported no significant outcome of supplementation with LCPUFA on their cognitive development (Schulzke et al., 2011).

Since brain development continues through childhood, there have been much interest in the association between cognitive development and omega-3 fat levels through diet and/or supplementation in children. Ryan et al. (2010) reviewed the available epidemiological studies and supplementation trials to date and concluded that, although the results were inconsistent, it appears that those studies that supplemented with higher doses and longer durations of DHA achieved a favorable positive outcome in cognitive development in childhood.

Vitamin B12, folic acid, and choline

B12 and folate deficiency resulting in anaemia is rare around the world. However, it can occur in both developing and developed countries especially in older people, in those with absorption problems and in vegetarians (De Benoist, 2008). Folate fortification of bread products has been made mandatory in Australia and in many other countries, which has reduced this deficiency significantly (Brown et al., 2011). In recent years, there has been an increasing interest in the association between vitamin B12, folic acid, choline metabolism, and cognitive development. Folate affects neural stem cell proliferation and differentiation, decreases apoptosis, alters DNA biosynthesis, and has an important role in homocysteine and S-adenosylmethionine biosynthesis (Zeisel, 2009; Zhang et al., 2009). It is believed that choline has similar roles in brain development as folate (Meck and Williams, 2003; McCann et al., 2006; Zeisel, 2006a,b, 2009; Signore et al., 2008). Furthermore, folate, choline and vitamin B12 metabolism are interconnected at the homocysteine-methionine-S-adenosylmethionine pathway (Zeisel, 2009). S-adenosylmethionine is one of the main methyl donors in different metabolic methylation reactions, including DNA methylation. Therefore, choline and folate deficiency may result in DNA hypomethylation, thereby altering gene transcription (Zeisel, 2009). In addition, choline is a component of phospholipids in cell membranes and a precursor for the neurotransmitter acetylcholine (Zeisel, 2006b). Vitamin B12 has a role in axon myelination that is important for impulse conduction from cell to cell, and it also protects neurons from degeneration. Vitamin B12 may also alter the synthesis of different cytokines, growth factors and oxidative energy metabolites such as lactic acid (Dror and Allen, 2008).

In children, the association between vitamin B12 and cognitive development has been mainly observed in infants born of vegetarian/vegan mothers or mothers on a macrobiotic diet. These diets can result in vitamin B12 deficiency, as vitamin B12 is largely found in animal products. A pooled analysis that included 48 case studies of infants with vitamin B12 deficiencies reported a variety of abnormal clinical and radiological signs, including: hypotonic muscles, involuntary muscle movements, apathy, cerebral atrophy, and demyelination of nerve cells (Dror and Allen, 2008). After vitamin B12 treatment, a rapid improvement in neurological symptoms is reported in deficient infants, but many of these infants remained seriously delayed in cognitive and language development in the longer term (Dror and Allen, 2008). The long-lasting effect of vitamin B12 deficiency is supported by the findings of Louwman et al. (2000). These researchers investigated the cognitive functioning of adolescents who consumed a macrobiotic diet until the age of 6 years, compared to children with an omnivorous diet. Those adolescents who consumed a macrobiotic diet until 6 years of age had lower levels of fluid intelligence, spatial ability and short term memory (even with currently normal vitamin B12 status) than the control subjects. Although vitamin B12 deficiency is not likely to occur in non-vegetarian people in western countries, Pepper and Black (2011) raised concern about the more frequent gastric bypass surgeries in obese women and the increased incidence of coeliac disease and inflammatory bowel diseases (such as Crohn's and ulcerative colitis). In these conditions, the absorption of vitamin B12 is substantially decreased in the intestine, thereby potentially adversely affecting the development of future children born to these women.

The association between maternal blood folate status and cognitive development has been investigated in several studies (Tamura et al., 2005; Veena et al., 2010). Tamura et al. (2005) did not find any relationship between maternal blood folate status ("low" vs. "normal") during the second half of the pregnancy and cognitive development of their children at the age of 5–6 years on different cognitive tests including Differential Ability Scales, Visual and Auditory Sequential Memory, Knox Cube, the Gross Motor Scale and the Grooved Pegboard. These researchers conducted their study among African-American women of low socioeconomic status and their disadvantaged children. These children nevertheless did not present with signs or symptoms of any overt clinical deficiency (such as megaloblastic anaemia). On the other hand, Veena et al. (2010) reported that higher maternal blood folate concentration—but not folate status ("low" vs. "normal", similar to the previous study)—was associated with better cognitive performance on a wide range of tests (Atlantis, Word Order, Pattern Reasoning, Verbal Fluency, Koh's Block Design and WISC-III) in 9–10-year-old Indian children. Interestingly, most of the women (96%) in this study manifested blood folate levels within the normal range. However, the only positive association found between maternal vitamin B12 status and cognitive

performance in these children was on verbal fluency, although almost half of the mothers (42.5%) manifested moderately low vitamin B12 level. It is possible that vitamin B12 affects some cognitive functions only if the person is severely deficient, as can be seen in vegetarian mothers and their children. Another reason for the limited findings may be that the tests conducted were not sensitive enough to detect small changes in different functions. Another research group in India showed that lower vitamin B12 levels during pregnancy impaired short term memory (Digit Span Test) and sustained attention (Color Trail Test) in 9 years old children after adjusting for covariates (Bhate, 2008) while non-verbal intelligence on Raven's Cultured Progressive Matrices and visual perception on a Visual Recognition Test were unaffected in this study.

Although there is no sufficient data about the requirements of choline in humans, choline does not seem to be deficient in the general population, with the exception of experimental conditions (Commonwealth of Australia, 2006). Evidence from numerous animal studies indicates that dietary choline has an important impact on the cognitive development of offspring (Meck and Williams, 2003; McCann et al., 2006). Choline in animal models alters the development of the hippocampus, which has a central role in memory and learning (Zeisel, 2006a). Like folate, choline also has a role in the closure of the foetal neural tube. A study among Californian mothers found an increased risk of neural tube defects of their children with lower maternal dietary choline intake, as identified from a food frequency questionnaire (Shaw et al., 2004). There is only one study to date that has evaluated the impact of maternal blood choline (represented across a wide range of concentrations) on intelligence (measured on the Wechsler Preschool and Primary Scale of Intelligence-Revised) in 5 year old children. However, the authors did not find a significant correlation between the two (Signore et al., 2008). In summary, the impact of vitamin B12, folate and choline on children's cognitive development has not been adequately researched to date in humans.

Zinc

Zinc deficiency appears to be a major problem worldwide, affecting 40% of the global population (Maret and Sandstead, 2006). Recent research suggests that toddlers, adolescents, older people and individuals with diabetes are possibly at a higher risk of zinc deficiency in Australia (Gibson and Heath, 2011). Animal studies have established a relationship between zinc and neurodevelopment (Shah and Sachdev, 2006; Summers et al., 2008; Coyle et al., 2009). It is believed that zinc is a vital nutrient for the brain, with important structural and functional roles (Bhatnagar and Taneja, 2001; Black, 2003a; Bryan et al., 2004; Shah and Sachdev, 2006; Georgieff, 2007). More specifically, zinc is a cofactor for more than 200 enzymes that regulate diverse metabolic activities in the body including protein, DNA and RNA synthesis. In addition, zinc plays a role in neurogenesis, maturation, and migration of neurons and in synapse formation (Bhatnagar and Taneja, 2001; Black, 2003a; Bryan et al., 2004; Shah and Sachdev, 2006; Georgieff, 2007). Zinc is also found in high concentrations in synaptic vesicles of hippocampal neurons (which are centrally involved in learning and memory), and seems to modulate some neurotransmitters including glutamate and gamma- aminobutyric acid (GABA) receptors (Bhatnagar and Taneja, 2001; Levenson, 2006).

Zinc supplementation has a positive effect on the immune status of infants and may prevent congenital malformations (Shah and Sachdev, 2006). However, the relationship between maternal zinc status and the child's cognitive development has not been fully investigated. In an observational study, low maternal zinc intake in Egyptian mothers was associated with lower levels of focused attention in newborns, measured with the Brazelton Neonatal Behavior Assessment Scale (Kirksey et al., 1994). Surprisingly, a placebo controlled randomized trial undertaken on poor Bangladeshi mothers found that 13 months old infants of zinc supplemented mothers scored less on the Bayley scales of infant development than infants born to mothers who received a placebo (Hamadani et al., 2002). In trying to explain their findings, these researchers argued that zinc supplementation alone may cause imbalances or even deficiencies of other micronutrients that are important for brain development, as micronutrients interact with one another (Hamadani et al., 2002)—a point which we will return to later in this review. Another double-blind randomized controlled trial of maternal zinc supplementation among African-American mothers showed no difference in the cognitive development of 5 years old children between the intervention and control groups, measured on the Differential Ability Scales, Visual and Auditory Sequential Memory, Knox Cube, Gross Motor Scale, and Grooved Pegboard (Tamura et al., 2003). In both studies, the mothers were supplemented only in the second half of their pregnancy. Overall, there are only a limited number of studies on this topic. Taken together, the findings do not consistently show a positive relationship between maternal zinc status and cognitive development of children.

Two articles that reviewed earlier observational and randomized control trials in children on zinc and cognitive development concluded that zinc deficiency can negatively influence cognitive development. Conversely, more recent randomized control trials from India (Taneja et al., 2005) and Bangladesh (Black et al., 2004), where malnutrition is common among children, did not find that zinc supplementation alone affects infants' cognitive development on the Bayley Scales of Infant Development test. Nevertheless, in the Bangladeshi trial, when zinc was combined with iron supplementation, it showed an improvement in cognition (Black et al., 2004). Additional studies are therefore needed to examine the long term benefit of zinc on brain development.

Iron

One of the most common nutritional deficiencies in both developing and developed countries is iron deficiency. In some parts of the world, such as in Sub-Saharan Africa and South-East Asia, the prevalence is more than 40%. In developed countries—including Australia—it could be as high as 20%, particularly in pregnant women and in children (World Health Organization, 2008). Over the past decades, a considerable literature has been published on the association between iron status/anaemia and cognitive development in children, as well as in animal models (Grantham-McGregor and Ani, 2001). It is believed that iron is

involved with different enzyme systems in the brain, including: the cytochrome c oxidase enzyme system in energy production, tyrosine hydroxylase for dopamine receptor synthesis, delta-9-desaturase for myelination, and fatty acid synthesis, and ribonucleotide reductase for brain growth regulation (Deungria, 2000; Lozoff and Georgieff, 2006; Georgieff, 2007; Rioux et al., 2011). In addition, iron appears to modify developmental processes in hippocampal neurons by altering dendritic growth (Jorgenson et al., 2003; Lozoff and Georgieff, 2006).

There are a limited number of studies that have examined the connection between maternal iron status or maternal iron supplementation and cognitive development. (In the below, treatment refers to anaemic individuals, and supplementation to non-anaemic children.) Tamura et al. (2002) found significantly inferior performance in language skills, fine motor skills and attention (and lower but not significant scores in every other test) in 5 years old children whose cord ferritin levels lay in the lowest quartile. Cognitive performance in this study was measured on the WISC-R, the Test for Auditory Comprehension of Language, fine and gross-motor scales of the Peabody Developmental Motor Scales and the Yale Children's Inventory for attention and tractability. The mothers who took part in the study were of African-American descent and low socioeconomic status, and a high proportion of the children were born small-for-gestational-age. However, a randomized placebo controlled iron supplementation trial in a representative sample of Australian pregnant women failed to find any difference between an iron supplemented vs. placebo group in the IQ of children at 4 years of age on the Stanford–Binet Intelligence Scale, despite maternal iron status having improved due to supplementation (Zhou et al., 2006). The authors suggested that supplementing pregnant women who are generally well-nourished with iron may not confer any additional health benefits, while Tamura's study was undertaken in disadvantaged mothers and small-for-gestational-age infants (Tamura et al., 2002; Zhou et al., 2006). A recent trial in Canada by Rioux et al. (2011) also found no evidence that better maternal iron and DHA status enhanced cognitive development in six months old infants, measured on the Brunet-Lezine Scale of Psychomotor Development of Early Childhood and the Bayley Scales of Infant Development. These researchers recruited a small sample size of mothers from a higher socioeconomic background and with better feeding practices, consistent with the methodology and findings of the Australian study cited above.

In children, the relationship between iron and cognitive development has been well researched. In addition, these investigations have been reviewed many times during the last decade. Grantham-McGregor and Ani (2001) reviewed a range of longitudinal studies and reported that anaemic infants had poorer cognitive and school performance in the long term, and that short-term iron treatment trials in anaemic children did not show benefits in cognitive development. A Cochrane review based on seven randomized controlled trials reached a similar conclusion, i.e., that short term iron treatment for anaemia in children less than 3 years old did not improve cognitive development (Logan et al., 2001). Sachdev et al. (2005) included 17 randomized controlled trials in their meta-analysis, and did not find convincing evidence of an association between iron supplementation and treatment for anaemic and cognitive development. However, treating older children with iron deficiency increased IQ significantly. A more recent review and meta-analysis on children (aged 6 years and older), adolescents and adults found that iron treatment increased IQ in anaemic individuals, but iron supplementation did not improve IQ in non-anaemic children (Falkingham et al., 2010).

In summary, there is a lack of epidemiological evidence or data from well-designed intervention trials demonstrating the impact of maternal iron supplementation on the cognitive development of healthy children. There is evidence that older anaemic children benefit from iron treatments. However, cognitive performance tests including the Bayley Scales of Infant Development and the Denver Developmental Screening Test may not be sensitive enough to detect small changes in short-term supplementation or treatment in young children (Armstrong, 2002). Furthermore, if iron deficiency occurs in very early life, the damage may be irreversible, and it may not be possible to reverse this damage with iron treatment (Beard, 2008).

Iodine

Iodine deficiency is a significant worldwide public health issue, especially in children and during pregnancy (World Health Organization, 2004). In Australia, the majority of children and pregnant women are mildly deficient in iodine, with some groups reaching moderate to severe deficiency (Gallego, 2010). Iodine deficiency in the soils in many countries has led to food fortification, most commonly the use of iodized salt (World Health Organization, 2004). The relationship between iodine and cognitive development is extensively researched. It is well known today that severe iodine deficiency during pregnancy may cause "cretinism" in children (Forrest, 2004; Zimmermann, 2007, 2009, 2011; Melse-Boonstra and Jaiswal, 2010). The clinical manifestation of cretinism depends on the severity of iodine deficiency; the features may include mental retardation, speech and hearing impairment, upper motor neuron and extrapyramidal lesions (Delong et al., 1985). Iodine is necessary for the production of thyroid hormones in the body; 70–80% of it is found in the thyroid gland (Melse-Boonstra and Jaiswal, 2010). Iodine deficiency manifests in hypothyroidism, causing underproduction of thyroid hormones including triiodothyronin (T3) and thyroxin (T4). Thyroid hormones play an important role in neurodevelopment and numerous neurological processes including neuronal cell differentiation, maturation and migration, myelination, neurotransmission, and synaptic plasticity (Zimmermann, 2009, 2011; Melse-Boonstra and Jaiswal, 2010). In addition, in animal models hypothyroidism alters neurogenesis and the development and functions of synapses in the hippocampus (Desouza et al., 2005; Gong et al., 2010).

Qian et al. (Qian, 2005) conducted a meta-analysis on studies from different locations in China where the soil is severely iodine deficient, and found a 12.3 point decrease in the IQ of those children whose mothers lived in iodine deficient areas compared to those living in iodine sufficient locations. The association between mild-moderate maternal iodine deficiency and cognitive development is not as clear as it is when iodine deficiency is severe (Forrest, 2004; Zimmermann, 2007, 2009, 2011; Melse-Boonstra and Jaiswal, 2010). In mild-moderate iodine deficiency, maternal

thyroid stimulating hormone (TSH) and the thyroid hormone T3 level are unaffected, such that hypothyroidism is not clinically or even sub-clinically diagnosed. In such situations the level of maternal T4 may not be sufficient for the appropriate neurological development of the foetus (Melse-Boonstra and Jaiswal, 2010).

A number of observational studies from iodine sufficient or mildly iodine deficient areas of USA, Russia, The Netherlands, Italy and Spain have shown a significant association between mild maternal thyroid deficiency and cognitive impairment in children. The tests that were reviewed in these studies included the WISC, Neonatal Behavioral Assessment Scale, Bayley Scale of Infant Development, McCarthy Scales of Children's Abilities and the Gnome Mental Development Scale (Haddow et al., 1999; Pop, 2001; Pop et al., 2003; Vermiglio et al., 2004; Riano Galan et al., 2005; Kasatkina et al., 2006; Kooistra et al., 2006). By contrast, one study did not find any relationship between maternal T4 levels and cognitive development in children at 6 months (visual recognition memory) and 3 years of age (PPVT and Wide Range of Visual Motor Ability). However, this study included only a very small number of women with abnormal thyroid function (Oken et al., 2009). Berbel et al. (2009) carried out a trial in Spain that showed better gross and fine motor coordination and socialization (Brunet-Lezine Scale) in 18-month-old children whose mothers were supplemented with iodine from early pregnancy, compared to those who took the supplement from late pregnancy. Velasco et al. (2009) also found that those infants whose mothers took daily iodine supplements from the first trimester of pregnancy exhibited better psychomotor development (measured on the Bayley Scales of Infant Development), compared to those whose mothers were not supplemented with groups evaluated at different ages (5.5 and 12.4 months, respectively). Contrary to expectation, another study from Spain reported lower psychomotor development (measured on the Bayley Scale of Infant Development) in infants (especially in girls) born to mothers with maternal multivitamin supplementation that contained high amounts of iodine (100–149 μg/day), when compared to those infants with lesser amounts of maternal iodine supplementation (<100 μg/day) (Murcia et al., 2011). It is possible that the optimum dose of iodine for those mothers who are manifesting only mild iodine deficiency is less; further research is needed to determine the safe level of iodine intake for mildly deficient pregnant women.

The vast number of studies on the iodine status and supplementation in children and its relationship to cognitive development in mild-moderate iodine deficient areas of the world has been reviewed several times. An earlier review and meta- analysis of 18 studies found a 13.5 point difference in IQ between iodine sufficient and iodine deficient children (Bleichrodt and Born, 1994). Other reviews reported that most observational studies on iodine deficient children found some degree of cognitive impairment (when compared to children from iodine sufficient areas), and iodine supplementation trials in school age children have provided some promising results with respect to improvement of some cognitive processes (Zimmermann, 2007, 2011; Melse-Boonstra and Jaiswal, 2010). More recent iodine supplementation trials from Albania and New Zealand found that supplementation of mildly iodine deficient 10–13 years old children improved matrix reasoning in both studies. In addition, fine motor skills and visual problem solving were improved in the Albanian trial (Zimmermann, 2006; Gordon et al., 2009). Relatively few studies have been conducted in very young children to support the significance of iodine in cognitive development (Melse-Boonstra and Jaiswal, 2010).

In conclusion, the above literature suggests that iodine is important for the cognitive development of older children. Furthermore, although iodine supplementation is critical for severely iodine deficient pregnant women, there is no general consensus about the effectiveness of iodine supplementation during pregnancy in countries with mild iodine deficiency.

Multivitamin and mineral supplementation

Although it is important to investigate nutrients individually, deficiencies of nutrients rarely occur in isolation, and an inadequate diet typically causes multiple micronutrient deficiencies. In addition, nutrients interact with each other and do not work separately (Benton, 2010a). Thus, it is important to investigate the association between multiple mineral and vitamins supplementation or deficiencies and cognitive development.

A recent systematic review of prenatal maternal micronutrient supplementation and children's cognitive and psychomotor development considered 18 studies, including six multiple-micronutrient supplementation trials. This review found some evidence that multivitamin and mineral supplementation might positively influence certain aspects of brain development in children (Leung et al., 2011). The review included six trials on multiple-micronutrient supplementations conducted in Peru, rural Taiwan, Tanzania (on HIV infected mothers), and in rural China, Indonesia and Bangladesh, where mothers were poorly nourished (Joos et al., 1983; Schmidt et al., 2004; McGrath et al., 2006; Tofail et al., 2008; Li et al., 2009; Caulfield et al., 2010). A very recent randomized controlled trial in Indonesia found that multiple micronutrient supplementation in undernourished pregnant mothers resulted in improved motor development, visual attention and spatial ability in pre-schoolers (Prado et al., 2012). All the above-mentioned trials are from low income countries, it is currently unknown whether the cognitive development of children of well-nourished mothers from higher income countries would benefit from multiple-micronutrient supplementation.

More consistent results from trials supplementing children with multiple-micronutrients have been shown. A meta-analysis investigated 20 randomized controlled trials published from 1970 to 2008 in developed as well as developing countries, and found that multiple-micronutrient supplementation may result in higher fluid intelligence (Eilander et al., 2010). However, this increase was only marginal, and no association was observed with crystallized intelligence in children. The finding of this review (i.e., that fluid intelligence, but not crystallized intelligence, may be influenced by multiple-micronutrient supplementation) is consistent with conclusions drawn from other studies (Benton, 2001, 2012). Fluid intelligence refers to reasoning ability that reflects the individual's current neurological potential (indexed by measures such as speed of processing)

rather than their level of past attainment and acquired, crystallized knowledge (which is measured by abilities such as depth of vocabulary). Fluid ability is typically measured via non-verbal cognitive tests, while crystallized intelligence is more usually measured by administering verbal cognitive tests (Eilander et al., 2010; Benton, 2012).

OVERALL DIET, FOOD, AND COGNITIVE PERFORMANCE

Breastfeeding

A considerable amount of literature has been published on the possible connection between breastfeeding and cognitive development. Many of these studies demonstrate significantly positive associations between the two; however, the associations typically diminish or are no longer significant after controlling for confounders including maternal IQ, which is believed to be the strongest predictor of children's intelligence (Rey, 2003; McCann and Ames, 2005; Michaelsen et al., 2009). Furthermore, it remains unclear whether the remaining, diminished associations between breastfeeding and child cognitive development are further confounded by factors that have not been controlled for (Michaelsen et al., 2009). A meta-analysis of 20 studies undertaken in the late 1990's found that breastfeeding in normal birth weight infants increased IQ by 2.7 points and in low birth weight children by 5.2 points, but only six of the studies controlled for maternal IQ (Anderson et al., 1999). Three critical reviews conducted in the early 1990's concluded that the evidence linking breastfeeding and cognitive development has not yet been comprehensively demonstrated (Drane and Logemann, 2000; Jain et al., 2002; Rey, 2003). However, a more recent review by Michaelsen et al. (2009) concluded that the majority of studies found an association between breastfeeding and cognitive development, even after adjusting for confounders, and the difference in IQ related to breastfeeding is around 2–5 points at any age. This finding is supported by a large randomized control trial, where breastfeeding mothers were randomized into a breastfeeding promotion intervention trial that resulted in a higher breastfeeding rate up to 12 months after birth (43.3 vs. 6.4%). Intelligence tests were conducted at age 6½ years on the children in both groups (i.e., intervention vs. control) and associations between longer exclusive breastfeeding and improved cognitive development were found (Kramer et al., 2008). As noted earlier in this review, it has been suggested that one of the reasons behind the advantage of breastfeeding over formula feeding concerns the concentration of LCPUFA in breast milk, especially DHA (Michaelsen et al., 2009).

More recently, some studies have directly examined the effect of breastfeeding on brain development and structure. A study by Kafouri et al. (2012) reported that longer breastfeeding duration is positively associated with cortical thickness in the parietal lobe in adolescents, and in the same study they also found an association between intelligence (measured on WISC) and longer breastfeeding after adjusting for relevant confounders, which included maternal education. Herba et al. (2012) used cranial ultrasound in 2 months old infants; those infants who were breastfed exclusively had larger gangliothalamic diameter and head circumference, and smaller ventricular volume compared to bottle fed babies. Furthermore, breastfeeding has been associated previously with not only higher IQ (measured on the WISC) in adolescents but with increased white matter volume, especially in boys (Isaacs et al., 2010).

In summary, the debate concerning whether breastfeeding and cognitive development have a positive association appears to continue, but with more advanced neuroimaging technologies now available, future research may offer greater insights. Nevertheless, as Gökçay (2010) pointed out, breast milk provides the best nutritional intake for infants, regardless of its putative association with cognitive development.

Malnutrition

The number of malnourished (undernourished) children continues to rise in some regions, such as in Sub-Saharan Africa (De Onis, 2000). Every year, 20 million newborns (15.5% of all births) are low birth weight, most of them from developing countries (United Nations Children's Fund and World Health Organization, 2004). The effect of malnutrition on brain structures has been extensively researched in animal models. Malnutrition appears to alter cell numbers, cell migrations, myelinisation, synaptogenesis, hippocampal formation and neurotransmission in rats (Debassio et al., 1996; Mathangi and Namasivayam, 2001; Granados-Rojas et al., 2002; Alamy and Bengelloun, 2012). In a human study, researchers described fewer numbers of neurons with shorter dendrites and abnormal dendritic spines in individuals with malnutrition; however, this study was carried out just on 13 severely undernourished infants, compared to seven adequately fed babies (Benítez-Bribiesca et al., 1999). Because of small sample size, this study cannot provide definitive evidence of the effect of malnutrition on brain structure (although the fact that significant differences were observed even with a relatively small number of participants is potentially revealing, in terms of statistical power considerations). Moreover, malnourished children have less energy and interest for learning that negatively influences cognitive development (Engle, 2010).

Malnutrition can develop *in utero*, when the mother is malnourished (as often happens in low income countries). In Western countries, restricted foetal growth is often the result of a medical condition such as severe hypertension, or if the mother consumes higher levels of alcohol (Henriksen and Clausen, 2002; Feldman et al., 2012; Mustafa et al., 2012). For example, in uncontrolled severe hypertension during pregnancy the placental blood flow is restricted and there are placental abnormalities, which may prevent the foetus from obtaining the required oxygen and nutrients for development (Henriksen and Clausen, 2002). It has been shown that intrauterine growth retardation (IUGR) or small-for-gestational age (SGA) at birth is associated with cognitive developmental delays and a decrease of 4–8 points in IQ scores compared to infants with a birth weight that is appropriate-for-gestational-age (AGA; Pallotto and Kilbride, 2006). Apart from IUGR, stunting can be caused by a nutritional deficit (such as protein-energy malnutrition) during the rapid growth of young children. Most often intrauterine malnutrition is followed by poor postnatal nutrition, and the combined and persistent effect of malnutrition across both periods results in seriously stunted growth (Dewey and Begum,

2011). Indeed, evidence from developing countries shows that stunting in early childhood is associated with poorer cognitive development and academic performance in later childhood (Grantham-McGregor, 1995; Grantham-McGregor et al., 2007). A recent review concluded that even mild but persistent malnutrition in early life (i.e., during the first 2 years of life) negatively influences reasoning, visuospatial functions, IQ, language development, attention, learning, and academic achievement, while supplementation with food can improve cognitive performance (Laus, 2011). In an interesting study, researchers randomly assigned 425 preterm infants to a "standard nutrient" group (who received either breast milk or standard formula) and "high nutrient" group (who received a higher protein-energy and micronutrient diet). The cognitive development of the children was then measured at 7½–8 years of age, and it was found that IQ (measured on the WISC) was higher in the high nutrient group, especially with respect to verbal IQ in boys (Lucas et al., 1998). A subgroup of these children ($n = 76$) was assessed again at 16 years, and a persistent effect of the high nutrient diet on verbal IQ was demonstrated. At this stage, brain MRIs were also undertaken and showed a larger volume of the caudate (which was correlated with higher verbal IQ), but only in males (Isaacs et al., 2008, 2009).

Obesity is a special form of malnutrition (overnutrition, as opposed to undernutrition which has been previously considered here), because the diet is likely to have low nutrient-density in conjunction with a high fat and carbohydrate content (Tanumihardjo et al., 2007). Obesity is of growing concern worldwide, with the number of overweight and obese children dramatically increasing over the past two decades. It was estimated in 2010 that 43 million children worldwide (including 35 million from developing countries) were overweight or obese, and this number is expected to continue to grow to 60 million by 2020 (De Onis et al., 2010).

Animal studies suggest that there may be a biological link between obesity and impaired cognitive performance that is related to insulin resistance and altered glucose metabolism (Jurdak et al., 2008). Furthermore, when rats were fed a high fat diet, it decreased neurogenesis in the hippocampus (Lindqvist et al., 2006). In addition, a maternal high-fat diet in mice altered the development of hippocampus in the foetus (Niculescu and Lupu, 2009), which may mediated by a decrease in the level of brain-derived neurotrophic factor (Molteni et al., 2002). A recent literature review concluded that overweight and obesity may result in poorer academic performance (measured as literacy, numeracy, and school grades; Burkhalter and Hillman, 2011), but only a few studies have researched a possible connection between obesity/overweight and cognitive performance. Li et al. (2008) described an association in 8–16-year-old children and adolescents between increased body mass index (BMI) and reduced cognitive performance, specifically visuospatial functioning as measured on the block-design test (a subtest of the WISC), but not attention, working memory (digit-span subtest) and academic performance (Wide Range Achievement Test). This association remained after adjusting for a range of covariates and potential obesity mediating factors. Palermo and Dowd (2012) did not find a similar association between increased body weight and cognitive performance as measured by the Woodcock Johnson Revised Test of Achievement and the Memory for Digit Span test. Bisset et al. (2012) examined weight status trajectories in 4–7-year-old children; overweight was not associated with cognitive outcomes as measured on the Kaufman's Assessment Battery for Children. The inconsistent findings in humans between obesity and cognitive development may be the result of the complexity of factors underlying these outcomes (Li et al., 2008). Moreover, associations between obesity and cognition that have been reported may be mediated by sociodemographic factors that include discrimination and isolation rather than through biological mechanisms (Palermo and Dowd, 2012).

Breakfast

The level of glucose metabolism in children's brains increases from birth until 4 years of age, reaching twice that of the adults' metabolic rate. This rate of glucose metabolism in children remains elevated until 9–10 years of age, before it declines to the adult level by late adolescence (Chugani, 1998). Therefore, regular meals and continuous glucose supply (to provide the brain with the required glucose for its high metabolism) is more important in children than in adults (Bellisle, 2004). Accordingly, children are more prone to the adverse effect of overnight fasting, and breakfast is a very important meal to provide fuel to the brain in the morning (Bellisle, 2004). A systematic review concluded that having breakfast is beneficial for cognitive function and development, especially in malnourished children. A lack of studies conducted into the optimal breakfast including type, composition and portion size exists but (Hoyland, 2009) carbohydrate rich, low-glycaemic food for breakfast that provides a continuous supply of glucose is known to facilitate better cognitive performance (Bellisle, 2004; Ingwersen et al., 2007; Micha et al., 2011).

A recent study showed that the brain's gray and white matter volume differed in various parts of healthy children's brain, according to the type of breakfast they ate (Taki et al., 2010). The researchers suggested that the difference may be due to the different glycemic index of the different breakfast staple types. The authors also proposed that if different breakfast types affect gray and white matter volume in the brain, they may in turn influence cognitive function. Therefore, the type of breakfast children eat can potentially have a long-term influence on cognitive development (Taki et al., 2010).

Dietary pattern and diet quality

Since individuals consume combinations of foods (which may contain other bioactive compounds that could act synergistically or antagonistically within or between food groups; Tangney and Scarmeas, 2011), it is important to investigate diet as a broadly encompassing variable in association with cognitive outcomes. Furthermore, as Tangney and Scarmeas (2011) state, if research shows an association between diet (as comprehensively measured) and better health outcomes, it may be easier to implement changes in terms of dietary interventions.

Some researchers have investigated the influences of overall diet on neurocognitive development during childhood. Gale et al. (2009) considered dietary patterns in infancy in relation to cognitive development and found higher full-scale IQ (measured on the Wechsler Preschool and Primary Scale of Intelligence test) at 4 years of age in children who consumed higher amounts of fruit, vegetables and food prepared at home during infancy (i.e., between 6 and 12 months). The association remained significant after adjusting for a wide range of factors, including socioeconomic status, maternal IQ and education. A cross-sectional study reported by Theodore et al. (2009) examined the association between (i) the intake of specific food groups in 3½ years old children and in the same children again at 7 years of age and (ii) their cognitive development measuring on the Stanford–Binet Intelligence Scale (at 3 years) and on the WISC-III (at 7 years). These researchers found that a higher level of consumption of fish at 7 years of age and bread and cereals at 3½ years of age was associated with higher IQ scores, whereas those children at the age of 3½ who consumed margarine every day scored significantly lower on IQ. Northstone et al. (2011) reported that higher scores on the "health conscious" dietary pattern (which included more salad, rice, pasta, and fruits) at 3 years of age were associated with higher IQ score on the WISC-III when these same children were tested aged 8½ years, compared to those children on the "processed" dietary pattern (with high fat and sugar content), after adjusting for a wide variety of potential confounders. In the same study, Smithers et al. (2012) examined six different dietary patterns and found negative associations between (i) the "discretionary pattern" (which contains biscuits, sweets, soft drinks, and snacks) at 6, 15, and 24 months of age, and ready-prepared baby foods at 6 and 15 months of age and (ii) IQ scores at 8 years of age (measured on the WISC). Smithers et al. (2012) also reported positive associations between children's IQ at age 8 years and "breastfeeding pattern" (measured at 6 months), "home-made contemporary" (legumes, fruits, fruit juices, cheese, egg) at 15 and 24 months, "home-made traditional" (vegetables, meat, sauces) at 6 months (but not at 15 and 24 months), and "ready-to-eat" food pattern (biscuits, breads, cereals, yoghurt) at 24 months.

CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

The majority of studies, which have investigated the association between nutrition and cognitive development, have focused on individual micronutrients, including omega-3 fatty acids, vitamin B12, folic acid, zinc, iron, and iodine. The evidence is more consistent from observational studies, which suggest these micronutrients play an important role in the cognitive development of children. However, the results from intervention trials of single nutrients are inconsistent and inconclusive, prompting the need for better controlled and more adequately powered studies in the future. It is plausible that children living in poor countries may encounter more multiple micronutrient deficiencies, as opposed to children living in rich countries who are reasonably well nourished (and where a small deficiency in one nutrient may not result in measurable, long-term change in cognitive outcomes, due to compensation over time). These are important considerations, because nutrients do not act alone; rather, they have in some contexts synergistic and in other contexts antagonistic effects with each other.

Individuals consume combinations of food and poor overall diet can cause multiple macro-and micronutrient deficiencies and imbalances. If an overall healthy diet synergistically enhances cognitive development in children, then public health interventions should focus on the promotion of overall diet quality rather than isolated micronutrients or dietary components consumed by children and adolescents.

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Nutrition as an important mediator of the impact of background variables on outcome in middle childhood

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Adequate nutrition is fundamental to the development of a child's full potential. However, the extent to which malnutrition affects developmental and cognitive outcomes in the midst of co-occurring risk factors remains largely understudied. We sought to establish if the effects of nutritional status varied according to diverse background characteristics as well as to compare the relative strength of the effects of poor nutritional status on language skills, motor abilities, and cognitive functioning at school age. This cross-sectional study was conducted among school-age boys and girls resident in Kilifi District in Kenya. We hypothesized that the effects of area of residence, school attendance, household wealth, age and gender on child outcomes are experienced directly and indirectly through child nutritional status. The use of structural equation modeling (SEM) allowed the disaggregation of the total effect of the explanatory variables into direct effects (effects that go directly from one variable to another) and indirect effects. Each of the models tested for the four child outcomes had a good fit. However, the effects on verbal memory apart from being weaker than for the other outcomes, were not mediated through nutritional status. School attendance was the most influential predictor of nutritional status and child outcomes. The estimated models demonstrated the continued importance of child nutritional status at school-age.

Keywords: nutritional status, school-age children, structural equation modeling, direct and indirect effects, co-occurring risk factors, cognitive outcomes

INTRODUCTION

While the literature provides evidence that the negative effects of early malnutrition persist to school-age (Pollitt et al., 1996), there are several significant knowledge gaps. First, despite evidence that the impact of nutrition varies across different neurocognitive domains, there have been few studies investigating this area, especially in middle childhood. And yet at school age, children are exposed to more differential experiences and acquire more sophisticated abilities across various cognitive domains (Fischer and Bullock, 1984). Second, there is a complex inter-related relationship between poverty, nutritional status and neurocognitive outcomes. Not only do the constraints of low income in deprived settings create practical barriers to good nutrition; additional socio-environmental factors reinforce the effects of this deprivation (Engle and Black, 2008). Poor nutritional status at this age may have long-term negative consequences and restrict development of a child's full potential. This is therefore a critical period for investigating the link between malnutrition and developmental outcomes, especially within a multiple risk context.

In many developing countries, particularly in sub-Saharan Africa, linear growth retardation, or stunting, a manifestation of chronic protein-energy malnutrition (PEM), is highly prevalent, with rates as high as 38% (de Onis et al., 2012). Various individual and environmental variables have been associated with an elevated risk of experiencing poor nutritional status. Important differences have been highlighted in the prevalence of stunting among boys and girls (Badenhorst et al., 1993; Lwambo et al., 2000; Semproli and Gualdi-Russo, 2007; Acham et al., 2008; Omigbodun et al., 2010; Goon et al., 2011; Senbanjo et al., 2011) although there are substantial variations in regional trends. Moreover, patterns observed among school-age populations are similar to those reported at younger ages (Wamani et al., 2007). With regard to age, several studies have reported a dramatic increase in stunting among older children (Stoltzfus et al., 1997;

Lwambo et al., 2000; Goon et al., 2011; Senbanjo et al., 2011) demonstrating that linear growth continues to falter throughout the school-age years (The Partnership for Child Development, 1998). Mendez and Adair (1999) found that children who started school at earlier ages (5 or 6 years) were substantially taller than children who started school later (7 or 8 years) so it may be that better-off children enrol in school at earlier ages. And although children in low income settings may all suffer the effects of deprivation, those from the least wealthy households in low income settings are more likely to be malnourished (Sigman et al., 1989; Brooks-Gunn and Duncan, 1997; Bradley and Corwyn, 2002; Abubakar et al., 2008; Ndukwu et al., 2013). Rural residence (Hautvast et al., 2000; Nabag, 2011) and a reduced likelihood of attending school (Ivanovic et al., 2012) have also been related to poor nutritional status. Over childhood, these risk factors have been known to alter the profile of undernutrition (protecting against or accentuating the risk of undernutrition) in a population (Pollitt et al., 1996), as well as being recognized as adversely affecting cognitive functioning independently of nutritional status.

Undernutrition has been shown to negatively impact on various developmental and cognitive domains including motor development (Pollitt et al., 1994; Chang-Lopez, 2007; Olney et al., 2007), language functioning (Wachs, 1995; Duc, 2009), IQ (Mendez and Adair, 1999) as well as memory and executive functions (Kar et al., 2008). This latter study observed that malnourished children showed poor performance on tests of higher cognitive functions but not on motor performance. Moreover, the impact of malnutrition on specific skills seems to vary according to diverse child-related and environmental variables. For instance, among the various gender-patterned deficits documented through an Indian study (Bhandari and Ghosh, 1980), malnutrition affected a wider range of aspects of immediate memory of boys than that of girls.

The effects and outcomes of nutritional status are correlated with environmental factors, the most salient of which is socioeconomic status (Bradley and Corwyn, 2002). Low SES leads to poor dietary intake which in turn impacts on brain and mental development eventually causing developmental deficits. School attendance has also been associated with better cognitive scores among both stunted and non-stunted children (Mendez and Adair, 1999). And as we have reiterated earlier on, rural children have a substantially higher risk of poor nutrition (Fox and Heaton, 2012) as well as poor cognitive outcomes.

In recent times, there have been efforts to investigate the complex relationship between background variables, nutritional status and developmental outcomes (Wachs, 1995). And in Kenya, a recent study investigated the direct and indirect effects of economic poverty on child outcomes (Abubakar et al., 2008). The results suggested that in infancy, impaired psychomotor development is associated directly with undernutrition, while the effect of poverty is mediated entirely through nutritional status (Abubakar et al., 2008). These results are similar to what had been earlier reported from Indonesia where nutritional influences mediated the relationship between poverty-related variables (e.g., SES) and child outcomes (Pollitt et al., 1994). As far as our literature search has revealed, the majority of studies exploring the relationship between undernutrition, co-occurring risk factors and other aspects of impaired child outcome has largely concentrated on children under the age of 5 years (Kariger et al., 2005; Abubakar et al., 2008; Olney et al., 2009, 2007; McDonald et al., 2013). We would like to build up on earlier work and extend the lines of research by focussing on school-age children.

Given the co-occurrence of malnutrition and multiple risk factors within this setting, are the adverse effects of these variables on neurocognitive outcomes related to their impact on nutritional status? Based on a model modified from Wachs (1995), we hypothesized that, (a) sociodemographic and biological factors make a unique contribution to nutritional status, and, (b) nutritional status is a strong predictor of various outcomes in school-age children. Because cognitive skills are more differentiated at this stage, we were able to explore the relationship between chronic malnutrition and developmental outcome across several outcomes. To delineate these effects and to investigate these relationships simultaneously required advanced statistical modeling. The main aim of this study was therefore to establish if diverse background characteristics created variations in nutritional status. We also sought to compare the relative strength of the effects of poor nutritional status on language skills, motor abilities, and cognitive functioning at school age. This information will enable the identification of points of intervention for those most at risk.

MATERIALS AND METHODS

The study was cross-sectional in nature.

STUDY SETTING

The study was conducted in Kilifi District, Kenya, among a predominantly rural community. The majority (66.8%) of the population lives below the poverty line and is therefore unable to access basic needs due to geographical, economic, and sociocultural barriers (Kahuthu et al., 2005). The district is a food deficit region relying on trade with other districts to meet the food gap—however, income-generating opportunities are few and unsustainable (FAO Kenya, 2007). Malnutrition remains rampant due to variability in crop production; and high illiteracy levels increase the population's vulnerability to food insecurity [Kenya National Bureau of Statistics (KNBS) and ICF Macro, 2010].

STUDY SAMPLE

Children between the ages of 8 and 11 years were recruited from the catchment areas of five local primary schools distributed across neighborhoods ranging from sparsely populated rural areas to more densely populated semi-urban areas. The total sample of 308 children comprised both schooling and non-schooling children. Their first language was Mijikenda, the local vernacular or Kiswahili, the lingua franca and national language.

The Ten Questions Questionnaire (Mung'ala-Odera et al., 2004) was administered to parents to determine the presence of any impairments or serious health problems in children. When the parent was not able to determine if the child had any impairments (visual, auditory, or motor) or in cases where only milder concerns were reported, testing was attempted. Children who were physically unable to perform the tasks were excluded.

ETHICAL CONSIDERATIONS

The Kenya Medical Research Institute/National Ethics Review Committee (KEMRI/NERC) provided ethical clearance for the study. Permission to visit schools was obtained from the District Education Office. We explained the purpose of the study to the head teachers of selected schools and then sought their permission to recruit children. We also held meetings with community leaders, elders, and parents (and guardians) of selected pupils to explain the purpose of the study. After each meeting, a screening questionnaire was administered to establish if selected children met the study's eligibility criteria. We presented information on the study to parents in the language with which they were most familiar. We then obtained written informed consent for their children's participation. All the selected children assented to their participation in the study.

MEASUREMENT OF VARIABLES

Building on the extant research literature, our analysis included age, gender, area of residence, school attendance and household wealth as underlying biological and environmental influences, nutritional status as a mediating variable and language skills, motor abilities and two factor scores of cognitive function as child outcomes. In order to test the various hypothesized relationships, we developed the model presented in **Figure 1**.

In the full model which included all the explanatory variables, the use of structural equation modeling (SEM) allowed the disaggregation of the total effect of the explanatory variables into direct effects (effects that go directly from one variable to another) and indirect effects (effects between two variables that are mediated by at least one intervening variable) (Bollen, 1989). We hypothesized that the effects of area of residence, school attendance, household wealth, age, and gender on child outcomes are experienced directly. Additionally, we hypothesized that the influence of these variables has an indirect effect on child outcomes through their influence on nutritional status. The model also took into account possible correlations among the five background variables. We fitted separate models for language skills, motor abilities, verbal memory, and executive function to see if there were differences among the four child outcomes.

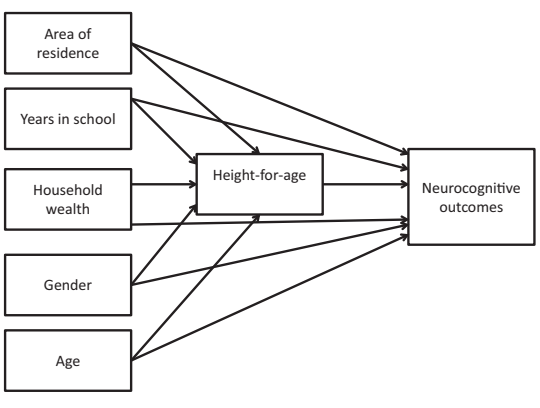

FIGURE 1 | Hypothesized model for testing the mediating influence of nutritional status on child neurocognitive outcomes.

Background characteristics

Information on child gender, age, school attendance (number of years that child has attended school), and household wealth was collected using a standard questionnaire. Birth records were used, where available, to confirm the child's date of birth. In the cases where records were not available, the procedure outlined by Kitsao-Wekulo et al. (2012) was followed. For the purpose of this study, an age variable in 6-month increments was created. An index of household wealth that divided the sample into three approximately equal groups—least wealthy (Level 1), moderately wealthy (Level 2), and the most wealthy (Level 3)—was derived from six socioeconomic indicators: maternal and paternal education, maternal, and paternal occupation, type of windows in the child's dwelling and ownership of small livestock. Area of residence was characterized as rural or peri-urban according to the most common settlement within the school catchment area.

Mediating factor

Children's heights were measured to the nearest centimetre using a stadiometer and height-for-age indices were calculated using EpiInfo (Centers for Disease Control, Atlanta, GA). Growth retardation was defined as height that was more than 2 standard deviations below levels predicted for age according to the World Health Organization reference curves for school-aged children (World Health Organization, 2007).

Child outcomes

A battery of neuropsychological tests was used to assess children's language skills, motor abilities, and cognitive functioning.

Language skills. The Kilifi Naming Test (KNT), a test of confrontation naming, was used to assess expressive vocabulary (Kitsao-Wekulo et al., in preparation). In the KNT, the child was asked to spontaneously give one-word responses when presented with a black and while line drawing of a familiar object. Correct responses were coded "1." A stimulus cue was provided when no response was given, the child stated that they did not know the name of the item or the item was perceived incorrectly. If the child did not provide a correct response after the stimulus cue, the word that was provided was recorded verbatim. The test was discontinued after six incorrectly named consecutive items. The final score was calculated by summing the number of spontaneously correct items and the number of correct items following a stimulus cue. These scores were standardized enabling the direct comparison of children's performance across tests.

Motor abilities. Children's motor abilities were assessed using five tests of gross motor abilities covering two areas of motor performance—static and dynamic balance—and three timed tests of fine motor coordination and manual dexterity (Kitsao-Wekulo et al., under review). Age-corrected scores were obtained by computing differences between observed and predicted scores in units of standard error of the estimate (i.e., in z-score units). Maximum likelihood factor analysis with oblique rotation was then applied to the z-scores to reduce the multiple motor scores to ability composites (Ackerman and Cianciolo, 2000). Factor analysis yielded

support for a two-factor solution; four tests loaded on the Motor-Co-ordination factor while the remaining four tests loaded on the Static and Dynamic Balance factor. Factor scores were defined as the mean of the z-scores for the tests loading on each factor. An Overall Motor Index was defined as the mean of the two factor scores.

Cognitive functioning. We administered eight tests of cognitive functioning. These included:

(a) a non-verbal Tower Test of executive function to measure problem-solving and planning ability;
(b) the Self-Ordered Pointing Test (SOPT) to assess verbal/visual selective reminding in terms of the capacity to initiate a sequence of responses, retain the responses and monitor the consequences of behavior;
(c) Verbal List Learning (VLL) in which five serial verbal presentations of a 15-item word list were used to test learning and working memory;
(d) Dots, a non-verbal test of memory where the child was required to point at a special dot on a sheet;
(e) a Contingency Naming Test of executive function designed to assess response inhibition, attentional shift and cognitive flexibility;
(f) Score, a test of auditory sustained and selective attention in which the child was required to place beads on one of two plates only after a special sound was heard from a cassette tape;
(g) the People Search, a test of visual sustained and selective attention in which the child was required to cross out compete figures as quickly as possible on a stimulus sheet comprising complete and incomplete stick figures;
(h) the Coloured Progressive Matrices (CPM) in which matrices of abstract patterns with a missing piece were presented and the child was required to complete the pattern with one from a choice of four pieces. This test assessed non-verbal reasoning and was administered to rule out impairment in global mental functioning.

A detailed description of the tests is presented elsewhere (Kitsao-Wekulo et al., 2012).

To reduce the test battery to a smaller set of ability composites, z-scores for each measure were subjected to principal component factor analysis with Varimax rotation. Based on factor content, skill composites were labeled Executive Function and Verbal Memory. Skill composites of the z-scores comprising each factor were computed based on factor weightings.

DATA COLLECTION PROCEDURES

All the tests were administered at a school near the child's home. Each child was tested individually in a quiet area within sight of other children, and in familiar surroundings to minimize test anxiety. Observations by the assessors suggested that none of the children was unduly anxious during the test sessions.

DATA ANALYSIS

Independent samples *t*-tests, Chi-square tests and univariate analysis were undertaken to determine group differences in nutritional status and outcomes. Pearson product-moment correlation coefficients were used to examine the relationship between the background variables and cognitive outcomes, language skills, motor abilities, and nutritional status. AMOS version 20 (SPSS) was used to test the fit of the overall model and to examine the relationships among the variables. SEM was used to examine the relationships between background characteristics, child nutritional status and child outcomes. We developed and tested a path analysis model (**Figure 1**) based on logic and theory about how background variables co-vary with nutritional status, and how they influence child outcomes directly and indirectly. In the full model which included all the explanatory variables, this format allowed us to test the mechanisms through which each of the background variables influenced various child outcomes directly and indirectly though a mediated path. An independent disturbance term that represented unexplained variance was estimated for each endogenous variable.

In fitting the Structural Equation Models, missing information was taken into account using the Maximum Likelihood (ML) Estimates. The ML technique assumes data are missing at random for continuous, binary, and categorical variables. All direct and indirect paths were tested and each of the four child outcomes was analyzed in isolation. Specific procedures for model development were to remove non-significant paths ($p = 0.05$) and use modification indices as suggested by the AMOS SEM program (Arbuckle, 1988) to add paths or correlations that would improve model fit. Chi-square analysis was conducted in initial examination of the goodness of fit to insure non-significance. However, because this method is sensitive to sample size, other indices of goodness of fit included the Tucker Lewis Index (TLI), Comparative Fit Index (CFI), and Root Mean Square Error of Approximation (RMSEA) (Bentler and Chou, 1987; Browne and Cudeck, 1993). Acceptable fit was defined as TLI and CFI >0.90 and RMSEA <0.08 and an excellent fit as TLI and CFI >0.95 and RMSEA <0.05.

RESULTS

DESCRIPTIVE STATISTICS

The study involved 308 boys and girls. The prevalence of linear growth retardation in this study population was high. Approximately 24% ($N = 74$) of all the children were stunted. **Table 1** portrays a summary of the sample characteristics. The proportion of stunted children residing in rural areas was significantly higher than that of their counterparts in peri-urban areas, χ^2 $(1, N = 308) = 4.12$, $p = 0.04$. A higher proportion of girls than boys was stunted but these differences were not significant, χ^2 $(1, N = 308) = 1.48$, $p = 0.22$.

More than one-third of the oldest children (aged 9.5 years or more) compared to 15.3% in the youngest group (aged 8 years or less) and 17.6% among those aged between 8.5 and 9 years were stunted. These differences were significant, χ^2 $(2, N = 308) = 12.98$, $p = 0.002$. Among children who did not attend school, a very high proportion was stunted compared to their counterparts who had attended school for at least 1 year and those

with more than 2 years of school exposure. These differences were highly significant, χ^2 (2, $N = 308$) = 32.89, $p < 0.001$. In terms of household wealth, the highest proportion of stunted children was found among those in the sample who were least wealthy (Level 1). The differences in prevalence of stunting among the three groups were significant, χ^2 (2, $N = 308$) = 7.85, $p = 0.02$.

CORRELATIONS

Variable intercorrelations are presented in **Table 2**. As can be seen from the table, more schooling and higher age were the most frequently correlated with household wealth, stunting, and child outcomes. These correlations provide some initial evidence that school attendance and age have moderate to strong associations with nutritional status, which in turn is associated with children's language functioning and motor skills.

Table 1 | Description of sample characteristics, *N* = 308.

Variable	Stunted		Not stunted	
	N	%	*N*	%
GENDER				
Boys	31	20.9	117	79.1
Girls	43	26.9	117	73.1
AREA OF RESIDENCE				
Rural	65	26.5	180	73.5
Peri-urban	9	14.3	54	85.7
AGE (YEARS)				
≤8.0	11	15.3	61	84.7
8.5–9.0	19	17.6	89	82.4
≥9.5	44	34.4	84	65.6
SCHOOL EXPOSURE				
0 years	22	62.9	13	37.1
1–2 years	21	20.8	80	79.2
>2years	31	18	141	82
HOUSEHOLD WEALTH				
Level 1	39	31.7	84	68.3
Level 2	21	22.3	73	77.7
Level 3	14	15.4	77	84.6

DIFFERENCES IN OUTCOMES

Children who were stunted performed more poorly than their counterparts who were not stunted on all the outcomes tested (**Table 3**). These differences were significant for the tests of language, $t_{(306equalvariances} = -2.627$, $p = 0.009$, and executive function, $t_{(100unequalvariances)} = -2.490$, $p = 0.014$. (Levene's test indicated unequal variances ($F = 5.572$, $p = 0.019$), so degrees of freedom were adjusted from 306 to 100 for executive function). Medium effect sizes were seen for language and executive function tests.

MODEL MODIFICATION

For each outcome, the initial model did not have a good fit. The steps in developing the individual path models involved making several revisions by deleting non-significant paths and covariances (**Table 4**). (Non-significant paths in initial models are indicated with dashed lines). Modification indices did not suggest the need for additional paths or correlations. The final models for the four child outcomes provided a good fit to the data. In order to simplify the output, only significant standardized path coefficients are shown in the final models (**Figures 2A–D**).

Language skills

The model for language skills (**Figure 2A**) fitted well, TLI >0.99, CFI >0.99, RMSEA <0.05. School attendance and age were related directly and indirectly (through nutritional status) to language skills. While more years of being in school were associated with both better nutritional status and higher language

Table 3 | Differences in outcomes.

	Stunted (*N* = 74)		Not stunted (*N* = 234)		Cohen's *d*
	Mean	*SD*	Mean	*SD*	
Language skills	−0.26	1.09	0.08	0.95	0.333
Motor abilities	−0.06	0.72	0.03	0.57	0.140
Verbal memory	−0.03	0.89	0.01	1.03	0.042
Executive function	−0.26	1.04	0.08	0.83	0.364

Table 2 | Correlations among variables in the models.

	1	2	3	4	5	6	7	8	9
Area of residence	1								
Gender	−0.012	1							
Age	−0.025	0.019	1						
Years in school	0.313**	−0.084	0.041	1					
HAZ	0.130*	−0.006	−0.300**	0.272**	1				
Household wealth	0.135*	−0.067	−0.240**	0.391**	0.146*	1			
Language scores	0.045	−0.166**	0.318**	0.427**	0.127*	0.048	1		
Motor scores	0.060	0.074	0.402**	0.318**	0.106	0.017	0.499**	1	
Verbal memory	−0.010	0.134*	0.182**	0.125*	0.043	−0.009	0.259**	0.311**	1
Executive function	0.213**	−0.082	0.28**	0.519**	0.240**	0.107	0.554**	0.614**	0.397**

*$p < 0.05$; ** $p < 0.01$.

Table 4 | Maximum likelihood estimates of covariances for initial model.

Covariance	Covariance estimate	Standard error	Correlation estimate	*p*-value
Years in school ↔ Area of residence	0.212	0.041	0.313	<0.001
Age ↔ Household wealth	−1.049	0.257	−0.240	<0.001
Area of residence ↔ Household wealth	0.214	0.091	0.135	0.019
Age ↔ Gender	0.011	0.032	0.019	0.738
Years in school ↔ Age	0.077	0.107	0.041	0.472
Household wealth ↔ Gender	−0.132	0.112	−0.067	0.238
Years in school ↔ Gender	−0.071	0.048	−0.084	0.141
Area of residence ↔ Gender	−0.002	0.012	−0.012	0.837
Age ↔ Area of residence	−0.011	0.026	−0.025	0.657
Years In school ↔ Household wealth	2.584	0.405	0.391	<0.001

scores, associations of nutritional status and outcomes with gender and age were less consistently observed. Younger children had better nutritional status while older children had better language outcomes. Boys had higher language scores than girls. The indirect path from gender through nutritional status was not significant. Direct paths from height-for-age Z-scores to outcome indicated associations of better nutritional status with higher scores on the language test. These results suggest that the influences of school and age (but not gender) on language scores were partially mediated through nutritional status.

Motor abilities

The model for motor abilities had an excellent fit, χ^2 $(1, N = 308) = 0.519$, $p = 0.47$; TLI >0.99, CFI >0.99, RMSEA <0.05 (**Figure 2B**). Paths linking longer attendance at school and higher age with outcome suggest that these two variables were directly and indirectly associated with motor abilities. Direct paths from height-for-age Z-scores to outcome indicated associations of better nutritional status with higher scores on the motor test.

Verbal memory

The model for verbal memory had a good fit, χ^2 $(5, N = 308) = 4.45$, $p = 0.49$; TLI >0.99, CFI >0.99, RMSEA <0.05, but it explained very little of the variance observed (**Figure 2C**). School attendance, gender, and age had a direct effect on verbal memory. As with the other models, the association between gender and nutritional status remained non-significant. Gender had a small effect on outcome and this effect favored girls. The path coefficient from nutritional status to verbal memory was not significant.

Executive function

The model for executive function also had a good fit, χ^2 $(1, N = 308) = 0.519$, $p = 0.47$; TLI >0.99, CFI >0.99, RMSEA <0.05 (**Figure 2D**). School attendance and age showed strong links with executive function indicating associations of more schooling and higher age with higher scores on executive function tests. Moreover, the direct and indirect effects were significant and the path coefficient from nutritional status to executive function was higher than for all other outcomes.

DISCUSSION

Although the direct effects of poor nutritional status on child neurocognitive functioning have been well-documented in the literature, very little is known about the complexities of that relationship in a multiple risk environment. Through the use of SEM, this study has attempted to elucidate some of the pathways through which nutritional status and other contextual characteristics may influence outcome in school-age children.

The risk factors for poor nutritional status in this population included older age, rural place of residence, low household wealth levels and not attending school. That younger children had a better nutritional status than their older counterparts was not unexpected; similar findings have been reported in earlier studies among infant (Powell and Grantham-McGregor, 1985) and school-age populations (Senbanjo et al., 2011). We also found that the prevalence of stunting was higher in rural than peri-urban areas. As rural areas tend to have high concentrations of people with low education and income levels, children are more likely to suffer the effects of these deprivations, though poorer nutritional status. Fotso (2006), in an effort to compare the magnitude of inequities in child malnutrition in urban and rural areas of selected countries in sub-Saharan Africa, reported similar findings. Moreover, in the current study, children from the least wealthy households faced the greatest risk of being stunted, compared to their counterparts in the most wealthy households, corroborating earlier findings in similar resource-restricted settings (Fotso, 2006). Our finding that levels of stunting were higher among children not attending school could be explained as follows. Children from poor families are more likely to end up with poor nutritional status (Abubakar et al., 2008), and consequently, less likely to attend school (Ivanovic et al., 2012).

In turn, poor nutritional status predicted poorer outcomes on all the tests. These findings are consistent with reports from studies among infants and school-age children living in similar and different contexts (Sigman et al., 1989; Abubakar et al., 2008; Kar et al., 2008; Bangirana et al., 2009). Poor nutritional status results in a wide range of cognitive deficits linked to structural abnormalities of different parts of the brain (Kar et al., 2008). Because stunting occurs in early childhood, these results provide evidence

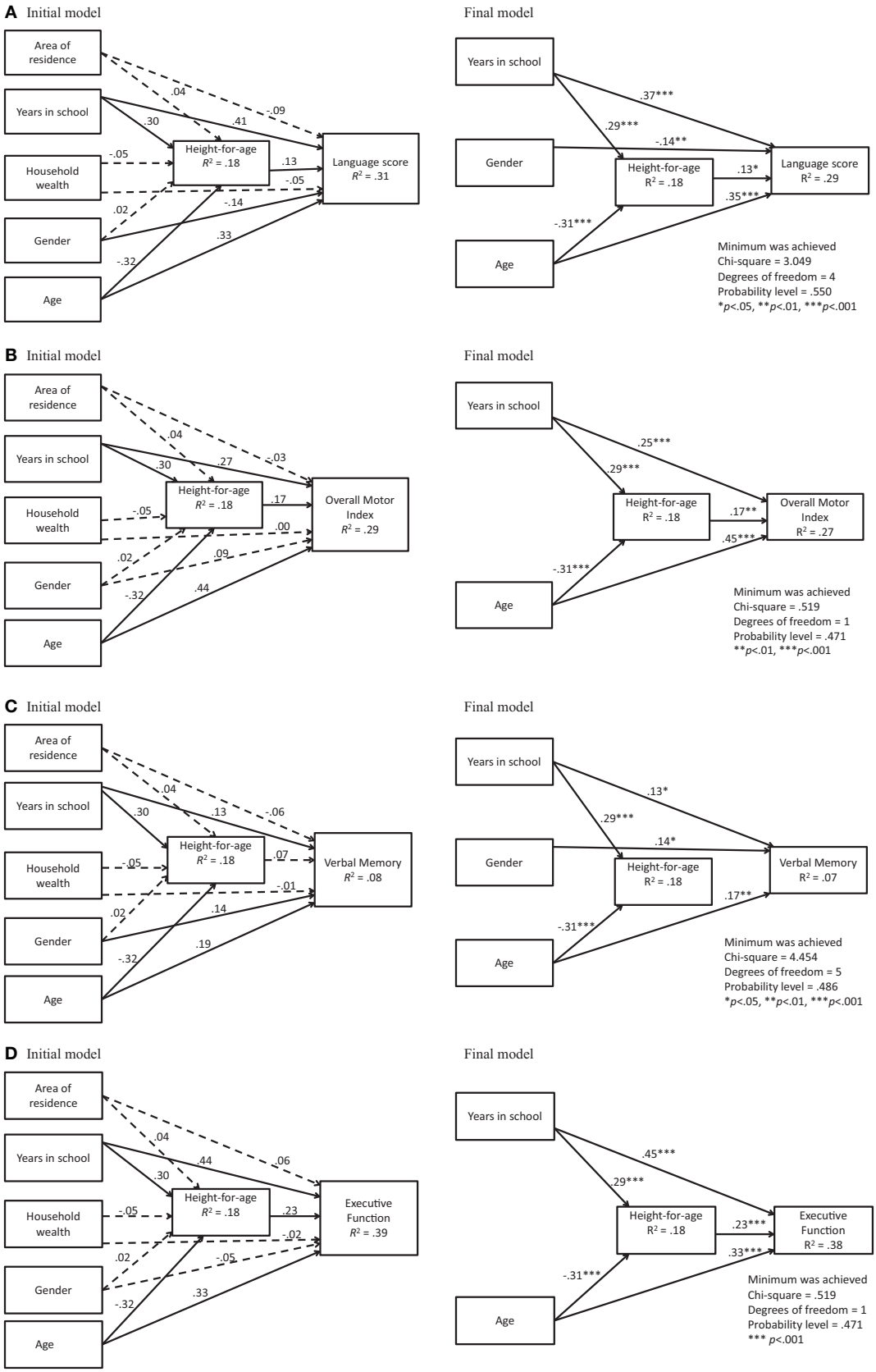

FIGURE 2 | (A) Initial and final models for language score. **(B)** Initial and final models for motor skills. **(C)** Initial and final models for verbal memory score. **(D)** Initial and final models for executive function score.

that the effects of poor nutritional status may be long-lasting, especially if appropriate interventions are not put in place.

The data show evidence for associations between background variables and nutritional status, and between nutritional status and multiple cognitive skills. As expected, the paths linking the variables to nutritional status and children's performance differed in magnitude for each outcome. The novelty, level of familiarity with and requirements of the various tasks could perhaps explain the differences observed. Mediated influences of nutritional status, as well as the direct effects of background variables were stronger for tests with a higher degree of novelty, which were less familiar and which had more complicated task requirements. For instance, the requirement to keep a shopping list in memory is a familiar common activity for school-age children. This may be a plausible explanation for the lack of sensitivity to nutritional status influences and weak direct effects observed on verbal memory.

Noteworthy in the current study is the negative relationship between age and nutritional status. Similar patterns have been reported in earlier studies which have recorded a dramatic rise in the prevalence of stunting with age among African children (Stoltzfus et al., 1997; Hautvast et al., 2000; Senbanjo et al., 2011). Stoltzfus et al. (1997) as well as Glewwe and Jacoby (1995) have postulated that, parents probably enrol the more healthy children in school at earlier ages. As a result, a pattern of higher prevalence of poor nutritional status among children who are older emerges. The same situation may pertain to the current study context. Strong age effects were seen on motor skills, language abilities and executive function, a finding which may be attributed to the following. Children's vocabularies expand as their semantic development takes effect (Zembar and Blume, 2009) hence older children do better than younger ones on vocabulary tests. A rapid increase of muscle strength and maturation of physical abilities related to balance and coordination also takes place in middle childhood (Zembar and Blume, 2009) resulting in better performance on motor tests among older children. Also, as this is a particularly active stage of maturation of executive function, children make significant cognitive advancements during middle childhood (Brocki and Bohlin, 2004).

Associations of gender with nutritional status and with motor skills and executive function did not reach significance. The literature on gender differences in nutritional status and gender influences on child outcomes illustrates a non-uniform pattern. Studies in sub-Saharan Africa, for example, report higher levels of stunting among boys (Semproli and Gualdi-Russo, 2007; Wamani et al., 2007; Goon et al., 2011), while studies from elsewhere have recorded higher levels for girls (Chowdbury et al., 2008). Although the literature on malnutrition seems to suggest that the differences in the manner in which boys and girls are treated may help one gender overcome early adversity, this did not seem to be the case in the current study. Our study also revealed that boys achieved higher scores on the language test while the reverse was true for verbal memory. Contrasting findings have, however, been reported in other studies where girls are found to consistently outperform boys on both measures (Kramer et al., 1997; Lowe et al., 2003). Perhaps in their day to day interactions, boys had more extensive prior experience with the objects that were represented pictorially on the language test hence they had an advantage over girls in naming the items. On the other hand, superior verbal memory scores for girls may be attributed to earlier maturation of their brains.

Our index for household wealth did not have significant direct or indirect effects on any of the child outcomes. On the contrary, several studies have reported that socioeconomic status is a strong predictor of both nutritional status (Brooks-Gunn and Duncan, 1997; Ndukwu et al., 2013) and outcomes in children (Bradley and Corwyn, 2002; Santos et al., 2008). The lack of an association between household wealth and child outcomes is not without precedence; an earlier study among infants living within the same context (Abubakar et al., 2008) has reported similar findings. We offer a couple of explanations for the non-significant direct effects of household wealth on nutritional status and child outcomes. First, we speculate that this finding may relate to the overwhelming influence of other factors, such as school attendance, among children at this age. This is evidenced by the moderate correlation seen between household wealth and school attendance. Secondly, our study was conducted within a context in which the majority of families live in economically depressed conditions. This may be the reason why, even though the indicators included in our SES measure distinguished one household from another, these differences were not significant in relation to the outcomes under study.

Although other studies have reported that children residing in rural areas have a substantially higher risk of poor nutritional status compared to their urban counterparts (Hautvast et al., 2000; Fox and Heaton, 2012), our study did not show evidence of such associations. The primary reason for this finding was that the current study was conducted within a predominantly rural context. Variations in children's area of residence may therefore have been too subtle to create any real differences in outcomes for children.

In the final trimmed models, school attendance had both direct and indirect (via nutrition) effects and was the most influential environmental predictor of nutritional status and child outcomes. The possibility that the nutrition-related benefits afforded by a school feeding program may explain this finding was negated by the fact that it was only in one school that children were offered food in school. When school attendance was taken into account, associations of nutritional status and cognitive functions with demographic factors like household wealth lost their significance; any bivariate associations washed out with the effects of going to school. This finding provides evidence that school attendance captures family resources more globally and meaningfully (such that there were no independent effects of area of residence and household wealth). Our models are also consistent with earlier studies that have demonstrated that where school attendance is not universal, even a little school exposure is associated with improved test-taking performance. In part, this may be due to increased test-taking awareness, as well as to methods of instruction, curriculum content or the types of questions that teachers ask, accelerating the development of cognitive skills over and above other factors (Holding et al., 2004; Alcock et al., 2008). Going to school thus offers opportunities for learning and practice, and also trains children to follow instructions,

hence the strong associations observed with tasks of higher order functioning.

Building up on previous similar work in this area, similarities were seen in the magnitude of the associations between background variables and nutritional status. However, the relationship between SES, stunting and outcome seen among infants (Abubakar et al., 2008) within the same context was not fully replicated in the current study population. This may have been because older children are exposed to more varied environments. Furthermore, as with the infant study, the direct path between household wealth and outcome in our study was not significant. As reiterated earlier on in this discussion, school attendance seemed to exert a greater influence than household wealth on nutritional status, and had strong direct associations with all outcomes (except verbal memory). A plausible explanation for this finding is that by the time children attain the age of going to school (around 6 years in the study context), the individual effects of socioeconomic status diminish as household wealth becomes an important determinant of whether or not a child goes to school (Mishra et al., 2007). Parents who are doing relatively well economically are able both provide more nutritious meals for their children as well as to retain their children in school. On the other hand, poor nutritional status may reflect limited economic resources. School attendance patterns of children from less wealthy households may be characterized by prolonged absenteeism or dropouts as their parents are unable to initiate and maintain their children's schooling (Mendez and Adair, 1999). Such children are thus likely to benefit less from the effects of school exposure. In light of these associations, school attendance could therefore be considered a proxy for household wealth, which in turn is strongly related to the nutritional status of the child. That there is a complex interactive relationship among the three factors is supported by the suggestion by Mukudi (2003) that the association between school attendance and nutritional status is a function of socioeconomic status. These associations could be more extensively explored through a longitudinal study.

Some of the major difficulties that emerge when comparing the effects of background variables on child development in different populations arise from the differences in environments to which they are exposed and in the outcomes tested. As noted by Goon et al. (2011), historical data such as birth weight, birth order, duration of breastfeeding and birth interval would likely provide a picture of previous states of malnutrition and provide further understanding of its aetiology within the current study population.

The estimated models demonstrated the continued importance of nutritional status as a powerful predictor of outcomes even as children grow older. Significant direct effects of the background variables on child outcomes suggest that the estimated models do not fully explain pathways through which they might influence child outcomes. The unexplained variance may be found in the home environment, an area which remains poorly investigated among rural African populations. Interventions to ameliorate the negative effects of poor nutritional status earlier on may mitigate the need for costly interventions later on, especially for those growing up in the contexts of poverty and poor nutrition.

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Perinatal iron deficiency and neurocognitive development

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Iron deficiency is the most common form of nutrient deficiency worldwide. It is highly prevalent due to the limited availability of high quality food in developing countries and poor dietary habits in industrialized countries. According to the World Health Organization, it affects nearly 2 billion people and up to 50% of women who are pregnant. Maternal anemia during pregnancy is especially burdensome to healthy neurodevelopment in the fetus because iron is needed for proper neurogenesis, development, and myelination. Maternal anemia also increases the risk of low birth weight, either due to premature birth or fetal growth restriction, which is associated with delayed neurocognitive development and even psychiatric illness. As rapid neurodevelopment continues after birth infants that received sufficient iron *in utero*, but that receive a low iron diet after 6 months of age, also show deficits in neurocognitive development, including impairments in learning and memory. Unfortunately, the neurocognitive complications of iron deficiency during critical pre- and postnatal periods of brain development are difficult to remedy, persisting into adulthood. Thus, preventing iron deficiency in the pre- and postnatal periods is critical as is devising new means to recapture cognitive function in individuals who experienced early iron deficiency. This review will discuss the prevalence of pre- and postnatal iron deficiency, the mechanism, and effects of iron deficiency on brain and cognitive development.

Keywords: nutrition, iron deficiency, cognitive development, learning, memory, hippocampus

INTRODUCTION

Iron deficiency is the primary cause of anemia, which affects roughly one-quarter of the world's population (McLean et al., 2009). As the most prevalent micronutrient deficiency in the world, iron deficiency affects all age groups, with the most common being children between the ages of 0 and 5 years (McLean et al., 2009). It was previously thought that neonates were protected from iron deficiency, in all but the most severe cases of maternal anemia, due to mobilization of iron stores accumulated *in utero* (Allen, 2000). However, it is now documented that even mild iron deficiency in the mother reduces iron stores in the fetus, resulting in a neonatal iron-deficient condition (Rao and Georgieff, 2002). Neonatal iron deficiency is also greater in infants born prematurely or of a diabetic mother (Lozoff et al., 2006). As the incidence of both these conditions is increasing worldwide (Beck et al., 2010; Martin et al., 2010; Danaei et al., 2011), it is likely that iron deficiency in infants will become an even greater concern in the future.

The impact of perinatal iron deficiency on human brain and cognitive development is of particular interest because the brain growth spurt that begins in the last one-third of pregnancy continues the first 2 years after birth due to dendritic growth, synaptogenesis, and glial cell proliferation (Courchesne et al., 2000; Gogtay et al., 2006). Neurogenesis in the hippocampal dentate gyrus also persists in the neonatal period (and throughout adulthood), and recent evidence indicates lingering neurogenesis in different cortical regions, including the prefrontal cortex in human infants (Sanai et al., 2011; Feliciano and Bordey, 2012). Total brain volume doubles the first year and reaches 80–90% of adult volume by age two (Knickmeyer et al., 2008). This phase of rapid growth represents a sensitive period, wherein environmental insults alter neurodevelopment (McEwen, 1999).

One such insult is iron deficiency. The hippocampus, a brain area important in learning, memory, and cognition, is highly susceptible to iron deficiency during the late fetal and early neonatal time period. For example, iron deficiency in the perinatal period is associated with altered expression of genes critical for hippocampal development and function (Carlson et al., 2007). Moreover, early iron deficiency causes neurocognitive dysfunction both during deficiency and after repletion (Beard and Connor, 2003; Jorgenson et al., 2005). Thus, preventing iron deficiency in the perinatal period is critical as is devising new means to recapture cognitive function in individuals who experienced early iron deficiency. Although iron deficiency is a major concern across the lifespan, this review will focus on the prevalence of pre- and postnatal iron deficiency, perinatal iron homeostasis, and the effects of perinatal iron deficiency on brain and cognitive development.

PREVALENCE OF IRON DEFICIENCY AND IRON DEFICIENCY ANEMIA

Of all micronutrient deficiencies, iron deficiency is most prevalent worldwide. It affects all age groups and demographics; however,

prevalence is higher in pubescent women, pregnant women, infants and preschool-age children. Rates of anemia in non-pregnant women of childbearing age reach approximately 40% in developing countries and 20% in industrialized countries (McLean et al., 2009). The incidence of iron deficiency anemia increases further during pregnancy, with rates in developing and industrialized countries reaching 59% and 24%, respectively. The large increase in iron deficiency in pregnant women in developing countries may be due to poor nutrition education and lack of iron supplements (Pasricha et al., 2013).

Infants born full term with an appropriate weight for gestation have iron stores that are adequate for about 6 months. This is vital because the neonatal gut is developmentally immature and infants are unable to regulate iron absorption until 6–9 months of age (Domellöf et al., 2002). Furthermore, iron content of breast milk is very low (approximately 35–40 μg/dL). Although information on iron deficiency in infants less than 1 year of age is lacking, the Pediatric Nutrition Surveillance System (PedNSS) reported hemoglobin in a national sample of infants from families participating in the Special Supplemental Nutrition Program for Women, Infants, and Children (i.e., Supplemental Nutrition Assistance Program (SNAP; Polhamus et al., 2004)). In 2003, 16.2% of infants aged 6–11 months, and 15% of children aged 12–17 months qualified as having iron deficiency anemia (Polhamus et al., 2004). In a Brazilian community, iron deficiency anemia in infants (average age 11.6 months) reached nearly 26% (Konstantyner et al., 2012); and in Canada, the rate of iron deficiency anemia in Cree Indian infants (average age 9 months) was nearly 32% (Willows et al., 2000). Sadly, in most countries including the U.S., the occurrence of iron deficiency worsens in preschool-aged children (CDC, 2011; see **Table 1**).

MATERNAL IRON DEFICIENCY AND IRON DEFICIENCY ANEMIA: EFFECTS ON NEONATAL IRON STATUS

As iron is concerned, the developing fetus was once considered a "perfect parasite," able to acquire sufficient iron from the mother even when she was iron deficient (Young et al., 2012). This notion has fallen by the wayside, however, and it is now clear that neonatal iron stores can be compromised when the mother is iron deficient or anemic. For example, under steady-state conditions serum ferritin concentration correlates with total body iron stores. New born infants from iron deficient mothers with low serum ferritin levels also had low serum ferritin indicating there is a limited capacity for the fetus to accumulate iron from low maternal stores (Jaime-Perez et al., 2005). Gestational iron deficiency also appears to have a programming effect on the physiologic mechanisms responsible for iron homeostasis resulting in offspring that are more likely to develop iron deficiency in the future regardless of adequate nutrition (De Pee et al., 2002; Georgieff et al., 2002; Emamghorashi and Heidari, 2004. Multiple studies have shown that iron status in infants born to iron deficient mothers is still abnormally low 9 months after birth despite being provided adequate dietary iron (Georgieff et al., 2002; Geltman et al., 2004).

The timing of iron deficiency during pregnancy is critical. First, there is an important need for iron early in pregnancy for neural development. In a recent study in rats, four dietary-feeding regimens were used to render the developing fetuses iron deficient at different stages of gestation. Maternal iron restriction beginning prior to conception and during the first one-third of pregnancy was associated with embryonic iron deficiency, postnatal anemia, reduced iron levels in the central nervous system, and decreased neural conduction velocities in an auditory brainstem response test conducted at postnatal day 45 (Mihaila et al., 2011). Importantly, the functional neural impairments were not induced when maternal iron restriction was initiated at the beginning of the last one-third of pregnancy. It's also noteworthy that in this study, the mothers were not anemic; indicating poorly timed iron deficiency is sufficient to disrupt neural development and function. Second, as the fetal liver continues to grow, most (> 66%) of the infants total body iron is acquired during the third trimester of pregnancy (Allen, 2000). Hence, infants born preterm and/or low birth weight have poorer iron stores and are a high risk population for iron deficiency (Scholl, 2011).

IRON HOMEOSTASIS IN THE BRAIN OF INFANTS

After 6 months of age, the blood-brain barrier is a major control point for the entry of iron into the brain, although the choroid plexus (the vasculature responsible for producing cerebrospinal fluid) is also a location of regulation for iron entry (Beard and Connor, 2003). The blood-brain barrier is important, as it prevents the brain from having direct access to the iron in the blood plasma, allowing for greater regulation (Piñero and Connor, 2000). Transferrin (Tf) receptors are present on the endothelial cells that make up the blood-brain barrier to allow for the binding and endocytosis of transferrin-bound iron into the brain (Beard, 2001). The rate of iron entry into the brain is increased during iron deficiency (Taylor et al., 1991), which is due to an increased amount of Tf receptors present on the cells of the blood-brain barrier (van Gelder et al., 1998), as well as a possible role for regulation by astrocytes (Beard and Connor, 2003).

Table 1 | Prevalence of iron deficiency anemia around the world.

		Iron deficiency anemia	Iron deficiency
Industrialized countries	**Pregnant women**	24% *	Iron deficiency is not commonly tested for and rates around the world are unknown at this time, but estimated to be much higher than for iron deficiency anemia
	Infant (0–12 months)	16.2% †	
	Children (1–5 years)	25% *	
Developing countries	**Pregnant women**	59% *	
	Infant (0–12 months)	40% ‡	
	Children (1–5 years)	67% *	

McLean et al. (2009), †Polhamus et al. (2004), ‡Chaparro (2008).

In the newborn infant, the story is a bit different. At birth, the blood-brain barrier is incompletely developed: it prevents iron transport proteins from diffusing into the brain but it lacks the ability to transfer iron from the blood into the brain parenchyma (Collard, 2009). Studies done in rat pups showed that iron regulatory proteins, which are involved with the regulation of Tf receptor, were not readily expressed until post-natal day 15, when peak myelination occurs in the rat (Siddappa et al., 2002). It is not known for human infants when the blood-brain barrier fully matures, but it has been estimated to occur by 6 months of age (Rice and Barone, 2000). During prenatal development iron accumulates in the brain so levels are highest immediately after birth (Siddappa et al., 2003). Due to poor bioavailability and low levels of iron in human breast milk, infant brain iron concentration decreases the first 6 months after birth, until the gastrointestinal tract and blood-brain barrier mature and are able to absorb dietary iron and regulate its entry into the brain, respectively. This coincides with the onset of myelination and an increase in transferring mRNA levels in brain (Connor et al., 1987; Connor and Menzies, 1996; Roncagliolo et al., 1998). Since oligodendrocytes synthesize Tf, the brain is the only organ in which Tf mRNA increases post-natally (Bloch et al., 1985). The developmental timeline for mechanisms responsible for iron homeostasis underscore the importance of maternal iron status during pregnancy.

Once iron penetrates the blood-brain barrier, much less is known about how it is distributed to different brain regions. Most likely Tf (secreted by the choroid plexus, which is another point of regulation) is the primary method for the distribution of iron in the brain (Beard, 2001); however, it is also possible for iron to be transported by binding to ferritin (Piñero and Connor, 2000). The iron can then be taken up by cells in various brain regions that express receptors for Tf and/or ferritin, with the expression of these receptors as the mechanism of regulation (Beard and Connor, 2003). This leads to an uneven distribution of iron between the various brain regions, which changes throughout the lifetime (Beard, 2001). In general, the globus pallidus, red nucleus, substantia nigra, and caudate putamen have higher iron concentrations throughout life, and the brain tends to accumulate iron with age (Piñero and Connor, 2000).

NEUROCOGNITIVE DEFICITS RESULTING FROM PERINATAL IRON DEFICIENCY

One of the first studies to look at the effect of iron deficiency on cognition in humans used the Bayley Scales of Infant Development, which assess motor, language, and cognitive development in infants and toddlers, and compared 9–26 month old infants who were given iron supplements to those given a placebo. Each group was tested and then re-tested within 8 days of the initial exam. The results showed an improved scores within the Mental Development Index for the iron supplemented group (Oski and Honig, 1978), which resulted in a surge of interest in this topic (Yehuda et al., 2010). Many different studies have looked at iron deficiency during various times of development; however, the most sensitive period (and the period that can cause the most irreversible damage) is the neonatal period, which is between 0 and 24 months of age (Pollitt, 1993). Although supplementation with iron has been shown to correct some of the cognitive deficits during this period, lower I.Q. and achievement test scores have still been found after treatment (Lozoff, 1989).

Many studies have investigated the impact of neonatal iron status at various times during this critical period and the effects that decreased iron levels have on cognition. In Papua New Guinea, infants who were given an iron dextran shot at 2 months of age had longer attention spans at 1 year of age when compared to control (Heywood et al., 1989). A study done in Costa Rica found that infants who had lower iron levels scored lower in the Bayley Scales of Infant Development tests in both cognitive and motor skill tests (Lozoff, 1989). In Guatemala, researchers found that an intramuscular injection of iron dextran improved Bayley Scales of Infant Development test scores in babies 6–24 months old after just one week, while oral supplementation did not show an effect within the week studied (Lozoff et al., 1982). In Chile, infants 3 months of age, were given either iron fortified formula or control diet for 12 months (Walter et al., 1983). At the conclusion of the diet intervention, Bayley Scales of Infant Development were administered. After the initial test, all infants received a trial of orally administered ferrous sulfate daily and then retested within 15 days (Walter et al., 1983). A similar relationship between low iron levels and lower Bayley Scales of Infant Development scores was found (Walter et al., 1983). Studies in Indonesia found that 8 weeks of oral supplementation of iron in anemic preschool aged children reduced deficits in visual attention and concept acquisition compared to children who were not given supplementation (Soewondo et al., 1989). Also in Indonesia, when 12–18 month old infants that were diagnosed with iron deficiency anemia were given an oral iron intervention, Bayley Scales of Infant Development scores significantly improved compared to those given a placebo, even after only 4 months of supplementation (Idjradinata and Pollitt, 1993). Nine and twelve month old infants were tested for their ability to discriminate a highly familiar stimulus, their mother's face, from a stranger's face using an electroencephalogram (Burden et al., 2007). At 9 months infants that were iron sufficient showed greater attentional response to the mother's face and greater updating of memory to the stranger's face, while iron deficient infants did not show this response until 12 months of age, suggesting a delay in cognitive development (Burden et al., 2007). It has also been demonstrated that infants with low serum ferritin concentrations have abnormal auditory recognition memory (Siddappa et al., 2004). The infants in this study did not discriminate a familiar stimulus (mother's voice) from a novel stimulus (stranger's voice) with the same vehemence as an iron sufficient infant (Siddappa et al., 2004). These findings suggest abnormalities in structures that mediate recognition and memory function, including the hippocampus (Georgieff, 2008).

Being iron deficient at birth seems to cause long term deficits as well. Five year old children, who were born either iron deficient, scored lower on tests of language ability, fine-motor skills, and tractability, when compared to children who were iron sufficient at birth (Tamura et al., 2002). In Israel, it was found that children who were born premature and had low ferritin levels at birth performed significantly worse on tests involving spatial cognition and processing of auditory signals when tested at 9 to 10 years of age, even though their hemoglobin levels had returned to normal (Armony-Sivan et al., 2004; Yehuda and Yehuda, 2006). Another

study showed similar results in that Costa Rican teens that were severely iron deficient during infancy, despite resolution of anemia while an infant, showed deficits when given neurocognitive tests (Trail Making test, Intra-Extra-dimensional Shift, Stockings of Cambridge, Spatial Working Memory, Rapid Visual Information Processing, Pattern Recognition Memory, and Spatial Recognition Memory) at the age of 19 years old, when compared to teens that were iron sufficient during infancy (Lukowski et al., 2010).

Of course, studies in animals have greatly advanced the knowledge of iron deficiency and its effects on cognitive performance. The fact that the avoidance response is affected by iron deficiency was one of the first cognitive aspects studied in rodents. Rats placed on an iron deficient diet at an early age showed deficits in both passive avoidance, where the rat had to inhibit its activity (i.e., not leave a platform and enter a chamber) in order to avoid shock, and active avoidance (i.e., where rats had to move into another chamber of the testing area in order to avoid shock) (Weinberg et al., 1979, 1981). Fear conditioning (as measured by heart rate deceleration in a cage where the rat had been shocked previously, as well as exposure to a tone that was played during the shock in a novel cage) was also impaired in iron deficient rats (McEchron et al., 2005).

Iron deficient diets also resulted in cognitive deficits in the Morris Water Maze, a hippocampal-dependent task which requires the rat to develop a spatial map of the area surrounding the pool in order to reach a hidden platform (Yehuda and Youdim, 1989). Similar deficits were observed in a water Y-maze (where the rat had two choices and a dry platform was placed at the end of the arm of the correct choice as a reward), including increased incorrect arm entries and a longer time to reach the platform (Yehuda et al., 1986), both of which indicate cognitive deficits. A recent study with piglets also demonstrated the effects of early-life iron deficiency on hippocampal-dependent learning (Rytych et al., 2012). Neonatal piglets placed on liquid diets of varying iron content (adequate, mildly deficient, and a severely deficient) 2 days after birth underwent repeated cognitive testing beginning about 2-weeks later using a T-maze task to measure spatial learning and memory. Severely iron deficient piglets were not able to successfully learn the task, while mildly deficient piglets took longer to learn compared to controls. In addition to poor performance, both sets of iron deficient piglets had less iron present in the hippocampus compared to controls (Rytych et al., 2012). These rodent and piglet studies confirmed that the magnitude of the cognitive effects observed correlated with the duration and severity of the iron deficiency.

The timing of iron deficiency during the perinatal period can also affect learning and memory. A pre-natal, post-natal, and pre+post-natal iron deficiency paradigm was used with mice pups to examine the effects of iron deficiency on learning and memory (Ranade et al., 2013). Mice pups that were pre-natal iron deficient or pre+ post-natal iron deficient performed poorly in a radial arm maze task; performance was better for pups in the post-natal deficient group, although their performance did not match that of pups provided adequate iron in both the pre- and postnatal periods. Even when pre-natal iron deficient pups became iron sufficient after birth, they still exhibited a poor ability to utilize reference memory, suggesting the hippocampus is highly vulnerable to iron deficiency in the pre-natal period (Ranade et al., 2013). However, the fact that performance of the post-natal iron deficient group was intermediate to the other iron deficient groups and control confirms that the window of vulnerability to iron deficiency for the hippocampus does not close immediately at birth.

Iron deficiency decreases brain iron concentration, which leads to numerous behavioral symptoms, such as irritability, apathy, reduced ability to concentrate, and other cognitive deficits (Piñero and Connor, 2000) (**Table 2**). Other important behavioral problems have been reported in association with iron deficiency as well. For example, children with Attention Deficit Hyperactivity Disorder (ADHD) were found to have lower levels of serum ferritin, an indication of reduced iron storage (Konofal et al., 2004). Iron-deficient animals develop ADHD-like behavior that has been linked to the dopaminergic system (Lahat et al., 2011). Deficits in motor development are also a symptom of iron deficiency in the neonate. In addition to cognitive development, the Bayley Scales of Infant Development also assesses fine and gross motor skills. Numerous studies (Lozoff et al., 1982; Walter et al., 1983; Lozoff, 1989; Idjradinata and Pollitt, 1993) indicate iron deficiency is associated with poorer scores in the motor function assessment within of the Bayley Scales of Infant Development. Further, anemic infants had low Mental and Psychomotor Development Index scores, even after 3 months of iron therapy. The anemic infants also showed deficits in language capability and coordination (Walter et al., 1989). In rodents, iron deficiency is associated with delayed development of surface righting, bar hold, forelimb placing, and negative geotaxis (Beard et al., 2006).

IRON UTILIZATION WITHIN THE BRAIN

So what are some potential mechanisms underlying iron deficiency-related deficits in cognition? Iron is important for erythropoiesis, formation of hemoglobin and myoglobin, gene transcription, cellular enzyme reactions, and important oxidation-reduction actions (Lieu et al., 2001). All of these, of course, are important for proper brain function, so it is not surprising that iron deficiency results in behavioral disorders and deficits in learning and memory (Rao and Georgieff, 2007). In humans, the hippocampus matures most rapidly over a short period of time: from late gestation to 2–3 years of age. During this period, there is an increase in iron uptake and utilization as well as neurogenesis, dendrite growth, myelination, synaptogenesis, and neurotransmitter synthesis (Fretham et al., 2011). Hippocampal-dependent memory appears and matures between 3–18 months of age (Nelson, 1995). Because infants are unable to fully regulate iron transport across the blood-brain barrier the first 6 months after birth, it is paramount that infants have adequate iron stores at birth.

Although hippocampal neurogenesis continues into adulthood, it occurs at a much greater rate prenatally and in the early postnatal period (**Figure 1**). The newborn neurons integrate into the developing neural circuitry and are thought to be important for learning and memory. Thus, in critical developmental periods environmental insults that inhibit neurogenesis or alter neuron maturation will likely affect present and future behavior. Iron deficiency has been shown to inhibit

Table 2 | Neurobehavioral consequences of iron deficiency.

Affected process	Population	Measure	Iron supplement?	Supplement administration and timing	Result	Possible cause	Source
Behavior	Costa Rican infants 12–23 months, IDA (n = 52), IS (n = 139)	Observation in clinic and at home using bayley scales of infant development. Spatial relations, affective state, behavior in relation to toys and in relation to caregiver	Yes	Behavior assessed before and after 3 months of iron therapy	Marked differences in behavior were found between the IDA and IS group despite resolution of anemia from iron therapy. IDA infants remained close to caregivers at all times, and showed increased fearfulness, wariness, hesitance, unhappiness, and tension	Inhibited neurotransmitter function, hypomyelination, and delayed neuromaturation from ID may lead to behavioral differences when compared to IS infants	Lozoff et al., 1998
Behavior-ADHD	French children, age 4–15 years old, ADHD (n = 45), control (n = 27)	Conners' Parent Rating Scale (CPRS). Serum ferritin, hemoglobin, hematocrit, and iron levels in blood were measured	No	NA	Serum ferritin was significantly lower in children with ADHD compared to control, while other blood measure were the same. Serum ferritin levels inversely correlated with CPRS scores	Low ferritin may be responsible for altered dopaminergic neurotransmission which can affect brain dopaminergic activity in children and contribute to ADHD	Konofal et al., 2004
Motor development	Inner city infants, 9–10 months, ID (n = 28), IDA (n = 28), IS (n = 21)	Peabody Developmental Motor Scales, Infant Neurological International Battery (IFANIB), toy retrieval task	No	NA	Poorer motor function found in IDA and ID infants, compared to IS. 34% of IS infants were standing alone, if not walking (19%) by 9 months age, while only 19% of ID and IDA infants could stand alone. ID infants showed deficits in toy retrieval task as well	Impaired myelination in the corticospinal tract may delay/alter normal development and refinement of motor skills. ID induced changes to dopamine function within the basal ganglia may explain poor performance in toy retrieval task	Shafir et al., 2008

(Continued)

Table 2 | Continued.

Affected process	Population	Measure	Iron supplement?	Supplement administration and timing	Result	Possible cause	Source
Sensory systems	Chilean children, ~4 years old, IDA ($n = 41$), IS ($n = 43$)	Auditory Brainstem Response (ABR), and Visual Evoked Potentials (VEP)	Yes	Children were supplemented for 6 months to a year when 6 month–18 months old. These are formerly deficient children	Formerly IDA children had significantly longer latencies for all ABR and VEP waves compared to IS children. Amplitudes were not different between groups	ID effects pathway transmission in both visual and auditory systems. May be due to hypomyelination. Differences in latency, but not amplitude, support this hypothesis. Latency changes relate to increases in conduction velocity during axonal myelination	Algarín et al., 2003
IQ	Egyptian children, age 6–12, IDA ($n = 22$), and IS ($n = 16$)	Weschler intelligence scale. Revised behavior problem checklist: conduct disorder, socialized aggression, attention problem-immaturity, anxiety-withdrawal, psychotic behavior, motor excess	No	NA	The mean IQ of the IDA group was significantly lower than the IS group. IDA children showed significant differences in motor control compared to the two other groups, and attention problems were higher in both anemic groups compared to IS, but highest in IDA	Iron deficiency can cause changes to hemoglobin concentrations, and mean corpuscular volume. In this study, both measures were predictive of attention deficit or motor excess	Mubarak et al., 2010
Memory	Chilean children, 10 years old, Formerly Iron Deficient (FID; $n = 19$) and control ($n = 23$)	Recognition memory task using Electrophysiological Recording and Processing (ERP)	Yes	Children were supplemented for 6 months to a year when 6 month–18 months old. These are formerly deficient children	Although accuracy within the task was the same for both groups, FID children took significantly longer to complete the task compared to controls. The FID group also had longer latency in the FN400 and P300 components of ERP testing, suggesting a delay in crucial memory searching processes and neural circuitry, respectively	Iron deficiency during formative years may have long lasting effects and cause deficiencies in neural circuitry and hypomyelination. The differences seen in the FID group in the FN400 component measure may also be due to difficulty accessing semantic memory to complete the task at the same level as the controls	Congdon et al., 2012.

Abbreviations: ID = Iron Deficiency, IDA = Iron Deficiency Anemia, IS = Iron Sufficient

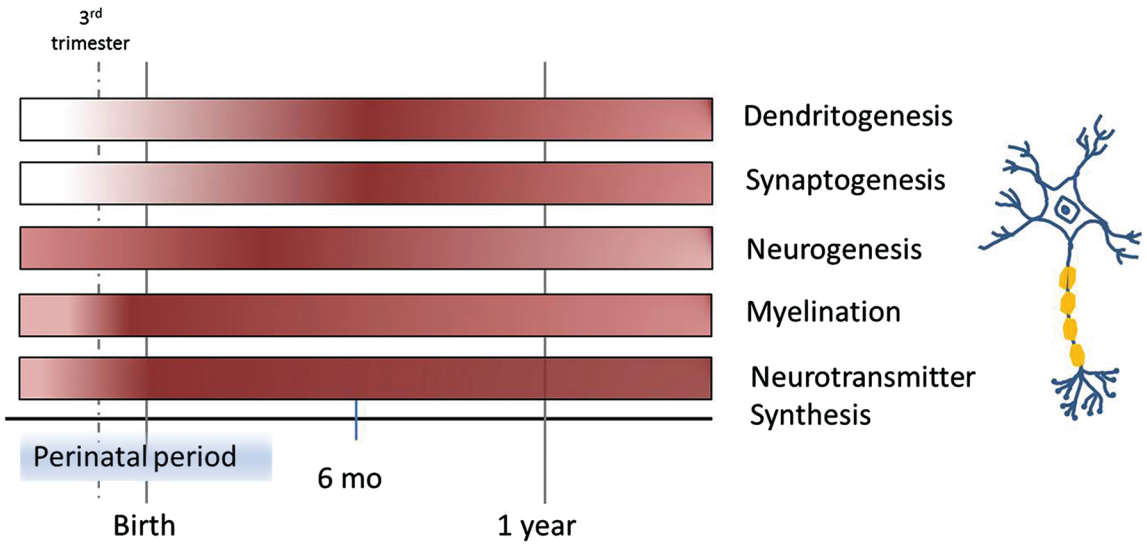

FIGURE 1 | Time course of different processes in brain development involved in learning and memory over the first year of life, highlighting periods of vulnerability to iron deficiency. Color intensity corresponds with the start, as well as peaks within each process.

neurogenesis in the developing hippocampus. Young rats whose mothers were fed an iron-deficient diet and who were born with iron deficiency, showed decreased brain-derived neurotrophic factor (BDNF) levels, down-regulation of BDNF target genes, and altered neuronal differentiation (Tran et al., 2008). Another study was carried out to investigate the effects of perinatal iron deficiency on hippocampal development in mice (Ranade et al., 2013). Iron deficiency in the pre- or postnatal period reduced BDNF and neurogenesis in the hippocampal dentate gyrus of pups. The impact of iron deficiency persisted, as the numbers of hippocampal pyramidal and granule cells were reduced in adults. Moreover, the structural and molecular defects in the pups were correlated with performance in hippocampal-dependent behavioral tasks, with pups from dams that were iron deficient throughout pregnancy and lactation displaying a broad array of defects, while pups from dams that were iron deficient only during pregnancy or during lactation displaying subsets of defects. These findings suggest that iron homeostasis is critical for the expression of neurotrophic factors that support brain development and provide a molecular basis for behavioral deficits related to perinatal iron deficiency (Lozoff et al., 2006).

Iron deficiency can cause changes to neuronal morphology. Neuronal shape is directly related to the computations performed by the cell and is crucial to information processing. Two key morphological features of neurons are dendritic arbor structure and spine density and geometry (Spruston, 2008). The developing arbor requires external inputs to stimulate and support branching morphogenesis. The arbor relies on external cues BDNF that signal via their respective transmembrane proteins. These in turn modulate factors that facilitate elongation and branching by promoting or reducing actin polymerization (Ackermann and Matus, 2003; Sekino et al., 2007). This process is not restricted to early developmental periods because it is also necessary for reshaping neural circuitry during experience-dependent learning throughout life (i.e., synaptic plasticity; **Figure 1;** Bagot et al., 2009). Like dendritic arbor structure, a spine's morphology can impact its function (Spruston, 2008). At post-natal day 15, CA1 pyramidal neurons in rats that underwent a period of iron deficiency *in utero* showed reduced dendritic branching and smaller spine head diameters (Brunette et al., 2010). Smaller spine heads could reduce conduction velocity resulting in less coordinated input to the soma as well as represent smaller post-synaptic density which could also affect signal transmission (Hodgkin, 1954).

Consistent with the structural changes noted above, iron deficiency also impacts synaptic plasticity in the developing hippocampus of rats (Jorgenson et al., 2005). For example, prenatal iron deficiency disrupted synaptic plasticity in the developing CA1 region of the hippocampus, but these differences were also apparent in adulthood, after iron repletion. When iron was replete, rat pups demonstrated no developmental increase in synaptic strength, as seen in control animals. It is hypothesized that major developmental events related to proper dendrite outgrowth and synaptogenesis were thwarted due to the unavailability of adequate iron (Jorgenson et al., 2005). This certainly may contribute to the lasting effects of iron deficiency on hippocampal structure and function.

In addition to marked changes in dendrite out growth and synaptogenesis, hypomyelination occurs when iron availability is limited. Proper myelination is important for rapid impulse transmission along axons. Myelination begins in the third trimester of the fetal period and progresses rapidly through infancy (**Figure 1;** Nakagawa et al., 1998). In the central nervous system, oligodendrocytes are responsible for myelination of axons. As mentioned before, oligodendrocytes synthesize Tf to mobilize needed iron, assuming it is readily available. Studies in humans and rats have demonstrated that iron deficiency can severely affect myelination. Increased latency of auditory brain stem potentials and visual evoked potentials (indirect markers of myelination) has been reported in iron deficient children (Roncagliolo et al., 1998; Algarín et al., 2003). Oligodendrocytes synthesize fatty acids and

cholesterol for myelin (Mackler et al., 1979; McKay et al., 1983). In a rat model, restriction of dietary iron during gestation and the early post-natal period resulted in significantly less myelin proteins, lipids, and cholesterol in the spinal cord, brain stem, and cerebellar white matter (Yu et al., 1986; Ortiz et al., 2004). Additionally, rats that were iron deficient in the perinatal period displayed deficits in myelinogenesis at adulthood, even though iron stores were replete (Ortiz et al., 2004). Finally, placing rats on an iron-deficient diet post-weaning lead to a significant decrease in myelination indices in the hindbrain and cerebrum (Beard and Connor, 2003). This suggests that the need and usage of iron by oligodendrocytes does not end during the perinatal period and that the adult brain still requires adequate iron.

Iron is also essential for a number of enzymes involved in neurotransmitter synthesis, including tryptophan hydroxylase used to produce serotonin, and tyrosine hydroxylase used to synthesize norepinephrine and dopamine. Neurotransmitter synthesis begins during embryogenesis (**Figure 1;** Herlenius and Lagercrantz, 2001). Dopamine is important for regulating cognition and emotion, reward and pleasure, movement, and hormone release (Dunnett et al., 2005). Striatal networks, with dopamine as the major neurotransmitter, relate to higher order cognitive and emotional processes, motivated behavior, positive affect, and reward-related processing, as well as motor function (Dunnett et al., 2005). Studies in humans have shown that young adults, who were iron deficient while an infant, tended to perform more poorly on tasks that involved inhibitory control, set-shifting, and planning, all of which are classified as executive functions that utilize striatal networks that rely on dopamine (Lukowski et al., 2010). Studies in rodents have determined that dopaminergic neurons co-localize with iron throughout the brain, that extracellular dopamine and norepinephrine are elevated in brains of iron-deficient rats, and that the density of dopamine receptors are altered in iron deficiency. These alterations are tightly connected to the extent of iron loss in each brain region (Beard and Connor, 2003). Other studies demonstrate that serotonin transporter (SERT) and norepinephrine transporter densities are also altered by iron deficiency (Burhans et al., 2005). Serotonin is particularly important for proper wiring of neural circuits and is highly implicated in neurodevelopmental disorders, such as autism, anxiety, or depression (Calabrese et al., 2013). The SERT, which is responsible for re-uptake of serotonin within the brain, is the predominant mechanism controlling strength and duration of serotonergic neurotransmission (Gaspar et al., 2003). SERT is expressed more during development than in adulthood (Daws and Gould, 2011). Iron deficiency leads to decreased expression of SERT which in turn exacerbates the decreased expression of BDNF. As previously noted, a reduction in BDNF can have serious consequences for hippocampal structure and function leading to deficits in learning and memory.

CONCLUSION

Iron deficiency is the leading micronutrient deficiency in the world. It affects people of all ages, but is most detrimental to infants and children. In developing countries, approximately 12% of children under 5 will die from a micronutrient deficiency (Ahmed et al., 2012). Children who survive will likely be iron deficient, if not anemic. In industrialized countries like the United States, the incidence of iron deficiency is increasing, probably due in part to the increase in the number of children and adults who are overweight or obese (inflammation disrupts iron homeostasis) (Del Giudice et al., 2009). As iron deficiency inhibits learning as well as motor and emotional development, individuals exposed to perinatal iron deficiency are at high risk for failing to reach educational milestones later in life. Moreover, as adults they are more likely to bear children who also experience iron deficiency. Hence, iron deficiency in one generation can beget iron deficiency in the next generation, and so on. Given the consequences of developmental delays in neurocognitive function, such as decreased motor development, lower IQ, difficulties with learning and memory (refer to **Table 2** for more evidence), strategies to prevent perinatal iron deficiencies and to promote neural plasticity in individuals exposed to poor iron status during key developmental periods, warrants more attention.

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Does docosahexaenoic acid supplementation in term infants enhance neurocognitive functioning in infancy?

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The proposal that dietary docosahexaenoic acid (DHA) enhances neurocognitive functioning in term infants is controversial. Theoretical evidence, laboratory research and human epidemiological studies have convincingly demonstrated that DHA deficiency can negatively impact neurocognitive development. However, the results from randomized controlled trials (RCTs) of DHA supplementation in human term-born infants have been inconsistent. This article will (i) discuss the role of DHA in the human diet, (ii) explore the physiological mechanisms by which DHA plausibly influences neurocognitive capacity, and (iii) seek to characterize the optimal intake of DHA during infancy for neurocognitive functioning, based on existing research that has been undertaken in developed countries (specifically, within Australia). The major observational studies and RCTs that have examined dietary DHA in human infants and animals are presented, and we consider suggestions that DHA requirements vary across individuals according to genetic profile. It is important that the current evidence concerning DHA supplementation is carefully evaluated so that appropriate recommendations can be made and future directions of research can be strategically planned.

Keywords: neurocognitive, development, n-3 LC-PUFA, DHA, infant

LC-PUFA IN THE CURRENT HUMAN DIET AND THE IMPORTANCE OF DHA FOR EARLY BRAIN DEVELOPMENT

The omega-3 (*n*-3) long chain polyunsaturated fatty acid (LC-PUFA), docosahexaenoic acid (DHA; 22:6 *n*-3), is involved in several critical brain functions (Bradbury, 2011). DHA accretes within the brain during gestation and the first year of life during the "brain growth spurt" (Innis, 2008). During this time, the developing brain is sensitive to extreme variations in the supply of DHA (Karr et al., 2011). DHA accretion in the fetal brain can be influenced by maternal diet, DHA stores, placental transport, and genetic polymorphisms (McCann and Ames, 2010). Dietary DHA is obtained during the postnatal period via breast milk or infant formula. The concentration of DHA within breast milk can vary depending on maternal DHA stores and diet (Innis, 2008). The average DHA intake of Australian women during pregnancy and lactation is below global recommendations; this is likely to impact the provision of DHA to the offspring (Bourre, 2007; Meyer, 2011).

Formula fed infants in many countries, including Australia, may also be at risk of receiving insufficient dietary DHA, since the inclusion of pre-formed *n*-3 and *n*-6 LC-PUFAs (including DHA) in formula is not mandatory. It is argued that infants are capable of synthesizing *n*-3 and *n*-6 LC-PUFAs endogenously from their shorter chain precursors (Guesnet and Alessandri, 2011). However, there is considerable debate around human capacity and ability to synthesize DHA. Research has found single nucleotide polymorphisms (SNPs) in the fatty acid desaturase genes (FADS1 and FADS2) modulate individual capacity for LC-PUFA synthesis (Glaser et al., 2011). Subsequently, it is now recognized that dietary requirements for DHA and other LC-PUFAs may vary across the population and are somewhat dependent on individual genetic profile (Koletzko et al., 2008). The need for LC-PUFA supplementation in term infants therefore remains unknown. This article summarizes the major animal studies and clinical trials pertaining to fatty acid supplementation during infancy, and evaluates the current level of evidence for LC-PUFA supplementation in term infants.

The *n*-3 and *n*-6 families are distinct groups of PUFAs important for human health, growth, and development. All PUFAs within the *n*-3 family are derived from alpha linolenic acid (α-LA; 18:3 *n*-3), while all *n*-6 PUFAs are derived from linoleic acid (LA; 18:2 *n*-6). (**Figure 1**) The (short chain) parent molecules of the *n*-3 and *n*-6 families, α–LA and LA, are described as "essential" fatty acids, since humans and other mammals cannot synthesize them endogenously (Kris-Etherton et al., 2000). The three most biologically active members of these families are the LC-PUFAs: DHA, eicosapentaenoic acid (EPA; 20:5 *n*-3) and arachidonic acid (AA; 20:4 *n*-6). These three fatty acids all carry out separate,

complex functional roles in the body (McNamara and Carlson, 2006).

For Western nations, low dietary intake of EPA and DHA is a concern as these substances are not widely available in contemporary diets (Calder, 2012). There are few plant sources of DHA and EPA, and they are found almost exclusively in oily, cold water fish, fish oil supplements, breast milk, and supplemented infant formula. α–LA is available in oils such as canola and soybean, as well as in walnuts and flaxseed (**Table 1**). Converting α–LA into EPA and DHA is a variable and inefficient process; stable-isotope tracer studies have shown that dietary α-LA accounts for between 0.2 and 8% of EPA and <0.05 and 4% of DHA (Burdge and Calder, 2005; Plourde and Cunnane, 2007). Similarly, less than 0.1% of LA is converted into AA. Furthermore, conversion levels vary according to the intake of other fatty acids and the ratio of *n*-6: *n*-3 PUFAs consumed in the diet (Bokor et al., 2010). *n*-6 PUFA intakes in Western diets are typically high, supplying ∼16 times more *n*-6 PUFA (including AA) than *n*-3 PUFA due to high intakes of beef, pork, poultry, wheat germ, and various cooking oils (Simopoulos, 2001b). In contrast, the diets of our paleolithic ancestors are thought to have contained roughly equal ratios of *n*-3: *n*-6 (Simopoulos, 2001a). Subsequently, there has been some debate as to whether modern diets contain sufficient quantities of *n*-3 fatty acids (in particular the *n*-3 LC-PUFAs, DHA and EPA) to support optimum health across the lifespan, especially during pregnancy and lactation (Eaton, 2000). It is possible that typical current levels of DHA intake are sufficient to achieve optimal neurocognitive functioning in childhood. This is the focus of the current review.

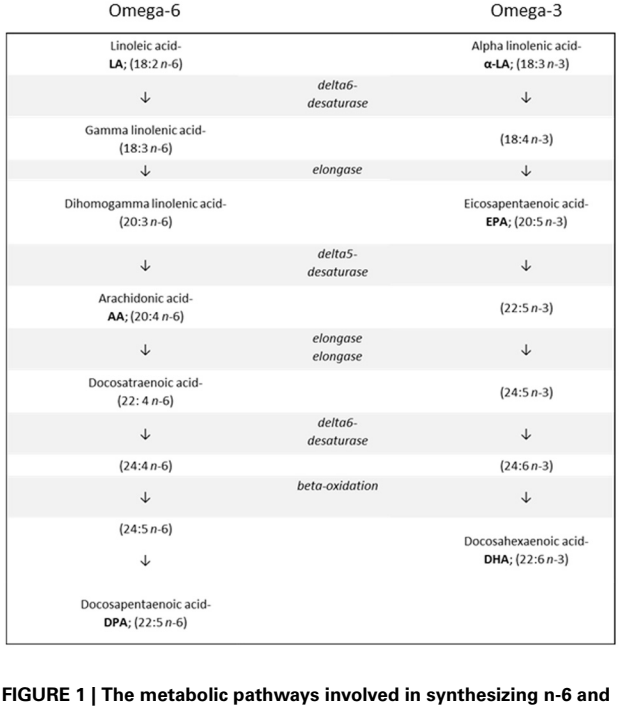

FIGURE 1 | The metabolic pathways involved in synthesizing n-6 and n-3 long chain polyunsaturated fatty acids from their respective shorter chain precursors.

DHA WITHIN THE BRAIN AFFECTS NEURONAL DEVELOPMENT

There have been several lines of evidence that suggest that LC-PUFAs (particularly DHA) are important for the function of the CNS at the cellular and neurobiological level. The concentration of DHA and other LC-PUFAs within the brain can alter the neuronal membrane fluidity and physical structure of neurons (Youdim et al., 2000). *n*-3 and *n*-6 LC-PUFAs are also involved in the production and activity of several neurotransmitters such as dopamine and serotonin (Zimmer et al., 2002; Aid et al., 2005; Chalon, 2006), affecting synaptic transmission and substrate binding to membrane receptors (Horrocks and Farooqui, 2004). Furthermore, DHA has been shown to affect neural functioning via its influence on gene expression in mammalian brain tissue (de Urquiza et al., 2000; Kitajka et al., 2004). The process of neurite outgrowth in hippocampal neurons is enhanced by DHA, which may in turn promote learning (Calderon and Kim, 2004) as the hippocampus is a critical brain region for memory formation (Rolls, 2008; Berger et al., 2012). DHA may also improve learning and memory through its role in the development of pre- and post-synaptic proteins which enable synaptic transmission and long-term potentiation (Cao et al., 2009). It is apparent that DHA plays numerous important biophysical roles in brain structure and function, and has the potential to influence neurocognitive development and subsequent performance (McNamara and Carlson, 2006; Parletta et al., 2013).

During the last trimester of gestation and for the first 18 months after birth, AA, and DHA are deposited within the cerebral cortex at a rapid rate (Martinez et al., 1974; Clandinin et al., 1980). As noted above, this stage of human development is known as the brain growth spurt (Martinez and Mougan, 1998), when neuronal development is particularly vulnerable to nutritional insufficiencies (Nyaradi et al., 2013). Further evidence from studies on young non-human primates has revealed that once brain DHA depletion has occurred it is physiologically difficult to reverse (Ikemoto et al., 2001).

Table 1 | Common dietary sources of *n*-3 and *n*-6: short chain essential fatty acids (α-LA and LA) and long chain polyunsaturated fatty acids (DHA and AA) (Howe et al., 2006; Russo, 2009).

		Omega-3 PUFA		Omega-6 PUFA
Dietary sources: EFA	α-LA	Vegetable oils: linseed and canola Flaxseed and walnut Fish: herring, salmon, and tuna Green leaves	LA	Vegetable oils: Corn, sunflower, and safflower Pork Walnut, peanut, and wheat Fish: herring, salmon, and tuna
Dietary sources: LC-PUFA	DHA & EPA	Fish: herring, salmon, trout, tuna, and fish oil supplements Breast milk	AA	Beef, pork, and poultry Whole-grain wheat

DIETARY RECOMMENDATIONS FOR DHA DURING PREGNANCY AND THE NEONATAL PERIOD

Considering the rapid accretion of DHA into the brain during the last trimester of pregnancy and into the first year of life, it is important to consider whether optimal DHA intake is occurring during this period. Numerous expert and government authorities worldwide agree that dietary DHA requirements are increased during pregnancy and lactation when a minimum of 200 mg of DHA per day is recommended (Van Elswyk and Kuratko, 2009). This dose can be achieved by eating 1–2 portions of fish per week or taking fish oil supplements (Koletzko et al., 2008). Recent figures indicate that 91% of Australian women are failing to meet DHA recommendations during pregnancy and lactation (Cosatto et al., 2010), with similar trends noted in many other Western countries (Meyer, 2011). In Australia, the median daily intake of DHA during pregnancy is ∼96 mg per day, ranging from 8 to 632 mg per day across individuals (Cosatto et al., 2010). Since the dietary intake and maternal stores of DHA during pregnancy are known to be key determinants of infant blood DHA concentrations at birth (Bourre, 2007), low DHA consumption by women eating Western diets has prompted some concern for the neurological and neurocognitive development of their offspring (Rogers et al., 2013).

With respect to intake of DHA via breastfeeding, findings from the 2010 Australian National Infant Feeding Survey have revealed that while around 96% of Australian women initiate breastfeeding after birth, there is a rapid decline in breastfeeding rates during the weeks and months following birth (AIHW, 2011; Burns et al., 2012). Despite international recommendations for exclusive breastfeeding for the first 6 months postnatally (Kramer and Kakuma, 2001), in Australia an average of only 56% of women breastfeed for this recommended amount of time (AIHW, 2011).

Moreover, the concentration of DHA within women's breast milk appear to be decreasing over time (Makrides et al., 1995). A comprehensive analysis of human breast milk LC-PUFA compositions have revealed the current worldwide average DHA concentration is approximately 0.32% of total fatty acids (TFA) (Brenna et al., 2007). This corresponds to ∼60 mg of DHA per day for the first 6 months postnatally, assuming an average breast milk intake of 750 mL per day (Cunnane et al., 2000). Analysis of Australian breast milk concentrations in 1981 and 1993 found that the amount of DHA decreased by 27% over this time period while concentrations of AA remained the same (Makrides et al., 1995). A similar decline in human milk DHA has been reported in a Canadian population (Innis, 2003). The decline in breast milk DHA may plausibly be explained by dietary shifts over time, the use of different FA analytical techniques (Makrides et al., 1995) or through differences in maternal FADS genotype across studies (Xie and Innis, 2008; Moltó-Puigmartí et al., 2010)—an issue that will be discussed further below.

Formula-fed infants may also be at risk of sub-optimal DHA supply during this period. DHA brain content has been analyzed in autopsy studies of human infants conducted in Australia (Makrides et al., 1994) and the United Kingdom (Farquharson et al., 1995). Both studies found that the brain tissue of breast-fed infants contained higher concentrations of DHA compared to their standard formula-fed counterparts. These findings are consistent with animal studies, where dietary restriction of *n*-3 PUFA decreased the amount of DHA within the brain (Diau et al., 2005; Brenna, 2011; Luchtman and Song, 2013). One of the compelling arguments for including DHA and other LC-PUFAs in infant formula is to render its composition more similar to breast milk, which is commonly cited as the "gold standard" for infant nutrition (Burns et al., 2012). DHA enriched formula can enable formula-fed infants to attain DHA levels that are equivalent to their human milk receiving counterparts (Cunnane et al., 2000). However, clear functional benefits to infant development need to be demonstrated before DHA enriched formula can be unequivocally recommended.

THE FUNCTIONAL EFFECTS OF DHA ON NEUROCOGNITION: EVIDENCE FROM STUDIES OF DEFICIENCY IN ANIMALS

The evidence that DHA deficiency is detrimental to neurocognitive development is well established in animal models. The functional consequences of lower DHA concentrations within the CNS in baboons include visual (Neuringer et al., 1986) and motor deficits (Champoux et al., 2002). Studies have found that *n*-3 PUFA deficient rodents exhibit poorer performance in the Morris water maze test compared to their *n*-3 PUFA-sufficient counterparts (Sheaff et al., 1999; Moriguchi et al., 2000; Lim et al., 2005). DHA deficient mice exhibit a range of neurocognitive impairments, including problems with learning and memory (Catalan et al., 2002) and can have a heightened stress response (Fedorova and Salem, 2006). Detailed reviews in this area have been conducted by Davis-Bruno and Tassinari (2011) and Luchtman and Song (2013).

Animal studies allow more flexibility with respect to study design and potentially offer greater insight into the neurological mechanisms influenced by DHA deficiency in human infants (Romijn et al., 1991). However, there are limitations in the extrapolation of findings obtained in animal studies to the study of nutrition in humans. Research undertaken in non-human primates offers certain advantages over rodent studies in terms of transferability to humans, related to similarities in relative brain size, retinal microarchitecture and other anatomical, physiological and genealogical homologies (Brenna, 2011). However, human neurocognitive functioning is more complex and relies to a greater degree on higher cognitive capacities such as language and executive functioning (Luchtman and Song, 2013). A degree of caution should be applied when generalizing findings from animals to humans (Innis, 2007). Nonetheless, there is a strong and consistent body of evidence obtained from animal studies linking *n*-3 LC-PUFA deficiency to impaired neurocognitive functions. These links warrant further investigation through clinical research in humans.

MATERNAL DIETARY DHA INTAKE AND SUPPLEMENTATION

Epidemiological studies of maternal DHA intake provide insight into the potential value of DHA on neurocognitive outcomes. A very large observational study ($n = 11,875$) in pregnancy found a significant association between low maternal seafood consumption (<340 g per week) and suboptimal neurocognitive outcomes in childhood (Hibbeln et al., 2007). Children aged

6 months to 8 years whose mothers consumed low seafood diets during pregnancy had lower verbal IQ, displayed less prosocial behavior (defined as voluntary behavior intended to benefit another) and had poorer social and communication skills compared to those whose mothers consumed high seafood diets (Hibbeln et al., 2007). Furthermore, in a study of Canadian Inuit people (who typically consume a DHA-rich diet) DHA concentrations in umbilical cord plasma were positively associated with longer gestation, better visual acuity, higher scores of novelty preference on the Fagan test at 6 months and higher cognitive scores on the BSID-II of mental and psychomotor performance at 11 months (Jacobson et al., 2008). Most published observational epidemiological studies have recognized a positive association between maternal intake of *n*-3 LC-PUFA rich foods during pregnancy and neurocognitive development of offspring (Oken et al., 2005, 2008; Hibbeln et al., 2007; Mendez et al., 2009; Boucher et al., 2011). The observational study by Gale et al. (2008), however, detected no association between the frequency with which mothers ate fish in pregnancy and full-scale or performance IQ of their offspring at 9 years of age. Nevertheless, these researchers did find that children whose mothers had eaten oily fish had higher verbal IQ and a reduced risk of hyperactivity compared to those children whose mothers did not eat oily fish, after adjustment for potential confounding factors. However, the findings of these studies are somewhat weakened due to potentially confounding variables, including social and economic differences that may independently affect neurodevelopmental outcomes in childhood (Drane and Logemann, 2000; Boyd et al., 2013).

It is well established that maternal fish oil supplementation during pregnancy substantially increases fetal DHA concentration at the time of birth (Larqué et al., 2012). Furthermore, two randomized controlled trials (RCTs) have shown that DHA supplementation during pregnancy offers significant benefit to infant neurocognitive development (Judge et al., 2007; Dunstan et al., 2008). However, the RCTs in this area are not easily comparable and positive effects have not been identified in all studies (Lo et al., 2012). One long term follow-up found that maternal supplementation enhanced neurocognitive outcomes up to 4 years later (Helland et al., 2003) but the effect did not persist after 7 years (Helland et al., 2008). A relatively large RCT ($n = 249$) from Bangladesh found that maternal DHA supplementation from 25 weeks gestation until delivery had no effect on infant BSID-II mental and psychomotor performance outcomes at 10 months (Tofail et al., 2006). Such findings may not be directly comparable to an Australian population since maternal nutrition and anthropometric status in developing countries are often low (Karim and Mascie-Taylor, 1997; Dhar et al., 2003). It is also possible that the control oil used in this study (which contained α-LA: 2700 mg and LA: 2250 mg per day) may have inadvertently promoted infant neurocognitive status, thereby attenuating any treatment effect from the intervention (Tofail et al., 2006).

A meta-analysis and systematic review of maternal *n*-3 LC-PUFA supplementation undertaken by Gould et al. (2013) highlighted numerous potential areas of bias in the current literature and remarked on the relatively poor quality of most RCTs in this field. Of major concern was the consideration that publications seldom reported the randomization process and/or method(s) used to conceal treatment allocation from participants. According to this review, the only RCT of maternal DHA supplementation and infant neurocognitive outcomes that was considered to be genuinely free from bias was conducted in Australia by Makrides et al. (2011a). This RCT found no significant association between moderate DHA supplementation (800 mg per day) during pregnancy and BSID-II scores of language or cognitive development at 2½years ($n = 2399$). However, this RCT revealed that children in the treatment group manifested significantly lower incidence of cognitive delay compared to their un-supplemented counterparts.

In summary, RCTs and epidemiological studies evaluating the potential neurocognitive impact of maternal DHA supplementation during pregnancy have revealed somewhat heterogeneous results in healthy term infants. Consequently, it may be premature to make unequivocal recommendations about any neurocognitive benefits of DHA supplementation in healthy term infants based on the currently available research findings.

CORRELATIONS BETWEEN INFANT NEUROCOGNITION AND BREASTFEEDING

Over the years, many prospective observational studies have indicated that breastfed infants have a significant neurocognitive advantage over their formula fed counterparts (Anderson et al., 1999; Agostoni et al., 2001; Oddy et al., 2003, 2011; Kramer et al., 2008). It has been theorized that this is due to the higher presence of DHA in breast milk, relative to formula milks. Some studies have found positive associations between DHA concentrations within breast milk and/or infant blood levels and better outcomes of visual acuity (Innis et al., 2001; Jørgensen et al., 2001). However, it is likely that breastfeeding enhances infant development due to a number of inter-related factors as reviewed in Jain et al. (2002). Specifically, observational studies are potentially confounded by the heterogeneous composition of breast milk (both within and between lactating individuals), environmental factors such as maternal/infant bonding and other influences (Jain et al., 2002). One particularly significant potentially confounding factor is social economic status (SES), which is positively associated with both maternal and infant IQ, along with the decision to breastfeed (Der et al., 2006; de Jager et al., 2013). Furthermore, the breastfeeding act itself may be indicative of maternal attentiveness and nurturing which may independently foster long term positive effects on infant neurocognitive outcomes (Morley et al., 1988). Despite appropriate statistical techniques used to try to control for the influence of confounders, observational studies can be subject to systematic bias and results should therefore be interpreted with some caution.

A study by Caspi et al. (2007) tested the association between breastfeeding and child IQ with respect to FADS2 genetic profile, specifically in terms of the SNP rs174575. Breastfed infants who were rs174575 C-dominant carriers achieved higher scores on standardized IQ tests compared to the C-carriers who were not breastfed. Meanwhile, children homozygous for the minor allele (GG genotype) were found to have similar IQs, irrespective of feeding method. These findings remained statistically significant after accounting for potential confounding variables including intrauterine growth, family social economic status, and maternal cognitive ability. While potentially important in terms of the

possible interaction between breastfeeding and the genetic status of the infant, these findings have yet to be replicated by other research studies.

CORRELATIONS BETWEEN INFANT NEUROCOGNITION AND DHA STATUS

Many trials have found that higher plasma or RBC DHA concentrations (often as a result of LC-PUFA supplementation) are positively correlated with infant neurocognitive outcomes (Agostoni et al., 1995, 1997; Gibson et al., 1997; Birch et al., 2000; Innis et al., 2001; Helland et al., 2003; Innis, 2003; Jensen et al., 2005; Drover et al., 2011). Some studies, on the other hand, have found no significant relationships (Lucas et al., 1999; Makrides et al., 2000; Auestad et al., 2001), while conversely two studies have found that higher infant DHA blood concentration has a negative neurocognitive effect (Scott et al., 1998; Lauritzen et al., 2005). It should be cautioned that associations between DHA status and infant neurocognitive status do not necessarily demonstrate causality, nor do they demonstrate the effectiveness of the intervention alone—as this may be confounded by other nutrients (Innis, 2003).

RCTs OF LC-PUFA SUPPLEMENTATION IN TERM AND PRETERM POPULATIONS

In order to confirm whether dietary LC-PUFA is responsible for the enhanced neurocognitive outcomes associated with breastfeeding and higher DHA status, RCTs of LC-PUFA supplementation are necessary. Several of these trials have been conducted, usually in formula-fed infants. The most common methodology involves comparing the neurodevelopmental outcomes of infants randomized to receive infant formula with DHA (either alone or in combination with AA and/or other PUFAs) or placebo (unsupplemented formula). The majority of trials in healthy term infants have shown little or no consistent, beneficial effects on neurocognitive outcomes as a result of dietary LC-PUFA supplementation. However, infant LC-PUFA supplementation has resulted in no negative effects on growth, development or morbidity (Koletzko et al., 2005). There is, therefore, currently no compelling argument either for or against LC-PUFA supplementation in term infants with respect to neurocognitive outcomes. This conclusion has been re-iterated in three consecutive versions of the Cochrane review (Simmer, 2001; Simmer et al., 2008, 2011) that have evaluated 9, 14, and 15 relevant RCTs, respectively. Interestingly, in the authors' conclusions for both the (2008) and (2011) Cochrane reviews, Simmer et al. refer to the positive results found by the Dallas group (Birch et al., 2005) and state that these results need to be replicated in other settings. These authors also propose that future RCTs should explore the use of DHA derived from single cell microalgae and supply higher doses of DHA, in line with typical human milk (DHA 0.32%) concentrations throughout the world.

Hoffman et al. (2009) reviewed 20 RCTs within this field including several studies not included in the Simmer et al. (2008) Cochrane review and elaborated on some methodological factors including dosage, source, and duration of supplementation. Hoffman et al. (2009) concluded that trials which supplied term infants with DHA in concentrations greater than 0.3%TFA (in addition to AA >0.3%TFA) were more likely to identify a significantly positive effect on neurocognitive and visual outcomes. In another meta-analysis utilizing individual patient data (IPD) with a considerable sample size ($n = 870$), Beyerlein et al. (2010) combined the raw scores from four methodologically similar RCTs (Lucas et al., 1999; Fewtrell et al., 2002, 2004; Bouwstra et al., 2005) which each assessed BSID-II outcomes at 18 months. The analysis concluded that LC-PUFA supplemented formula conveys no significant benefit on neurodevelopment at 18 months, as assessed using the BSID-II. However, this meta-analysis was unable to access IPD from all relevant trials (Birch et al., 2000; Clandinin et al., 2005).

Evidence that infant DHA supplementation conveys significant benefit on visual acuity is derived from a systematic review of 12 clinical studies from the Harvard School of Public Health (SanGiovanni et al., 2000). SanGiovanni et al. (2000) incorporated the results from both randomized and non-randomized studies of DHA supplemented formula and concluded that increased dietary DHA improved visual acuity in term infants at two and four months of age. It should be cautioned that analyses that combine findings from both randomized and non-randomized trials have a higher risk of incorporating selection bias in the recruitment for the trial (Szajewska, 2011). More recent reviews in this field suggest that more research is required before definitive recommendations can be made concerning whether term infants would benefit from *n*-3 LC-PUFA supplemented formula (Agostoni, 2008; Benton, 2008; Belkind-Gerson et al., 2008; Makrides et al., 2011b; Campoy et al., 2012).

The majority of RCTs of infant DHA supplementation (as described above) use infant formula and are constrained by the consideration that their study samples have chosen not to breastfeed, thereby reducing external validity of the findings (Gibson and Makrides, 1998; Smithers et al., 2008a). There have been a small number of studies which have supplemented infants with DHA directly, thereby bypassing the need to employ formula-based supplementation. To the best of our knowledge, only one such direct supplementation RCT has addressed the effect of DHA on infant neurocognitive functioning (Meldrum et al., 2012). In this recent double-blinded, placebo-controlled trial conducted in Australia (Meldrum et al., 2012), healthy term infants ($n = 287$) were randomized to either very high dose fish oil (incorporating >250 mg DHA plus 60 mg EPA) or placebo (olive oil) per day from birth to 6 months. The study determined that while infants within the fish oil group had significantly higher DHA concentrations in erythrocyte and plasma phospholipids at 6 months of age relative to the placebo group, there was no significant difference between standard or composite scores of the BSID-III (third edition) at 18 months or on outcomes from the Child Behavior Checklist. In a subtest which explored the development of infant communication skills ($n = 185$), the study found that scores for later developing gestures and total number of gestures were significantly higher in the fish oil group compared to the placebo group at both 12 and 18 months. This finding is interesting since gestural skills in infancy are associated with visual recognition memory, deferred imitation and turn-taking skills (Heimann et al., 2006). Furthermore, these skills are understood to predict language and communicative ability in later life

(Acredolo and Goodwyn, 1988). Direct supplementation of the oil emulsion allowed participation of breast- and/or formula-fed infants alike. However, a potential criticism of this study is that the odor of the fish oil may not have been adequately masked, and therefore parents were frequently able determine which treatment their child was receiving. This, in turn, may have affected parents' ratings of their child's gestural abilities.

While the focus of the current paper is on term infant neurocognitive response to DHA supplementation, valuable information can be gleaned from investigation into the preterm population. Preterm infants are especially vulnerable to DHA deficiency as they have not had access to maternal lipid stores for the normal period of gestation (Haggarty, 2002). Similar to full term infants, preterm infants fed formula milk without DHA have lower DHA status compared to those fed human milk (Carlson et al., 1986) or LC-PUFA supplemented formula (Koletzko et al., 1989; Lapillonne et al., 2000). It has been identified that children born preterm have higher rates of learning disabilities, language impairment, attention deficits, hyperactivity, and reduced cognitive test scores compared to (gender- and age-matched) children born at term (Bhutta et al., 2002; Perricone and Morales, 2011). Although there are many factors associated with preterm delivery, it is possible that lower DHA status during the critical period of brain growth may contribute toward impaired neurocognitive development (McNamara and Carlson, 2006).

The DINO (DHA infant neurodevelopmental outcomes) double-blind RCT provided supplementation to lactating mothers ($n = 657$) in order to increase breast milk DHA (Makrides et al., 2009). Lactating women were either supplied six 500 mg DHA-rich tuna oil capsules per day or placebo soy oil capsules. Mothers were encouraged to breastfeed; however, if the mother chose not to provide breast-milk or if additional milk was required, infant formula was provided. The DHA concentrations of the formula matched the typical milk DHA concentrations of the two groups. The study found that preterm infants in the DHA group had better visual development (as determined through sweep VEP acuity) at 4 months corrected age [i.e., chronological age corrected for the degree of prematurity; (Smithers et al., 2008b)]. A higher mean mental development index of children in the DHA supplemented group (as assessed using the BSID-II) was found. However, after adjusting for confounding factors this benefit was not statistically significant ($p = 0.2$). Yet the number of children in the DHA group with low cognitive scores (indicative of mildly delayed development) was significantly smaller in the treated group compared with the control group. In pre-planned secondary analyses, the DINO study found DHA supplementation had a significantly positive effect on cognitive outcomes in girls compared to boys. The reason for differences in response to DHA as a function of gender remains unclear but may be related to the higher rate of LC-PUFA synthesis that has been identified in females (Burdge and Nagura, 2002). The authors proposed that the dose of DHA chosen for this study may not have been sufficient to elicit equivalent neurocognitive benefits in males and they considered whether enhancing DHA concentrations may evoke neurocognitive advantages (Makrides et al., 2009). Furthermore, the authors concluded that the DHA concentration in standard human breast milk of Australian women is sub-optimal for the visual development of preterm infants (Smithers et al., 2008b).

There have been numerous LC-PUFA supplementation trials in preterm infants which have reported greater visual acuity following enhanced dietary DHA relative to placebo (Birch et al., 1992; Carlson et al., 1993; Smithers et al., 2008b). Similarly, numerous RCTs in preterm populations have found that DHA supplementation positively affects neurocognitive outcomes including language comprehension (O'Connor et al., 2001), memory (Henriksen et al., 2008) and mental and psychomotor development (Clandinin et al., 2005). However, the review by Qawasmi et al. (2012) points out that there are also many RCTs which have consistently shown no neurocognitive effect, both in preterm and term infant populations. Similarly, Schulzke et al. (2011) concluded that there is insufficient evidence to recommend DHA supplementation with respect to routine neonatal care in preterm infants. The most recent Cochrane review of LC-PUFA supplementation of preterm formula concludes that there are no clear long-term benefits on visual or intellectual development after pooling data across studies of preterm infants (Schulzke et al., 2011). Finally, two recently published reviews reflect uncertainty as to whether preterm infants should be supplemented with dietary DHA with respect to potential long term neurocognitive or developmental benefits (Molloy et al., 2012; Lapillonne et al., 2013). Further investigation is clearly warranted concerning the role of DHA supplementation in modulating optimal neurocognitive outcomes in preterm infant populations.

GENETIC FACTORS MODULATING INDIVIDUAL DIETARY REQUIREMENTS FOR *n*-3 LC-PUFAs

A relatively new area of investigation concerns how genetic differences may modulate individual LC-PUFA requirements. Such research has focused on the genes FADS1 and FADS2. These genes are known to act upon the enzymes delta-5 desaturase (D5D) and delta-6 desaturase (D6D) and influence the efficiency with which shorter chain *n*-3 and *n*-6 PUFAs are converted into LC-PUFA products (Nakamura and Nara, 2004; Schaeffer et al., 2006; Rzehak et al., 2009; Bokor et al., 2010; Glaser et al., 2011). The D5D and D6D enzymes are present in the human liver from early gestation (Innis, 2005). The fetus is capable of synthesizing *n*-3 LC-PUFAs from shorter chain *n*-3 precursors from 26 weeks gestation (Uauy et al., 2000).

SNPs within FADS1 and FADS2 have been associated with the ratio of desaturation precursors (i.e., α-LA, LA, eicosadienoic acid (EDA; 20:2 *n*-6) and dihomogamma linolenic acid (DGLA; 20:3 *n*-6) to desaturation products, including the LC-PUFAs (EPA, DPA, and AA) (Schaeffer et al., 2006; Gillingham et al., 2013). In a very large German study conducted in an adult population, the authors found that up to 28% of AA variability was associated with 11 common SNPs (and 5 SNPs of reconstructed haplotypes) from the FADS1 and FADS2 gene clusters (Schaeffer et al., 2006). Yet, the FADS polymorphisms tested by Schaeffer et al. (2006) could only explain ∼7% of EPA and 3% of DHA levels. Few subsequent studies have been able to identify a significant association between FADS polymorphisms and DHA concentrations in human populations (Koletzko et al., 2011; Lattka et al., 2013).

However, Koletzko et al. (2011) identified significant associations between fetal and maternal FADS genotypes and DHA concentrations in fetal circulation, irrespective of maternal diet. While it currently appears that DHA concentrations are primarily modulated though dietary supply, several FADS genetic polymorphisms are still under investigation internationally (Glaser et al., 2011; Gillingham et al., 2013) and are currently being considered in our laboratory.

INTERPRETING CURRENT RESEARCH FINDINGS

Inconsistent findings within this literature have been the subject of much consideration. suggestions as to why discrepancies may have occurred are outlined in the review published by Meldrum et al. (2011). Previous studies were identified as having considerable variability in: (i) inadequate sample sizes, (ii) doses of DHA utilized, (iii) source of DHA used (i.e., algal or fish sources), (iv) age at which supplementation was initiated, (v) duration of supplementation, (vi) type of neurocognitive/developmental assessments used to evaluate neurocognitive functioning, and (vii) variability in participant compliance across studies. It was also speculated in this review that genetic polymorphisms might represent a potentially relevant factor affecting the outcomes of these RCTs. It is now recognized that dietary requirements for DHA and other LC-PUFAs may be somewhat heterogeneous across individuals; for example, being somewhat dependent on an individual's genetic profile (Koletzko et al., 2011).

In addition to FADS genetic considerations, the lack of consistency across RCTs of DHA supplementation may also be due, at least in part, to gender based differences in LC-PUFA metabolism from shorter chain precursors (Guesnet and Alessandri, 2011). It is known that the capacity to convert α-LA into *n*-3 LC-PUFAs including DHA is significantly greater in females than in males (Burdge, 2004), resulting in higher DHA circulating plasma concentration in females (Giltay et al., 2004). This is thought to be due to the influence of estrogen and other hormones on the activity and expression of D5D and D6D in the liver (Extier et al., 2010; Decsi and Kennedy, 2011). Evidence from the Western Australian pregnancy cohort (Raine) study ($n = 1038$) has also found long term neurocognitive benefits from breastfeeding to be gender specific (Oddy et al., 2011). This study found that breastfeeding had a more pronounced neurocognitive benefit on male infants, as evident until 10 years of age (Oddy et al., 2011). A gender specific response to DHA enriched human breast milk was also observed by Makrides et al. (2009) in the aforementioned DINO study of preterm infants. Females manifest a greater capacity for LC-PUFA synthesis (Burdge and Nagura, 2002), such that males may have higher dietary DHA requirements during infancy (Makrides et al., 2009).

CONCLUSIONS AND FUTURE DIRECTIONS

DHA is known to play a critical role in the developing human brain and there is evidence of its neurobiological importance during infancy (McCann and Ames, 2005). DHA deficiencies in animals have proven to exert deleterious effects on a range of neurodevelopmental outcomes (McNamara and Carlson, 2006). High intake of oily fish during pregnancy appears to benefit children's neurocognitive development. Additionally, several studies have demonstrated positive associations between infant blood concentrations of DHA and neurocognitive status (Innis, 2003, 2007). Yet previous RCTs undertaken in this field provide conflicting evidence concerning the putative neurocognitive benefits of *n*-3 LC-PUFA supplementation in healthy full term infants. Despite the consideration that the majority of RCTs report little or no effect from supplementation (as cited in several Cochrane reviews and meta-analyses), many researchers suggest that further work should be undertaken in order to better define optimal DHA intakes before and during infancy.

It is possible that DHA deficiency during critical periods of brain growth and structural organization may render the brain vulnerable to neurological or neurodegenerative diseases later in life (Farquharson et al., 1995). The development of the brain's architecture prenatally and during infancy lays down the foundation on which the structure of the adult brain is based. It is therefore possible that early modification of LC-PUFA levels will have long-term structural and functional consequences which may be too long-term and/or subtle to detect in healthy infants and children (McNamara and Carlson, 2006). However, more noticeable effects may emerge in older individuals. In this context, the equivocal findings obtained in this field to date are heuristic, as they will stimulate further research which should more rigorously control for the potentially confounding variables that have been identified in this review. As proposed by Alderson and Roberts (2000), inconclusive results are potentially very informative in biomedical science as they stimulate further inquiry and ultimately improve health outcomes for future generations.

While RCTs are generally thought to provide the most robust indexes of causal mechanisms in human clinical research (Szajewska, 2011), the results from observational studies in humans and intervention studies in laboratory animals also deserve serious consideration. In future, larger and higher dose RCTs should be undertaken using more sensitive measures of infant and child neurocognitive capacity. A more careful approach to the design and analysis of observational epidemiological studies is also likely to yield tangible benefit (for example, through undertaking larger studies which are able to consider a wider range of potentially confound variables).

Debate about the usefulness of dietary DHA during infancy has been ongoing for over 30 years and a wealth of data have been collected by research groups around the world. It would be of far-reaching benefit for more international collaboration to be undertaken within this area of inquiry. Data access facilitated through computational platform/s that enable sharing of de-identified, IPD could provide researchers with the means to carry out statistically novel methods of data interpretation on a very large scale. Meta-analysis of IPD allows researchers to analyze the raw data of relevant original studies (using similar covariates) and thereby derive potentially more reliable conclusions (Stewart and Parmar, 1993). To the best of our knowledge, only one IPD meta-analysis on the neurocognitive effects of LC-PUFA supplementation during infancy has been conducted (Beyerlein et al., 2010). This meta-analysis was able to access the raw data from 4 out of the 6 relevant RCTs. The limitations imposed on this IPD meta-analysis by the non-availability of data from two relevant RCTs could

be prevented in future if guidelines and policies are implemented within a collaborative international scientific framework that is more sensitive to ethico-legal and data-ownership issues.

Despite the absence of a scientific consensus with regard to putative benefits (as identified in this review), many manufacturers of infant formula include DHA and AA in certain formulations and market them as "superior products" which provide a distinct neurocognitive advantage (Simmer et al., 2011). This shaping of public opinion through retail is controversial, considering that there is little concrete scientific evidence to support these claims. Australia is one of the leading markets for *n*-3 products and supplements and, consequently, consumers are at increased risk of being misled by current marketing approaches (McManus et al., 2011). It is important for future research to address these claims definitively, so as to either avoid unnecessary supplementation of infants' diets (and the associated economic cost) or to ensure that infants are universally provided with adequate dietary DHA to prevent suboptimal neurocognitive development.

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Gluten- and casein-free dietary intervention for autism spectrum conditions

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Dietary intervention as a tool for maintaining and improving physical health and wellbeing is a widely researched and discussed topic. Speculation that diet may similarly affect mental health and wellbeing particularly in cases of psychiatric and behavioral symptomatology opens up various avenues for potentially improving quality of life. We examine evidence suggestive that a gluten-free (GF), casein-free (CF), or gluten- and casein-free diet (GFCF) can ameliorate core and peripheral symptoms and improve developmental outcome in some cases of autism spectrum conditions. Although not wholly affirmative, the majority of published studies indicate statistically significant positive changes to symptom presentation following dietary intervention. In particular, changes to areas of communication, attention, and hyperactivity are detailed, despite the presence of various methodological shortcomings. Specific characteristics of best- and non-responders to intervention have not been fully elucidated; neither has the precise mode of action for any universal effect outside of known individual cases of food-related co-morbidity. With the publication of controlled medium- and long-term group studies of a gluten- and casein-free diet alongside more consolidated biological findings potentially linked to intervention, the appearance of a possible diet-related autism phenotype seems to be emerging supportive of a positive dietary effect in some cases. Further debate on whether such dietary intervention should form part of best practice guidelines for autism spectrum conditions (ASCs) and onward representative of an autism dietary-sensitive enteropathy is warranted.

Keywords: autism, brain, gastrointestinal, gluten, casein, diet, intervention, intestinal permeability

INTRODUCTION

Pervasive developmental disorders are a complex, lifelong, heterogeneous group of conditions that variably affect the way a person communicates and interacts with people and the environment around them. Autism, Asperger syndrome (AS) and Pervasive Developmental Disorder—Not Otherwise Specified (more commonly known as autism spectrum disorder, ASD) reflect the current primary diagnostic classifications of the condition (World Health Organisation, 1992) although likely to change in revised diagnostic descriptions (Mattila et al., 2011).

The clinical presentation of the autism spectrum conditions (ASCs) as they are becoming known includes primary impairment in areas of: verbal and/or non-verbal communication, the use of reciprocal social interaction (cumulatively known as social affect) and the presence of repetitive or stereotyped behaviors. Various other behaviors may also be present as peripheral features including sensory-perceptual issues (Tomchek and Dunn, 2007) and gait and motor co-ordination problems (Whyatt and Craig, 2012).

The symptoms of ASCs are thought to result from a complex, variable interaction between genetics and environment (Grafodatskaya et al., 2010) though there is currently no genetic or biological test to diagnose the condition. The assessment of an ASC is carried out by detailed observation of overt symptoms combined with an analysis of developmental history according to prescribed criteria.

Contemporary research efforts are being directed away from the search for a condition-specific genetic factor to embrace a more cumulative model based on elevated risk as a function of smaller gene point mutations (Klei et al., 2012) given the heterogeneity present. Included in such a model is the growing realization that the label of autism is not representative of just one condition, but rather the presentation of similar symptoms across various conditions (Novarino et al., 2012). Recent moves to establish specific endophenotypes of ASCs, based on combinations of symptoms and presentation history, influence of co-morbidity, effectiveness of various management strategies, etc., is also gaining popularity (Nordahl et al., 2011).

Abbreviations: ADHD, Attention-deficit hyperactivity disorder; AS, asperger syndrome; ASC, autism spectrum condition; ASD, autism spectrum disorder; CD, coeliac disease; CF, casein-free; CNS, central nervous system; GFCF, gluten-free, casein-free; GFD, gluten-free diet; GI, gastrointestinal; PKU, phenylketonuria; RCT, randomized controlled trial.

ASCs are categorized as life-long conditions although there is evidence to suggest differential patterns of development may be present among cases reflective of some diagnostic instability (Fountain et al., 2012). There is a gender disparity in ASCs (Whiteley et al., 2010c). Alongside other developmental conditions (Centers for Disease Control and Prevention, 2010), the numbers of cases being diagnosed has increased in recent years (Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators; Centers for Disease Control and Prevention, 2012), thought primarily to be the result of changing diagnostic criteria and better case ascertainment. The role of the environment as part of any real increase in cases has however not been ruled out (Rutter, 2005; Weintraub, 2011) and indeed continues to garner support.

ASCs carry elevated risk for various other comorbid conditions including epilepsy and learning disability (Steffenburg et al., 2003). Such co-morbidities highlight the importance of the brain and neuronal functions to ASCs. Study of these areas has dominated both psychological and neuropsychological theories of aetiology and pathology with a focus on both structural and functional changes to be present.

Various other co-morbidities have been detailed as being over-represented in cases of ASCs. Gastrointestinal (GI) co-morbidities expressing as both functional symptoms and chronic underlying symptoms including coeliac disease (CD) (Barcia et al., 2008; Genuis and Bouchard, 2010) and indications of inflammatory bowel disease-type conditions (Ashwood et al., 2003) have also been reported alongside various nutritional indicators of for example, functional iron deficiency (Latif et al., 2002) in some cases. Although some guidance exists for the systematic inspection of such GI disorders in cases of autism (Buie et al., 2010), continuing discussions on the nature of the GI comorbidity present coupled with ethical concerns on the use of invasive medical procedures in cases of ASCs mean no reliable estimates on population comorbidity presently exist. Importantly a diagnosis of ASC is not currently thought to confer protection against the development of any other health or psychiatric condition over a lifetime.

There is at present no universal intervention for reducing/minimizing the more disabling overt symptoms of ASCs and improving developmental outcomes and quality of life indicators. Society has an important role in the provision of appropriate health, education, and employment opportunities in such a process. Existing best practice guidelines for intervention and management strategies are aimed at ameliorating or managing core and peripheral symptoms based on specialized education and behavioral training (Volkmar et al., 2004). The individual, and their strengths and weaknesses, is an important focus. The emphasis lies in the application of early years intervention aimed at improving developmental outcome where optimal results have, in some cases been suggested to impact on neuronal functioning such as cortical activation (Dawson et al., 2012). Such research is reflective of the perceived plasticity of early development and brain function.

The use of various medications as part of pharmacotherapy is also relatively commonplace for ASCs (Francis, 2005). Such medical intervention provides an important service where comorbid features such as epilepsy are present, but with newer compounds also increasingly looking to address more core features too (Oberman, 2012). At the current time, there is however no single drug or universal medication strategy to treat the condition and its entire range of symptoms.

As with many other cognitive and/or developmentally-defined conditions, specific groups of people with ASCs seem to be at increased risk of various problems associated with eating and diet (Kalyva, 2009). Whether as a consequence of core symptoms based on the variable presentation of inflexible patterns of behavior, issues with fine and gross motor skills or as a result of underlying intolerances to various foodstuffs, several dietary-related issues can be apparent (Martins et al., 2008). Corresponding anthropometric growth measures of people with ASCs have not yet determined any consistent trend as being present as a result of such feeding issues. It has been reported that measures of weight and calculated body mass index (BMI) can present as aberrant in cases of autism (Whiteley et al., 2004; Curtin et al., 2010) seemingly echoing UK and other population trends.

Some people with ASCs have been reported to show an improvement in core and peripheral symptoms following the adoption of specific exclusion diets and/or the variable use of nutritional supplements such as vitamins (Adams et al., 2011), minerals and fatty acids. A diet devoid of gluten (the major protein in wheat, barley and rye) and/or casein (derived from mammalian diary produce) has been one of the more popular interventions suggested to show some effect. This document aims to: (1) summarize the main experimental research carried out on the use of a gluten- and/or casein-free (GFCF) diet for ASCs, (2) summarize the main effects reported following dietary exclusion, (3) highlight the various safety issues associated with dietary use, and (4) discuss the most current theories potentially explanatory of a dietary effect. Although it is beyond the scope of this document to examine all the research conducted on the use of GFCF diets for ASCs, specific studies will be highlighted on the basis of their importance to the research timeline, methodology employed and overall contribution to knowledge.

DIETARY STUDIES: WHAT IS THE EVIDENCE FOR EFFECT?

Notions regarding the potential for a gluten-free diet (GFD), casein-free diet (CFD), or combined gluten- and casein-free diet (GFCF) to affect the symptoms of ASCs have persisted for many years. Much of the impetus and scientific rationale for the use of such dietary interventions originally stemmed from: (1) models approximating a relationship between food and ASCs with that of dietary related in-born metabolic conditions such as Phenylketonuria (PKU) and (2) dietary investigations suggestive of amelioration of overt symptoms in conditions such as schizophrenia (previously linked to autism) and other psychiatric disorders (Dohan et al., 1969).

The first ever formal description of autistic symptoms contains reference to GI symptoms and dietary issues being present in some cases (Kanner, 1943). Early ideas speculating on a potential link between diet and ASCs were strengthened by some of the writings of Hans Asperger, who provided the initial descriptions of AS, and a suggestion of a relationship between AS and CD (Asperger, 1961). Notwithstanding such potential associations,

early research attempting to validate any universal link between ASCs and CD were in the most part unsuccessful (Pavone et al., 1997) although retaining the possibility of a connection between a proportion of cases of ASC and co-morbidity of CD (Barcia et al., 2008; Genuis and Bouchard, 2010). Contemporary use of a diet devoid of gluten and/or casein for ASCs is now considered to be widespread; despite no formal published guidelines yet accepting dietary intervention as a viable intervention strategy for the condition.

Meta-analyses of the specific findings of the various trials of such dietary intervention for ASCs published in the peer-reviewed scientific literature have been summarized by several authors (Knivsberg et al., 2001; Mulloy et al., 2010, 2011) including the Cochrane Library of Systematic Reviews (Millward et al., 2008). The main conclusions from such meta-analyses suggest caution in the universal adoption of GFCF dietary intervention for ASCs whilst stressing the need for further controlled research to ascertain any significant effect. A thorough examination of the individual evidence included in these texts is beyond the scope of this document. Several pertinent and additional studies published after the Cochrane review (post-2008) do, however, necessitate further description.

In the early 1990s Knivsberg, Reichelt and colleagues based at various sites in Norway published initial and follow-up behavioral and psychometric data for a small group of people ($n = 15$) with ASCs on a GFCF diet (Knivsberg et al., 1990, 1995). For many, these studies were the first primary evidence for the potential effectiveness of a GFCF diet for ASCs adding scientific validity to the array of anecdotal observations previously described, and strengthened by the long period of dietary exclusion between publications. The downside to these initial studies lay predominantly with the open, non-randomized methodology employed together with a lack of suitable blinding; thus introducing potential bias into the interpretation of results obtained.

The Norwegian team have subsequently been involved in further experimental studies of GFCF dietary intervention for ASCs. Two of these studies (Knivsberg et al., 2002; Whiteley et al., 2010a) were randomized controlled trials (RCTs) lasting for 1 and 2 years respectively. Both studies indicated significant positive group effects on several measures of behavior and development indicative of potential improvements to symptoms for some children with ASCs on diet.

One of these RCTs (ClinicalTrials.gov NCT00614198) published in 2010 (Whiteley et al., 2010a) was not included in the most recent Cochrane Library Review (Millward et al., 2008) given its publication date. This study known as "ScanBrit" used an adaptive study design responsive to intermediate analysis of results (a "drop-the-loser" design) to analyze any dietary effect ($n = 72$). The main findings indicated statistically significant changes to both core and peripheral behaviors in the diet group in the first 12 months of study followed by indications of a plateau effect of diet following 12 months further study. Results also indicated a substantial degree of variability in individual response to intervention.

Another recent single-blind investigation of potential dietary effect (Johnson et al., 2011) ($n = 22$) reported no overall difference between diet and non-diet groups following 3 months of study. This despite finding some gains in areas previously described by Whiteley et al. (1999, 2010a) and others related to dietary intervention. Anecdotal but numerous clinical observations leading up to the formal studies of Reichelt and Knivsberg (Knivsberg et al., 1990, 1995) indicated that GFCF dietary intervention needed to be implemented for at least 6 months before one could reasonably assess response or not and why the subsequent ScanBrit trial (Whiteley et al., 2010a) used a considerably longer implementation period.

Double-blind RCTs of a GFCF or individual CFD or GFD intervention for ASCs are currently few in number; primarily as a result of the cost involved to perform such a study and issues on how to ensure a double-blind methodology is implemented and adhered to. One group (Elder et al., 2006) has reported results from a double-blind trial; another group reported double-blind results following dietary challenge (Lucarelli et al., 1995). A further trial is cited as on-going (Diet, and behaviour in young children with autism, 2012), but at the time of writing has not been published in the peer-reviewed literature. The results from Elder et al. (2006) suggested no significant group effects as a result of dietary intervention in place. Whilst methodologically sound, this trial has however been criticized over the small participant group ($n = 15$) used, measures of dietary adherence and the short study period (6 weeks on diet and 6 weeks of no diet). The trial by Lucarelli et al. (1995) contained a double-blind element during dietary challenge and is one of two trials where investigations into whether a GFD or a CFD alone may have any effect for people with ASCs were carried out. Lucarelli et al. examined the effects of a CFD ($n = 36$). They reported an improvement in group behavior scores of autistic behaviors after 8 weeks of intervention. They also reported a worsening of autistic symptoms when a casein-challenge was introduced. Whiteley et al. (1999) measured response to a GFD alone over a period of 5 months during an open trial ($n = 22$). Results were slightly less clear in this study despite some indications of significant improvements to autistic symptoms in specific participants. Again, variability in response to intervention was reported amongst the participant group.

On the basis of these and other smaller trials, the experimental research base examining the use of a GFCF diet for ASCs can most accurately be described as mixed yet broadly suggestive of decreased autistic symptoms and improved developmental outcome for some individuals. These findings are complemented by other more survey-based research (Pennesi and Klein, 2012). The main caveat being that methodological issues associated with various forms of bias still persist to potentially confound experimental results. Such biases include: a lack of placebo conditions in trials, small participant numbers, short trial duration, problems associated with the outcome measures used and problems with the monitoring of dietary adherence.

An additional important issue not yet adequately covered by many of the dietary studies completed so far relates to the measurement of clinical *vs.* statistical significance; that is analyzing day-to-day performance of individuals on diet and determining what (if any) positive changes are present that increase quality of life and overall daily living and functioning for individuals rather than just providing statistical evidence of effect.

WHAT KIND OF EFFECTS ARE OBSERVED?

At the time of writing, there is no universal consensus on the type of effects experimentally observed following successful outcome from a GFCF diet for ASCs. Taking the various studies of diet into account, reported positive effects can be broadly categorized into several areas to include core autism and peripheral symptoms:

- Communication and use of language (Knivsberg et al., 1990, 1995, 2002; Lucarelli et al., 1995; Whiteley et al., 1999, 2010a; Johnson et al., 2011).
- Attention and concentration (Knivsberg et al., 1990, 1995, 2002; Lucarelli et al., 1995; Whiteley et al., 1999, 2010a).
- Social integration and interaction (Knivsberg et al., 1990, 1995, 2002; Whiteley et al., 1999, 2010a).
- Self-injurious behaviour/altered pain perception (Knivsberg et al., 1990, 1995; Lucarelli et al., 1995; Whiteley et al., 1999).
- Repetitive or stereotyped patterns of behaviour (Knivsberg et al., 1990, 1995, 2002).
- Motor co-ordination (Knivsberg et al., 1990, 1995; Whiteley et al., 1999).
- Hyperactivity (Whiteley et al., 2010a; Johnson et al., 2011).

There have also been suggestions of potential variable abatement of co-morbid conditions such as epilepsy and seizure-type disorders (Knivsberg et al., 1990, 1995) following dietary use for ASCs and coincidental seizure activity following reinstallation of a gluten-load (Whiteley et al., 1999). Similar case studies describing a reduction of seizure activity have also been reported in CD following use of a GFD (Pratesi et al., 2003). Changes to anti-epileptic or other medication as a result of the introduction of such dietary intervention for ASCs have not been advocated without consultation with the supervising medical physician. Whether such dietary intervention represents an alternative treatment modality for some forms of epilepsy independent of ASC co-morbidity has also not been investigated. The use of a ketogenic diet in respect to specific types of treatment resistant epilepsy (Lee and Kossoff, 2011) and also autism (Evangeliou et al., 2003) may potentially offer some clue to effect given the likely overlap between dietary regimes.

Within the spectrum of cases of ASCs, anecdotal reports of responders and non-responders to dietary intervention persist, although no universal criteria to account for response differences has yet been formulated. Given the heterogeneous spectral nature of ASCs, it is highly unlikely that everyone will benefit from such a dietary change.

Chronological age is thought to be a factor in response. Indeed, the experimental studies conducted thus far have predominantly looked at dietary response in children and young adults with ASC. Effects are thought to be similar to the ethos behind other more educationally and behaviorally-based interventions, where younger children are reported to show more pronounced effects from diet. Whether this is due to plasticity and maturational factors in brain function for example or purely coincidental as a function of known diagnostic instability at younger ages (Charman et al., 2005) is unknown at the current time.

Anecdotal reports of improvements to some of the symptoms of ASCs following introduction of a GFCF diet where functional bowel problems (diarrhoea, constipation, alternating stools) have emerged. There is some evidence to corroborate a potential connection between ingestion of specific dietary components such as dairy products and the presence of functional GI problems in ASCs (Afzal et al., 2003). Further investigations are however required, and indeed on-going, into whether this forms universal criteria for positive response to diet (ClinicalTrials.gov NCT01116388) (A study to assess the role of a gluten free-dairy free (GFCF) diet in the dietary management of autism associated gastrointestinal disorders, 2012).

RISKS AND SAFETY ISSUES

The use of a GFCF diet for ASCs carries a number of potential risks. Current, best evidence suggests that whilst the effects of dietary intervention may largely be apparent during the first year of intervention (Whiteley et al., 2010a), there appears to be a continued requirement for the diet to be in place for much longer assuming initial positive effects are witnessed (Knivsberg et al., 1995).

Whilst potential nutritional deficiencies in ASCs are a major cause for concern (Arnold et al., 2003)—for example, calcium intake following the exclusion of dairy products—the limited investigations completed so far suggest that with suitable support, dietary intake need not be adversely affected by introducing such a diet (Cornish, 2002; Adams et al., 2008). The increasing range and availability of GFCF foods may help alleviate the feeding problems described in ASCs based on limited product range and other personal preferences (taste, texture, etc.). Further investigations are however, required on the basis of nutritional value and fat, protein and sugar content of such alternative foods (Mariani et al., 1998) specifically where anthropometric measures of dieters may already be irregular.

Anthropometric information following GFCF dietary use in ASCs is sparse. Allowing for geographical and ethnic differences, case study reports suggest a trend toward normalization of growth parameters following dietary intervention (Hsu et al., 2009) although no large scale studies have yet been conducted.

Pathology following the use of a GFCF diet has been suggested; specifically related to bone health and use of a CF diet in ASCs (Hediger et al., 2008). There is continuing debate as to whether this is due to specific deficiencies as a function of dietary exclusion, a consequence of abnormal eating patterns in ASC generally or part of a broader physiological problem with the absorption of nutrients associated with the condition (Clark et al., 1993; Stewart and Latif, 2008; Herndon et al., 2009) particularly where bowel or malabsorptive issues may already be present. Accompanying issues with functional levels of important vitamins linked to calcium homeostasis such as vitamin D have also been identified (Neumeyer et al., 2012).

Whilst not specifically a safety issue of the GFCF diet, the use of various nutritional supplements as part of the dietary regime alongside dietary exclusion also requires comment. Children following a GFCF diet are perhaps more likely to be also following other complementary and medicine (CAM) approaches at the same time as their diet, particularly when GI comorbidity is also apparent (Perrin et al., 2012). Bearing in mind the often intricate

balance required between specific vitamins and minerals (e.g., calcium supplementation affecting iron absorption; Cook et al., 1991), professionals have been advised to be mindful of such adjunctive interventions.

Finally, as with any potential intervention for ASC, great thought is required into the "necessity" of such an intervention and the likely cost/benefit ratio to individual users given the current lack of formal best-responder data. Unlike more traditional conditions where such dietary interventions are employed, people with ASCs may not be able to readily understand why such a diet is being used or communicate any preference on its application or not. Indeed, food and established feeding patterns may be a great source of comfort, stability, routine and coping to some; use of a GFCF diet may likely upset some people with ASCs especially during the early days of intervention. In such cases, great care is required to involve all persons potentially affected by such dietary changes (person, family, school, support services, etc.) to ensure appropriate monitoring with regards to effectiveness and safety; also potentially including observations on dietary compliance.

POTENTIAL MODES OF ACTION

At the current time, no universal theory has been accepted to account for the effect (or non-effect) of GFCF dietary intervention on behaviour and development in ASCs. Given the heterogeneity observed in the presentation of overt symptoms in ASCs, it is likely that more than one model of dietary effect may pertain in different cases. Whiteley et al. (2010b) summarized the main hypotheses commonly ascribed to dietary success/non-success. As per previously, ASCs are not thought to be protective of co-morbid conditions that may have a dietary link where for example, low levels of co-morbid PKU and ASC have been reported (Baieli et al., 2003).

Indeed, an early analogy with PKU had been put forward (Seim and Reichelt, 1995) in relation to GFCF diets focusing on the cumulative effects of protein and peptide aggregates crossing the blood-brain barrier to exert a neuronal action, stressing a collective, chronic effect rather than an acute action.

The possibility of an underlying metabolic condition being connected to dietary response has been further extended (Shattock and Whiteley, 2002) from conditions such as schizophrenia (Dohan et al., 1969). The theory suggests that abnormal porosity of the intestinal wall (gut hyperpermeability or leaky gut) and potentially other membranes throughout the body, combines with inadequate hydrolysis of dietary proteins to produce onward effects to the central nervous system (CNS).

Some support for the model has been published; specifically preliminary indications of peptiduria (Reichelt et al., 2012) appearing in cases of autism coinciding with antibody production to peptides (Vojdani et al., 2004) and the effects of administration of specific dietary-derived peptides on behavior (Sun and Cade, 1999) and neuronal functioning (Sun et al., 1999) in animal models. A role for incomplete elimination of bovine-derived peptides impacting on psychomotor development and autism has also been reported (Kost et al., 2009).

Alongside, gut hyperpermeability has been reported in approximately a quarter to a third of children with an ASC examined (D'Eufemia et al., 1996; Boukthir et al., 2010; de Magistris et al., 2010) although not universally so in all investigations (Robertson et al., 2008) complemented by findings in other, more GI-related conditions (Cummins et al., 1991). Importantly also, there are indications of a reduction of GI permeability in those cases where a GFCF diet has been implemented in cases of autism (de Magistris et al., 2010) similar to processes described in CD (Cummins et al., 1991). This point in particular may also account for the findings reported by Robertson et al. (2008) of no abnormal permeability in their cohort, who crucially included participants already following a special diet at the time of sampling. A role for inflammation and inflammatory signaling and processes similar to those described in cases of schizophrenia (Severance et al., 2012a) requires further investigation as do potential issues governing the integrity of the intestinal barrier via sulphonation (Bowling et al., 2012), tight-junction modulators (Fasano, 2012) and any contributing role for pathogenic agents (Severance et al., 2012b).

The gut-brain model in its entirety however has not yet been fully validated, specifically with regards continuing dispute on the detection of dietary-derived peptides in biological fluids as evidence of abnormal protein metabolism (Cass et al., 2008). There is preliminary evidence suggestive of potentially relevant compounds present in urine correlating with suggested best responder characteristics (Wang et al., 2009) although further investigations are warranted. The implications of such findings for screening and recommendations of potential dietary effectiveness are therefore the source of continuing debate.

Focus has also shifted to more fundamental problems with carbohydrate metabolism as potentially being implicated in a dietary effect. Williams et al. (2011) reported on decreased mRNA expression for disacharidases and hexose transporters present in cases of ASCs. This follows on from earlier research hinting at reduced dissacharidase activity (Kushak et al., 2011) potentially indicative of underlying lactose intolerance to be present. Combined with a suggestion of some involvement for the composition of GI bacterial species in cases of ASCs (Parracho et al., 2005; Clayton, 2012) and possible effects from bacterial translocation, this remains an area in need of further investigation.

Various individual accounts of CD and ASCs have been documented (Barcia et al., 2008; Genuis and Bouchard, 2010). Genuis and Bouchard (2010) detailed the rapid resolution of GI symptoms and corresponding abatement of autistic symptoms following implementation of a GFD. Similar case reports have been highlighted with regards to schizophrenia and overlapping CD, together with documented brain imaging changes (De Santis et al., 1997). Likewise indications of specific allergies to foods such as gluten and casein in some people with ASCs have also been highlighted (Lucarelli et al., 1995; Jyonouchi et al., 2002). Additional studies incorporating the exclusion of dietary gluten and casein in related conditions such as attention-deficit hyperactivity disorder (ADHD) have also noted positive effects on symptoms (Pelsser et al., 2011) particularly in cases of overlapping CD (Niederhofer, 2011) where both somatic and psychological presentation were affected. Combined however, such co-morbidities are not thought to be able to account for all cases of success despite no commonplace screening for such potential issues in ASCs and the possibility of non-CD mediated sensitivities (Biesiekierski et al., 2011).

CONCLUSIONS

Experimental studies on the use of a GFD, CFD, or combinatorial GFCF diet for ASCs have suggested an amelioration of symptoms and improved developmental outcome for at least a proportion of people on the autistic spectrum. That being said, various methodological issues potentially biasing results remain which, combined with a lack of generalizable information on mode of action and best-responder data, have limited the impact of such findings over the years.

More recent controlled longitudinal studies examining group dietary effectiveness alongside an increasing recognition of individual cases of food-related co-morbidity and evidence of more consolidated biological mechanisms potentially at work, offer a favorable evidence base for at least a partial effect of diet on some cases of ASCs.

There is a continued requirement for further study on the potential role of dietary intervention for ASCs. Future controlled trials including blinded and placebo elements are necessary carrying appropriate power of study by sample size and duration. Based on the significant heterogeneity present in ASCs and the likelihood of various "autisms" manifesting similar presentation, further thought should also be given to the concept of best- and non-responders to this type of intervention. So for example, (1) screening for GI and/or potentially relevant pathogenic comorbidity, (2) measuring gut hyperpermeability, (3) examining gut microbial populations and food-related enzyme activities, and (4) ascertaining the presence of inflammatory processes, either peripherally in GI tissue or more centrally, might all be included as parameters for future dietary investigations. Similarly, measuring any relationship between behavior and GI function over the course of dietary intervention may offer some information about any connection between these factors.

Given the evidence hinting at neurological changes following the implementation of dietary intervention in related conditions, future research might also benefit from looking at brain structural and biochemical changes in cases of ASCs adopting dietary intervention. Indeed, the gut-brain relationship, seemingly so important to explaining the role of dietary intervention in best-responder cases, is a woefully under-researched area with ASCs in mind.

Finally but perhaps just as important, is a need to focus on the measurement of clinical changes to symptoms alongside statistical changes to psychometric or other assessment tools in view of the restrictiveness of the dietary regime. This point in particular reflects the fact that not everyone who might potentially benefit from dietary intervention will necessarily be able to implement such a restrictive regime, or indeed, want to.

The growing emphasis on various phenotypes for ASCs provides a template for conceptual changes to the way ASCs are viewed; where a 'diet-related autism phenotype' may be a target for future research and indeed a marker for efficacy of dietary intervention. Further discussions on whether such dietary intervention should form part of best practice guidelines for ASCs and onward representative of an autism dietary-sensitive enteropathy is warranted.

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Conflict of Interest Statement: Kevin Carr, Malcolm Hooper, and Paul Whiteley are directors of ESPA Research, a UK subsidiary organization which carries out research into ASCs including investigations on the use of a gluten- and casein-free diet as an intervention for autism and related conditions. Lynda Todd is an employee of ESPA Research. Paul Shattock is Chairman of ESPA (Education and Services for People with Autism) and has a son with autism. Malcolm Hooper is also a Trustee of ESPA. Kevin Carr, Paul Shattock, and Paul Whiteley are directors and shareholders of Analutos Ltd. in the UK which provides mass spectrometric and other analytical services to various sectors of the healthcare, chemical and pharmaceutical industries. Karl Ludwig Reichelt is an unpaid consultant to Biomedical Laboratory in Norway providing mass spectrometric and other analytical services to various healthcare industries. Ann-Mari Knivsberg and Anders Seim declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Subjective thirst moderates changes in speed of responding associated with water consumption

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Participants ($N = 34$) undertook a CANTAB battery on two separate occasions after fasting and abstaining from fluid intake since the previous evening. On one occasion they were offered 500 ml water shortly before testing, and on the other occasion no water was consumed prior to testing. Reaction times, as measured by Simple Reaction Time (SRT), were faster on the occasion on which they consumed water. Furthermore, subjective thirst was found to moderate the effect of water consumption on speed of responding. Response latencies in the SRT task were greater under the "no water" condition than under the "water" condition, but only for those participants with relatively high subjective thirst after abstaining from fluid intake overnight. For those participants with relatively low subjective thirst, latencies were unaffected by water consumption, and were similarly fast as those recorded for thirsty participants who had consumed water. These results reveal the novel finding that subjective thirst moderates the positive effect of fluid consumption on speed of responding. The results also showed evidence that practice also affected task performance. These results imply that, for speed of responding at least, the positive effects of water supplementation may result from an attenuation of the central processing resources consumed by the subjective sensation of thirst that otherwise impair the execution of speeded cognitive processes.

Keywords: cognition, water, performance, mood, thirst

INTRODUCTION

Does having a drink help you think? Anecdotal evidence suggests that water consumption can help cognitive performance and recent research has supported this folk wisdom. This paper reports a study that examines the effect of water supplementation on cognitive performance and mood in adults, and examines whether there is a moderating effect of thirst. While the literature on the effects of water supplementation on cognition is rapidly growing, it is currently not vast. Therefore, this introduction will review the complementary literature on the negative effects of dehydration on cognition and mood, and the effect of additional water on cognition and mood in both adults and children.

There is some, if conflicting, evidence to suggest that dehydration negatively affects cognitive performance in adults (Benton, 2011). For example, dehydration to more than 1% loss of body weight resulted in poorer performance on a visual vigilance task and slower reaction times on a working memory task (Ganio et al., 2011). Some have found evidence suggestive of a dose response effect, with performance decreasing with increasing levels of dehydration (Sharma et al., 1986; Gopinathan et al., 1988). However, others have found that dehydration due to water deprivation does not affect cognitive performance (Szinnai et al., 2005), perhaps because dehydration caused by water deprivation takes some time to develop and participants may adapt during this period. Subjective ratings of cognitive performance and mood have been shown to be affected by dehydration. For example, reported alertness, and concentration worsen in dehydrated individuals (Shirrefs et al., 2004; Szinnai et al., 2005). Furthermore, adults judged that they were more fatigued and anxious when dehydrated (Ganio et al., 2011).

There are fewer studies assessing the effects of dehydration on cognition and mood in children; this is in part because it is not viewed as morally acceptable to deliberately dehydrate children. Thus, those studies that have been conducted have focused on children who happen to be dehydrated because they live in a hot climate. Studies conducted in Israel (Bar-David et al., 2005) and Italy (Fadda et al., 2012) have reported that a large proportion of children arrive at school in a dehydrated state (63 and 84%, respectively), and that there is a relationship between hydration status and memory, with children who are dehydrated having shorter digit spans than those who are better hydrated. Recent evidence suggests that around two-thirds of children in more temperate climes, including the UK, France, and the USA, may arrive at school dehydrated (as measured by urine osmolality over 800 mOsmol/kg of water) (Bonnet et al., 2012; Friedlander, 2012; Stookey et al., 2012), but these studies did not examine associations with cognitive performance.

Given that dehydration negatively affects cognitive performance, one might expect that supplementing with fluid would improve performance. In contrast to the few studies examining the effect of dehydration on cognitive performance in children, the majority of research on the effects of water supplementation on cognition has examined children. Offering children additional drinking water has a positive effect on their cognitive

performance, particularly on tasks that require speeded processing or memory. For example, children performed better on tests assessing visual memory (measure by a consecutive spot the difference task) if they had consumed water (250 ml offered) (Edmonds and Burford, 2009). Similarly, children's verbal recall of objects was better on the occasion on which they consumed additional water (300 ml offered) (Benton and Burgess, 2009). Performance on tasks requiring visual attention and processing speed (letter cancellation) seem particularly sensitive to water supplementation (Edmonds and Burford, 2009; Edmonds and Jeffes, 2009; Booth et al., 2012). Reaction time has also been shown to be sensitive to water supplementation in children (Booth et al., 2012). One constant across the letter cancellation and reaction time tasks is that they both require speeded processing and fast responding. Thus, the current study includes a series of measures that assess these cognitive processes.

The cognitive performance of adults has also been shown to be improved by water supplementation. For example, performance on a rapid visual information processing task was improved by water consumption in a dose-dependent manner (120 or 330 ml offered), but only in those individuals who rated themselves as thirsty before drinking the water; if participants had low thirst initially, consuming water resulted in poorer performance (Rogers et al., 2001). Speeded processing has been shown to be improved by water consumption (200 ml offered), at both 20 and 40 min post consumption, while digit span and reaction time was not found to be affected by additional water (Edmonds et al., 2013). However, a third study found no relation between supplementation (120 ml or 300 ml offered) and performance on a range of cognitive tests, even when participants were grouped by initial thirst level (Neave et al., 2001). Water supplementation has not been found to impact on subjective measures of mood (Edmonds et al., 2013).

The present study investigated the effect of water supplementation on cognitive performance and mood in adults. We also considered whether subjective thirst moderates the relation between water supplementation and cognitive performance and mood. Given the range of cognitive processes shown to be affected by dehydration and water supplementation, a battery of tasks was administered via CANTAB. We controlled for baseline hydration status by having participants fast overnight before attending their test sessions, with no fluids being consumed since the previous evening. We expected water supplementation to result in improved performance on some of the cognitive test battery, and that thirst may mediate the effect of water.

METHODS

PARTICIPANTS

Thirty-seven participants (25 female) were recruited. One did not return for the second testing session and so their results were discarded. The mean age was 29 years (range 20–53 years; $SD = 8.3$ years).

MEASURES

Thirst scale

Our thirst scale asked, "how thirsty are you?" and participants marked a line to indicate their response. The line was labeled, "not hirsty at all" and "very thirsty" at opposite ends. A high score indicated greater subjective thirst.

Mood scale

The Visual Analogue Mood Scale (VAMS) (Stern, 1997) was used to assess the participant's mood. For each of 8 emotions, participants marked a line to indicate the extent to which they felt the emotion. The emotions assessed were afraid, confused, sad, angry, energetic, tired, happy, and tense; a higher score indicates a higher rating for each emotion.

CANTAB

The Cambridge Neuropsychological Test Automated Battery (CANTAB) (Sahakian and Owen, 1992) is a computer administered battery of tasks. After a brief practice exercise using the touch screen, nine tests (described below) were administered. Standardized instructions were read out before commencement of each test. The order was the same for all participants and parallel versions were counterbalanced across testing sessions.

Simple Reaction Time (SRT) measures participants' reaction time to a known stimulus at a known location. Using their dominant hand, participants pressed a button as quickly as possible after a square was presented on the screen. The analaysed output variable is mean reaction time.

Paired Associate Learning (PAL) assesses visual memory and visual learning. During a presentation phase, six boxes randomly opened and closed one by one to reveal either a pattern or nothing. During the test phase one of the patterns previously shown was presented. Participants touched the screen to match the pattern to the box where it was located previously. This was followed until all six patterns were identified. The number of patterns increased with each trial and terminated after ten consecutive fails. Output variables included in the analyses are total errors and stages completed.

Verbal Recognition Memory (VRM) assesses both immediate and delayed memory of verbal material under conditions of free recall and forced choice recognition. During a presentation phase, 12 words appeared one by one and participants read each aloud; they were instructed to remember the words. In the first test phase, participants recalled the words without feedback. In the second test phase, half of the words were those presented previously and the remainder were distractors (total $n = 12$). They were presented one at a time and participants touched the screen to indicate whether or not the word was in the original list. The output variable is the number of words correctly identified.

Big Little Circle (BLC) tests comprehension, learning, and reversal. It is a training test for the Intra/Extradimensional set shifting task (IED) and participants follow and then reverse a simple rule. Two boxes were presented, each containing a circle. Initially, participants were instructed to touch the box containing the little circle. After a number of trials, they were then instructed to touch the box containing the big circle. The analysed output variable is percentage correct.

Intra-Extra Dimensional Set Shift (IED) tests rule acquisition and reversal. Participants must make visual discriminations, maintain attentional sets and have shifting and flexible attention. In this task four boxes were simultaneously presented on the

screen, two of which contained a pattern. After touching one of the patterns, participants were given feedback that was used to ascertain the rule that was being used to present the patterns. The rule changes after several correct answers and participants must learn the new rule. The duration of the test varies per person, and the test ends if responses are continually incorrect. The output variables are total trials and total errors.

Rapid Visual Processing (RVP) is a test of sustained attention. Single numbers were presented rapidly in a box in the center of the screen. During the practice trials, participants pressed a button when they saw the last digit in a target sequence of numbers (i.e., 3, 5, 7). In the test phase, participants had to respond to three target sequences in the same manner. The test lasted for 4 min. The output variable is total correct hits.

Verbal Recognition Memory 2 (VRM2) is a forced choice recognition test. This task was presented approximately 20 min after the first VRM test with the same procedure as that used in the second test phase, but with a different set of twelve distracter words. The variable analysed is total correct.

Choice Reaction Time (CRT) is similar to SRT, but with the addition of stimulus and response uncertainty, brought about by two possible stimuli and two possible responses. It assesses motor speed and general alertness. In this task an arrow appears on the screen pointing to the left or the right. Participants had to rapidly press a left or right button to indicate which way the arrow was pointing. There are two outcome variables; mean reaction time for items appearing on the right and mean reaction time for those appearing on the left.

PROCEDURE

Participants took part in the "water" and "no water" condition one week apart. The order of conditions was counterbalanced. In both conditions participants fasted overnight and were instructed to consume no food or drink after 9.00 pm on the evening preceding testing. A pre-screening medical questionnaire was used to identify and exclude any participants for whom this may cause problems, including those with conditions such as kidney problems, diabetes, or pregnancy, or any other condition that would prevent them from doing an overnight fast.

On the day of testing, participants arrived in the morning. After informed consent was taken, all participants were offered a cereal bar to eat (two fruit options, each 117 kcal). When participating in the water condition, they were offered a 500 ml bottle of water to drink. They were encouraged to have a "big drink," and then the bottle was removed to prevent the participant from having any further water during the testing session. Any remaining water was measured in order to calculate the amount of water drunk. If participants drank the whole 500 ml and asked for more, they were offered a second bottle. When participating in the no water condition, participants were offered nothing.

Participants then filled in the VAMS and thirst scale[1], and completed the CANTAB tests. At the second testing session, participants took part in the other water condition and tasks were presented in the same order. At the end of the second test session, participants were debriefed and given a £30 gift voucher for their time. CANTAB testing and the mood scales took approximately 1 h to complete.

ETHICS

This study was approved by the University of East London ethics board. Informed consent was obtained from each participant prior to the study commencing.

STATISTICAL ANALYSIS

The primary aim was to investigate the effect of water supplementation on cognitive performance and mood, while taking into account potential effects of the order of water and no water conditions. The omnibus analyses consisted of a series of mixed model Analyses of Variance (ANOVAs) that were conducted for each outcome variable, for which WATER (water, no water) was a within subjects factor, and ORDER (water first, no water first) was a between subjects factor.

Follow up analyses were conducted that investigated whether thirst moderates the effect of water supplementation on cognition. Participants were grouped according to a median split of thirst ratings.

RESULTS

There were three exclusions; one participant did not complete the testing at both time points and two were administered the same CANTAB form at both time points in error. The final sample size was 34 participants (25 female). In terms of order, equal numbers had the water or no water test first. In terms of CANTAB version, 18 had version A first. There was no confounding: under each CANTAB order, half had the water test first.

All participants except one reported fasting since 9.00 pm the previous evening. The one participant who reported breaking the fast, had a small drink at 9.30 pm, and was not excluded because this was consumed very close to the 9.00 pm deadline. Participants drank a mean of 775.44 ml water ($SD = 464.00$; range 120–2500 ml) with breakfast when they were in the water condition.

WATER CONSUMPTION AND ORDER

The initial analyses employed a mixed model ANOVA of WATER (water/no water) × ORDER (water first/no water first), conducted separately for thirst, mood scales, and each CANTAB test.

Thirst and mood scales

Means and SDs for thirst and mood scale ratings are presented in **Table 1**, along with the results of the omnibus statistical analysis. Participants rated themselves as having greater subjective thirst on the occasion on which they were not offered water; there was no effect of ORDER and no interaction.

In the case of the VAMS, most of the responses were not affected by WATER or ORDER. There were effects of ORDER on scales that asked participants to rate whether they were confused, sad, or tense; participants rated themselves as more tense,

[1]Bioelectrical impedance was recorded at this point, with the aim of assessing hydration status. Unfortunately, these recordings were not reliable, and will not be reported further.

Table 1 | Thirst and mood scales means, SDs, and F ratios by water condition (water/no water) and order (water first/no water first).

Scale	Water first				No water first				Results from the omnibus statistical analysis; those with $p < 0.05$ in bold
	Water		No Water		Water		No Water		
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	
Thirst	6.44	4.21	12.48	3.41	5.07	4.02	11.64	3.68	**Water $F_{(1, 32)} = 54.95$, $p < 0.001$** Water × Order, $F_{(1, 32)} = 0.095$, $p = 0.760$ Order $F_{(1, 32)} = 1.20$, $p = 0.282$
Afraid	0.58	0.72	0.63	0.92	0.88	1.47	1.8	2.29	Water $F_{(1, 32)} = 3.03$, $p = 0.091$ Water × Order $F_{(1, 32)} = 2.46$, $p = 0.127$ Order $F_{(1, 32)} = 2.87$, $p = 0.100$
Confused	1.18	1.25	0.68	0.88	1.66	1.66	2.88	2.48	Water $F_{(1, 32)} = 1.42$, $p = 0.243$ **Water × Order $F_{(1, 32)} = 8.12$, $p = 0.008$** **Order $F_{(1, 32)} = 7.52$, $p = 0.010$**
Sad	0.98	1.20	0.81	1.13	1.34	1.41	2.30	2.69	Water $F_{(1, 32)} = 0.976$, $p = 0.331$ Water × Order $F_{(1, 32)} = 1.94$, $p = 0.17$ **Order $F_{(1, 32)} = 4.54$, $p = 0.041$**
Angry	0.92	1.18	0.86	1.24	1.62	2.18	1.81	2.01	Water $F_{(1, 32)} = 0.028$, $p = 0.865$ Water × Order $F_{(1, 32)} = 0.116$, $p = 0.736$ Order $F_{(1, 32)} = 3.26$, $p = 0.080$
Energetic	4.29	2.88	3.58	3.16	4.22	2.81	4.01	3.43	Water $F_{(1, 32)} = 0.87$, $p = 0.359$ Water × Order $F_{(1, 32)} = 0.25$, $p = 0.620$ Order $F_{(1, 32)} = 0.036$, $p = 0.751$
Tired	4.23	3.20	3.38	3.01	3.05	2.64	5.06	3.03	Water $F_{(1, 32)} = 0.69$, $p = 0.414$ **Water × Order $F_{(1, 32)} = 4.16$, $p = 0.050$** Order $F_{(1, 32)} = 0.103$, $p = 0.751$
Happy	7.02	2.02	6.88	2.57	6.43	2.47	6.06	3.24	Water $F_{(1, 32)} = 0.269$, $p = 0.684$ Water × Order $F_{(1, 32)} = 0.033$, $p = 0.856$ Order $F_{(1, 32)} = 1.02$, $p = 0.320$
Tense	1.55	1.33	1.09	1.41	2.06	1.81	2.75	2.18	Water $F_{(1, 32)} = 0.091$, $p = 0.764$ Water × Order $F_{(1, 32)} = 2.31$, $p = 0.139$ **Order $F_{(1, 32)} = 6.89$, $p = 0.013$**

more sad and more confused when they had the no water first order. In the case of confused, this was modified by whether they had a drink of water; they were less confused if they had a drink. All other effects of mood were not statistically significant.

CANTAB

Means and SDs for all CANTAB tests are presented in **Table 2**, along with the results of the omnibus statistical analysis. The most clear-cut findings were for SRT and Intra/Extra dimensional set shift.

On SRT there was a significant main effect of WATER and a significant interaction with ORDER. Participants had faster mean reaction times on the occasion on which they drank water, compared to the occasion on which they did not have a drink of water. The WATER × ORDER interaction was consistent with a practice effect. The water effect was only clearly observable in the condition where water was last. Follow up *t*-tests confirmed these impressions. Reaction times were shorter in the water condition compared to the no water condition, but only if the water condition came second, $t_{(16)} = 2.71$, $p = 0.016$; there was no difference between water and no water groups on the occasion on which they had the water condition first, $t_{(16)} = 0.53$, $p = 0.603$.

In the case of Intra/Extra dimensional set shift (IED; stages completed) there was also a main effect of WATER and WATER × ORDER interaction. Fewer stages were completed by the participants after they had consumed water, but this was only for those who had the water condition first. Follow up *t*-tests supported these impressions. Significantly fewer stages were completed in the water compared to the no water condition when the water condition came first, $t_{(16)} = 2.20$, $p = 0.043$, but there was no significant group difference when the water condition came second, $t_{(16)} = 1.0$, $p = 0.332$.

Performance on Rapid Visual Information Processing (RVP; total hits and misses) was consistent with the explanation that it

Table 2 | CANTAB tests means, SDs, and *F* ratios by water condition (water/no water) and order (water first/no water first).

Task	Water first				No water first				Results from the omnibus statistical analysis; those with $p < 0.05$ in bold
	Water		No Water		Water		No Water		
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	
SRT mean RT[1, 2]	263.94	53.03	267.58	50.37	242.63	24.80	278.09	61.32	**Water $F_{(1, 32)} = 6.99$, $p = 0.013$** **Water × Order $F_{(1, 32)} = 4.63$, $p = 0.039$** Order $F_{(1, 32)} = 0.126$, $p = 0.725$
IED stages completed	7.47	2.58	8.53	0.94	8.59	1.70	8.65	1.46	**Water $F_{(1, 32)} = 5.39$, $p = 0.028$** **Water × Order $F_{(1, 32)} = 4.26$, $p = 0.047$** Order $F_{(1, 32)} = 1.23$, $p = 0.275$
MOT mean error	9.31	2.93	10.65	2.64	11.44	2.67	9.74	2.63	Water $F_{(1, 32)} = 0.135$, $p = 0.716$ **Water × Order $F_{(1, 32)} = 10.02$, $p = 0.003$** Order $F_{(1, 32)} = 0.598$, $p = 0.441$
MOT mean RT	898.99	148.30	745.67	161.91	718.89	147.74	827.13	201.62	Water $F_{(1, 32)} = 0.58$, $p = 0.452$ **Water × Order $F_{(1, 32)} = 19.49$, $p < 0.001$** Order $F_{(1, 32)} = 1.02$, $p = 0.319$
PAL total errors	3.24	4.66	1.88	3.43	1.41	1.73	3.06	3.05	Water $F_{(1, 32)} = 0.094$, $p = 0.761$ **Water × Order $F_{(1, 32)} = 9.80$, $p = 0.004$** Order $F_{(1, 32)} = 0.094$, $p = 0.761$
PAL stages completed	5.00	0.00	4.94	0.24	5.00	0.00	5.00	0.00	Water $F_{(1, 32)} = 1.00$, $p = 0.325$ Water × Order $F_{(1, 32)} = 1.00$, $p = 0.325$ Order $F_{(1, 32)} = 1.00$, $p = 0.325$
VRM recall total correct	9.12	1.80	8.42	2.15	8.88	1.62	9.00	1.73	Water $F_{(1, 32)} = 0.74$, $p = 0.396$ Water × Order $F_{(1, 32)} = 1.45$, $p = 0.237$ Order $F_{(1, 32)} = 0.11$, $p = 0.741$
BLC % correct	100	0.00	100	0.00	99.71	0.83	100	0.00	Water $F_{(1, 32)} = 2.13$, $p = 0.154$ Water × Order $F_{(1, 32)} = 2.13$, $p = 0.154$ Order $F_{(1, 32)} = 2.13$, $p = 0.154$
RVP total hits	19.29	4.69	20.82	4.43	22.24	4.02	19.24	4.56	Water $F_{(1, 32)} = 1.23$, $p = 0.276$ **Water × Order $F_{(1, 32)} = 11.67$, $p = 0.002$** Order $F_{(1, 32)} = 0.244$, $p = 0.624$
CRT right mean RT	327.62	69.66	318.42	51.02	313.18	43.01	324.77	46.23	Water $F_{(1, 32)} = 0.021$, $p = 0.886$ Water × Order $F_{(1, 32)} = 1.57$, $p = 0.220$ Order $F_{(1, 32)} = 0.061$, $p = 0.806$
CRT left mean RT	351.71	86.74	333.06	65.66	315.28	50.34	328.72	44.63	Water $F_{(1, 32)} = 0.073$, $p = 0.789$ Water × Order $F_{(1, 32)} = 2.77$, $p = 0.106$ Order $F_{(1, 32)} = 1.07$, $p = 0.309$
PAL 8	8.18	11.64	7.06	9.44	4.29	4.48	4.77	2.88	Water $F_{(1, 32)} = 0.061$, $p = 0.807$ Water × Order $F_{(1, 32)} = 0.365$, $p = 0.550$ Order $F_{(1, 32)} = 1.67$, $p = 0.205$
VRM recall total novel	0.18	0.39	0.35	0.61	0.18	0.39	0.24	0.44	Water $F_{(1, 32)} = 1.12$, $p = 0.297$ Water × Order $F_{(1, 32)} = 0.281$, $p = 0.600$ Order $F_{(1, 32)} = 0.262$, $p = 0.612$
VRM 2 recognition immediate correct	11.82	0.39	11.59	0.62	11.29	1.05	11.71	0.69	Water $F_{(1, 32)} = 0.272$, $p = 0.606$ Water × Order $F_{(1, 32)} = 3.65$, $p = 0.065$ Order $F_{(1, 32)} = 1.28$, $p = 0.27$

[1] *The analysis of SRT median latency showed the same significant main effect and interaction as that observed for mean SRT.*

[2] *All reaction times are in milliseconds.*

was affected by practice such that more hits and fewer misses were made in the condition that went second.

Performance on the Motor Control task shows a significant WATER × ORDER interaction for both errors and latency, suggesting more errors and faster responding on the second test. This suggests a speed-accuracy trade off on the second test.

As can be seen from the data presented in **Table 2**, the remaining CANTAB tests were not affected by WATER or ORDER. Performance on PAL Stages Completed and Big Circle/Little Circle% correct seem to be at ceiling.

DID THIRST MODERATE THE EFFECT OF WATER SUPPLEMENTATION?

Follow up analyses investigated whether thirst moderated the effect of water on cognitive performance. Participants were grouped as thirsty or not thirsty by taking the median split of the thirst scale reported on the no water day. Mixed model ANOVAs were conducted on all CANTAB tests and mood scales, for which WATER (water/no water) was a within subjects factor and THIRST (thirsty/not thirsty) was a between subjects factor.

There were significant finding for only two CANTAB tests, SRT, and IED. In the case of SRT, there was a significant main effect of WATER, $F_{(1,\ 32)} = 7.03$, $p = 0.012$, and a significant interaction between WATER and THIRST, $F_{(1,\ 32)} = 4.85$, $p = 0.035$. The main effect of THIRST was not statistically significant, $F_{(1,\ 32)} = 0.494$, $p = 0.487$. The significant interaction is illustrated in **Figure 1**, which indicates that individuals in the low thirst group showed similar response times for both the water and no water testing days. Conversely, those participants who reported being thirsty showed elevated response times (slower) on the no water day, $t_{(16)} = 2.61$, $p = 0.019$. Furthermore, on the occasion on which they had water, their response times were as fast as the non-thirsty group, $t_{(16)} = 0.61$, $p = 0.551$.

In the case of IED, there was a significant main effect WATER, $F_{(1,\ 32)} = 4.79$, $p = 0.036$, with performance better on the no water day than the water testing day. This main effect is illustrated in **Figure 2**. The main effect of WATER did not interact with thirst, $F_{(1,\ 32)} = 0.65$, $p = 0.426$, suggesting that subjective thirst is not moderating this effect. The main effect of THIRST was not significant, $F_{(1,\ 32)} = 1.49$, $p = 0.231$.

In the case of the mood ratings, ratings of "tiredness" and "tense" were higher if individuals were thirsty compared to those who were less thirsty [Tiredness, $F_{(1,\ 30)} = 5.82$, $p = 0.022$; Tense, $F_{(1,\ 30)} = 6.23$, $p = 0.01$]. In neither case was there a significant interaction between THIRST and WATER. However, ratings of happiness did show a significant interaction between THIRST and WATER, $F_{(1,\ 30)} = 4.62$, $p = 0.040$. This was a rather counterintuitive finding in which those who were less thirsty had higher happiness ratings after having water ($M = 7.1$) compared to no water ($M = 6.2$), and those who were more thirsty had higher happiness ratings after no water ($M = 7.3$) compared to having water ($M = 5.9$); however, the follow up t-tests were not statistically significant, so not too much weight should be placed on these findings.

DISCUSSION

The results of the present study show that water supplementation has a positive effect on performance on a SRT task, and that this is moderated by participants' subjective feelings of thirst.

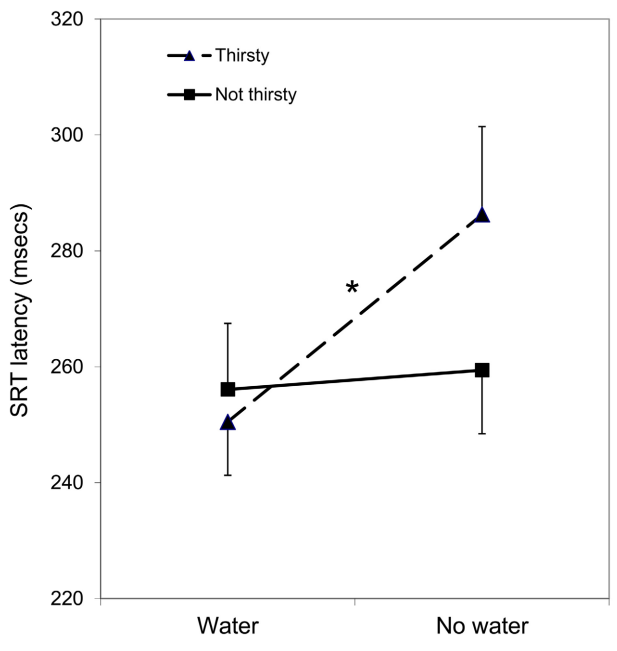

FIGURE 1 | Mean RT (correct trials) on the SRT task as a function of water condition (water/no water) and thirst (thirsty/not thirsty). Asterisk indicates a statistically significant simple main effect of WATER, restricted to the Thirsty group.

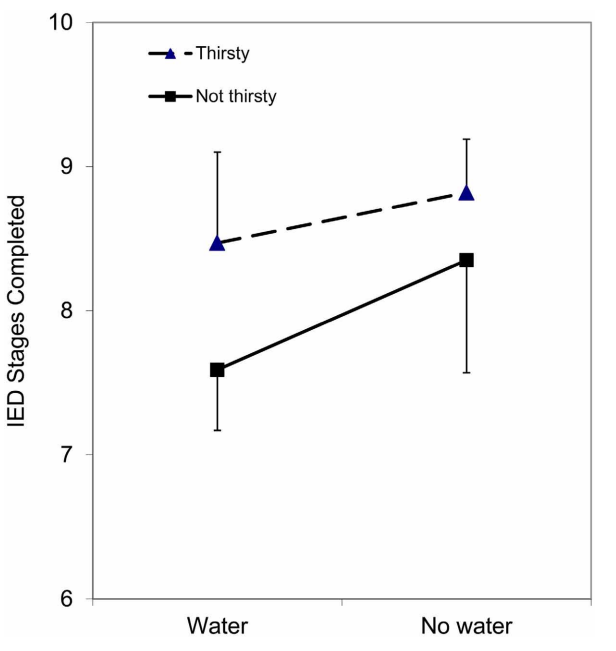

FIGURE 2 | Mean RT (correct trials) on the Intra-Extra Dimensional Set Shift task as a function of water condition (water/no water) and thirst (thirsty/not thirsty). Note that the main effect of WATER was statistically significant, and was not moderated by THIRST.

Participants who were not thirsty showed a similar speed of responding whether or not they had water to drink. In contrast, participants who rated themselves as thirsty performed at a similar level to non-thirsty participants after a drink of water, but were slower if they did not have a drink. Thus, in thirsty individuals, having a drink of water seems to bring reaction times to a level commensurate with those of non-thirsty individuals, rather than the water making them respond even more quickly. This is strikingly different from the results from the IED task, which showed that performance on the set shifting task was poorer after having water, compared to the occasion on which they did not have water, and that this was not moderated by subjective ratings of thirst.

Thirst moderates the effect of water on some aspects of cognitive performance. Cohen (1983) proposed that dehydration negatively affects cognitive performance, because thirst detracts attention from performance. This explanation is based on a capacity model of information processing, such as that described by Kahneman's (1973) model of attention, which suggests that attention is a finite resource and processing capacity used by one process will result in less being available for others. Thus, the findings of the present study imply that, for speed of responding at least, the positive effects of fluid supplementation may result from an attenuation of the central processing resources that are consumed by the subjective sensation of thirst that otherwise impair the execution of speeded cognitive processes.

While speeded processes were improved by water supplementation, particularly in the case of thirsty individuals, for the controlled processes required by performance on the IED task, performance was facilitated by thirst. Similarly, previous research has found that performance on some cognitive tests appears to be impeded by water supplementation. For example, Edmonds et al. (2013) found that backwards digit span improved from baseline in those offered no water, while showing a very small change in those supplemented with water. D'Anci et al. (2006) suggested that the positive and negative effects of water supplementation on cognitive performance may be explained by the underlying physiological processes, which can have excitatory or inhibitory effects. For example, vasopressin activates the thirst response and has been linked to attention and arousal (Van Londen et al., 1998). Thus, for some aspects of performance, thirst may lead to better performance.

Participants rated themselves as more tired and tense if they were thirsty, but there were minimal effects of thirst or water supplementation on the mood measures used in the present study. This may be because water supplementation and thirst have very little effect on subjective mood. This is in line with studies reported previously; while links have been reported between dehydration and self-rated mood (Shirrefs et al., 2004), previous studies on water supplementation have not found that supplementing with water affects mood (Edmonds et al., 2013). Alternatively, it could be that the measure chosen to rate mood was not sufficiently sensitive and this could be explored in future studies.

Our results showed significant interactions between water supplementation and order that were indicative of both water and practice influencing performance. Taking SRT performance as an example, participants that experienced the water condition second showed faster response times under the "water" condition than the "no water" condition. Whereas, participants that experienced the "water" condition first showed no significant difference between conditions, although the "water" group was slightly faster; this is likely to be because performance in the no water condition benefited from practice. Thus, these results suggest that both practice *and* water consumption played a role, with practice counteracting the effect of water consumption for the water first group. In order to avoid practice influencing results, future studies could adopt the baseline-test design used in other studies (Edmonds and Burford, 2009), and manipulate water supplementation as a between subjects variable.

In the studies conducted on water supplementation and cognition in both adults and children, there are few constants in the research design. This variation in study design is both problematic and prudent. It can be difficult to make comparisons across studies when variables such as the amount of water offered the interval between water supplementation and test, the age of the sample, and the cognitive tests used, all vary. However, in a developing research area, in order to avoid missing effects, it is important not to restrict the study design too soon. This is particularly important when considering the areas of cognition assessed. While there are many differences, there are some constancies that should be incorporated into further studies. For example, letter cancellation, as a test of visual attention and processing speed, has been used in many studies (Edmonds and Burford, 2009; Edmonds and Jeffes, 2009; Booth et al., 2012; Edmonds et al., 2013), and performance on this task reliably shows an improvement at 20, 30, and 40 min post supplementation. Future studies should seek to evaluate the study parameters outlined above in a systematic way.

In conclusion, the present study revealed water consumption to have contrasting effects on different cognitive processes. Water consumption was found both to impair set shifting performance, and to facilitate speed of responding, but in a manner that was dependent upon subjective thirst. More specifically, water consumption appeared to have a corrective effect on the response times for thirsty individuals, bringing their speed of responding up to the level of non-thirsty individuals. This moderating effect of subjective thirst occurred despite participants being asked to abstain from consuming fluids overnight, with the aim of ensuring that all participants arrived at the laboratory with a degree of mild voluntary dehydration. These results are consistent with the facilitative effects of water consumption arising from the freeing up of attentional resources that were otherwise occupied with processing the sensations of thirst. Practice effects also influenced performance, but there was an effect of water supplementation over and above the effects of practice. Further work should examine how this is mediated by thirst mechanisms, as well as determining why water consumption can have negative as well as positive effects on cognitive performance.

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Habitual fat intake predicts memory function in younger women

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High intakes of fat have been linked to greater cognitive decline in old age, but such associations may already occur in younger adults. We tested memory and learning in 38 women (25 to 45 years old), recruited for a larger observational study in women with polycystic ovary syndrome. These women varied in health status, though not significantly between cases ($n = 23$) and controls ($n = 15$). Performance on tests sensitive to medial temporal lobe function (CANTABeclipse, Cambridge Cognition Ltd, Cambridge, UK), i.e., verbal memory, visuo-spatial learning, and delayed pattern matching (DMS), were compared with intakes of macronutrients from 7-day diet diaries and physiological indices of metabolic syndrome. Partial correlations were adjusted for age, activity, and verbal IQ (National Adult Reading Test). Greater intakes of saturated and trans fats, and higher saturated to unsaturated fat ratio (Sat:UFA), were associated with more errors on the visuo-spatial task and with poorer word recall and recognition. Unexpectedly, higher UFA intake predicted poorer performance on the word recall and recognition measures. Fasting insulin was positively correlated with poorer word recognition only, whereas higher blood total cholesterol was associated only with visuo-spatial learning errors. None of these variables predicted performance on a DMS test. The significant nutrient–cognition relationships were tested for mediation by total energy intake: saturated and trans fat intakes, and Sat:UFA, remained significant predictors specifically of visuo-spatial learning errors, whereas total fat and UFA intakes now predicted only poorer word recall. Examination of associations separately for monounsaturated (MUFA) and polyunsaturated fats suggested that only MUFA intake was predictive of poorer word recall. Saturated and trans fats, and fasting insulin, may already be associated with cognitive deficits in younger women. The findings need extending but may have important implications for public health.

Keywords: cognition, memory, hippocampus, saturated fat, unsaturated fat, insulin, women

INTRODUCTION

Long-term maintenance of optimal cognitive function is becoming increasingly important, given the shift in demographics toward a growing elderly population. There is accumulating evidence for the importance of lifestyle factors in maintaining cognitive function into older age (Gillette Guyonnet et al., 2007; Lee et al., 2009). A recent longitudinal epidemiological study found evidence for cognitive decline in middle-aged adults: a cohort of 10,308 British civil servants aged 45–70 (known as the Whitehall II study) was assessed for cognitive function on three occasions over 10 years (Singh-Manoux et al., 2012). Cognitive decline was apparent in all age groups, even in those aged 45–49 at baseline; moreover, the effect was probably underestimated due to practice effects. Such cognitive decline in a relatively healthy group is most likely to be due to lifestyle factors including diet, perhaps interacting with genetic susceptibility.

Experimental studies in rodents have found that animals fed diets high in saturated fat for several weeks show impairments in learning and memory (Murray et al., 2009), and independently of obesity (Valladolid-Acebes et al., 2011). Various changes in the brain, particularly in the hippocampus, may underlie these effects, including a reduction in neurogenesis, increased inflammatory activity, altered blood–brain barrier permeability, reduced neuronal plasticity, and decreased hippocampal insulin sensitivity (Molteni et al., 2002; Kanoski et al., 2010; McNeilly et al., 2011; Davidson et al., 2012; Pancani et al., 2013). In human beings, diets high in saturated or trans fats, and low in polyunsaturated fats (PUFAs) have been linked with poorer cognitive function in older populations, both in cross-sectional epidemiological studies (Kalmijn et al., 2004) and in longitudinal studies in which cognition was tracked for several years (Morris et al., 2004; Gillette Guyonnet et al., 2007; Okereke et al., 2012). This includes the finding that the fat intake of 40- to 60-year-olds predicted cognitive function 20 years later, with relative deterioration associated with saturated fats and improvements associated with unsaturated fat intake (Eskelinen et al., 2008).

High circulating blood cholesterol has been linked to poorer cognition in some studies (Gendle et al., 2008), but findings are inconsistent and suggest that population cholesterol-lowering *per*

se should not be a priority for cognitive function (Solomon et al., 2009; Reynolds et al., 2010).

Although saturated and trans fats have been linked to impaired cognition, conversely several observational studies suggest associations between higher intake of PUFAs, especially n-3 fatty acids (Kalmijn et al., 2004), but also n-6 fatty acids (Roberts et al., 2010) perhaps by displacing intake of saturated fat (Devore et al., 2009), and better cognition or protection from cognitive decline. These essential fatty acids are known to be critical for optimum neuronal membrane function, and their intake during pregnancy benefits cognitive development of the offspring (Helland et al., 2003; Daniels et al., 2004). More recent studies also support beneficial effects of higher monounsaturated fats (MUFA) on cognition: a 3-year follow-up study of 482 women aged at least 60 found that higher MUFA (assessed by food frequency questionnaire, FFQ) was associated with better memory and visual learning, but there was no association with saturated and trans fats, after adjusting for confounders including energy intake (Naqvi et al., 2011). In another prospective study of older Italians, baseline intakes of PUFA and MUFA (FFQ assessed) were associated with protection against cognitive decline over an 8.5-year follow-up, although total energy intake was similarly protective (Solfrizzi et al., 2006). In a randomized controlled trial in 522 elderly Spanish men and women with cardiovascular risk factors, one group received a low-fat control diet, while two other groups received Mediterranean-style diets, one of which was supplemented with extra-virgin olive oil, and other with mixed nuts, both of which are rich in MUFA (PREDIMED-NAVARRA trial; Martinez-Lapiscina et al., 2013). Cognitive function was assessed after 6.5 years of dietary intervention: in fully adjusted analyses, cognitive performance was found to be superior in both MUFA-rich dietary intervention groups compared to the control diet group.

However, not all studies support a beneficial effect of MUFA on cognitive decline: in 2002, results from a large 6-year prospective study of 5,395 Dutch adults (the Rotterdam Study), which included a semi-quantitative FFQ, and monitoring for dementia, did not find any association between either high intakes of saturated and trans fats, and cholesterol, or low intakes of MUFA and PUFA, and risk of developing dementia, after adjusting for likely confounds (Engelhart et al., 2002). Yet, only 197 participants (3.6%) had been diagnosed with dementia over that period, and more subtle declines in cognitive function were not assessed. In another large epidemiological study in the USA (Nurses' Health Study; Samieri et al., 2013) in which women aged at least 70 were followed over 6 years, adherence to a Mediterranean diet, and MUFA:saturated fat ratio, were not significantly associated with cognitive decline. Furthermore, one recent report of 1528 60- to 64-year-old Australians assessed over 4 years found that the Mediterranean diet was not protective of cognitive decline, but instead, higher MUFA was actually associated with greater decline (Cherbuin and Anstey, 2012).

The evidence for chronic effects of diet on cognition has focused on the elderly, largely (though not always) relying on simple global cognitive tests (e.g., the Mini Mental State Examination – MMSE) that can be applied to substantial elderly populations and detect clinical cases or mild cognitive impairment. Moreover, nutrient intakes in these studies are usually assessed by FFQ, which are not accurate for measurement of absolute nutrient intakes (Flood et al., 2004). Given that detectable cognitive decline is thought to result from changes taking place over 20–30 years (Launer, 2005), it is possible that chronic dietary factors may already be affecting the cognition of younger adults. Thus, the question arises whether relationships between dietary fats, blood lipids, and cognition could be detected in younger ostensibly healthy populations, if more sensitive domain-specific cognitive tests and accurate dietary assessments, or trial designs, are used. Recently, two relevant experimental studies from the same group have been conducted in younger adult men: Edwards et al. (2011) asked 20 men aged 25–45 (mean age = 36 years) to eat a ketogenic very high-fat low-carbohydrate (Atkins-style) diet (74% energy as fat; 1.5% carbohydrate) for 7 days, following 3 days on a low-fat (17%) high-carbohydrate diet. Cognitive function over a range of domains was assessed by a sensitive computer-based test battery before and after this diet. There were no changes in memory function, but complex and simple reaction times were slightly longer after the high-fat diet, and calmness and alertness deteriorated. However, these results are difficult to interpret due to the absence of a parallel control group.

In a subsequent study with an improved randomized controlled cross-over design, 16 younger men, aged 19–28, ate a 70% fat, 4% carbohydrate diet for 5 days, and, on another 5 days, an equicaloric low-fat diet of 24% fat and 50% carbohydrate (both diets were high in protein, at 26% of energy), with a 2-week wash-out period in between, as well as 3 days on a standardized diet prior to each test diet (Holloway et al., 2011). Compared to the low-fat diet, the high-fat diet resulted in deterioration of focused attention and speed of memory retrieval, as well as slower information processing, although other measures of attention and episodic or working memory were not significantly affected. Mood was unaffected in this study. Similarly, in healthy overweight adults, after one week on a high-fat, low-carbohydrate weight-reducing diet, memory was impaired compared to participants following a more balanced reducing diet, though speed of responding on a vigilance task was faster (D'Anci et al., 2009). In contrast, a 6-week low-carbohydrate ketogenic diet improved memory relative to a high-carbohydrate diet, in elderly participants with mild cognitive impairment (Krikorian et al., 2012).

A limitation of those studies is the use of extreme differences in fat content, and the lack of information on the type of fat, although given the high protein content, it is likely that the high-fat diets were rich in saturated fats. It is also possible that this short term impairment of cognition would not occur over a longer dietary adaption period. One way to address this is to assess habitual diet and examine relationships between intake of fat type and cognition. This approach was taken in a study testing a heterogeneous sample of 498 men (32%) and women (68%) aged from 17 to 66 (mean = 20.5), with body mass index (BMI) ranging from 15 to 40 (Francis and Stevenson, 2011). Cognitive tests were chosen to be sensitive to hippocampal (or medial temporal lobe) function (story recall and verbal paired associate learning) as well as a digit span test included as a control for attention. Relevant aspects of habitual diet were assessed by a bespoke FFQ that included 24 typical dietary items known to contribute high levels of saturated

fat or refined carbohydrate or both (Dietary Fat and Sugar Short Questionnaire, DFS-SQ; Francis and Stevenson, 2013). In regression analyses adjusting for potential confounders, there were weak but significant associations (0.8–2.9% variance explained) indicating that higher saturated fat and refined carbohydrate intake predicted poorer performance on the memory tasks. In a second study, two groups ($n = 16$ per group) were recruited based on high and low scores on the DFS-SQ, but otherwise matched for confounders such as age, sex, BMI, and verbal IQ. A larger range of cognitive tests was employed; nevertheless, significant differences were restricted to story recall and visual pattern reproduction. Verbal associate learning scores did not differ between groups, nor did a range of other cognitive tests assessing attention and executive function.

Limitations of these latter studies are that effects of fat and carbohydrates were combined, and differences in energy and other nutrient intakes were not accounted for. In particular, participants consuming greater quantities of the sort of energy dense foods assessed by the DFS-SQ may have higher energy intakes, albeit BMI and activity ranges were apparently matched. The possible importance of overall energy intake for memory function was demonstrated in an experimental study in healthy elderly men and women stratified by age, sex, and BMI into three 3-month dietary intervention groups: caloric restriction (CR; $n = 20$), enhanced intake of unsaturated fats (UFA; $n = 20$), and usual diet control (CON; $n = 10$; Witte et al., 2009). Cognitive function was assessed before and after the 3-month trial using a verbal recall test, a digit span test of attention and a working memory (trail making) task. Although self-reported dietary measures did not show a significant reduction in energy intake in the CR group, they showed significant weight loss, indicative of actual energy restriction. The UFA group showed a significant increase in unsaturated fats to saturated fat ratio, which is independent of energy intake. Effects were specific to the verbal recall test: CR showed significantly improved memory after the 3-month trial, whereas scores for the other two groups did not change. In sub-analyses of nine participants in the CR group who had weight loss >1 SD above the mean weight loss for the control group, fasting insulin, and the inflammatory marker C-reactive protein levels were strongly inversely correlated with memory performance. No benefits were found for the UFA group. Interestingly, similar results were found when obese women were put on a 50% energy reduction diet for 15 weeks: while there were no changes in tests of attention, verbal recall was improved (Kretsch et al., 1997). However, simple reaction time was slowed after energy restriction in this study.

Therefore, despite some intriguing evidence suggesting the importance of dietary fat type for cognitive health, there are inconsistencies, and even opposing effects, in the literature. Thus, the present study was designed to capture reliable dietary data (7-day diet diaries), as well as detailed physiological measures and sensitive cognitive tests of memory and learning, in women aged 25–45. We tested the hypothesis that greater saturated and trans fatty acid intakes, and a higher saturated fat to unsaturated fat ratio, would predict poorer performance of younger women on memory tasks expected to be sensitive to medial temporal lobe or hippocampal function.

MATERIALS AND METHODS

PARTICIPANTS

Women were recruited for this study from a larger sample ($n = 68$) who had been invited to take part in a study of polycystic ovary syndrome (PCOS), diet, and health, which included both women diagnosed with PCOS and matched non-PCOS controls (Barr et al., 2008). Recruitment for the larger study was via a self-help PCOS charity (Verity) in the UK and by local advertisement. Participants attended a university research center on one occasion and were invited to take part in this subsidiary study. It was explained that the only addition to the laboratory tasks involved was a 30-min computerized cognitive test session, which would occur while they waited to give a blood sample following an oral glucose tolerance test (OGTT). Forty-two women consented to take part, from whom 38 had complete data ($n = 23$ PCOS; $n = 15$ matched controls) and have been included in the results presented here. The diagnosis of PCOS was initially self-reported but then confirmed by their general practitioner, based on the presence of two of the following three features with the exclusion of other endocrine disorders: oligo-ovulation leading to oligomenorrhea, or anovulation leading to amenorrhoa; hyperandrogenism, clinical (hirsutism, male pattern alopecia, acne), and/or biochemical; polycystic ovaries (Barr et al., 2008). However, in this sample, there were no significant differences in either physiological (including endocrine), anthropometric (e.g., BMI, % body fat, waist–hip ratio, WHR) or cognitive function variables between women with and without diagnoses of PCOS, with the exception of slightly fewer errors on the National Adult Reading Test (NART; i.e., better verbal IQ or education) for the PCOS women. Thus, analyses were run on the 38 women as one group, but results were adjusted for NART errors (see below). Although this sample was small for an observational study, it compares favorably with sample sizes found in several of the experimental or intervention studies described above (Kretsch et al., 1997; Witte et al., 2009; Edwards et al., 2011; Holloway et al., 2011; Francis and Stevenson, 2013). This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by Roehampton University and National Research Ethics Service (06/Q0104/130). Written informed consent was obtained from all participants.

MEASUREMENT OF HEALTH BEHAVIORS

In the week preceding their attendance at the laboratory, habitual dietary intake and activity were assessed using a 7-day food and activity diary. Pedometers (Yamax Digi-walker SW-200) were provided to participants to wear for the same 7 days in order to characterize physical activity levels alongside the activity diary. Physical activity levels were calculated from the 7-day diary in order to assess the mean daily physical activity level of the participants. Activities were categorized into metabolic equivalents of energy (METs) as a multiple of basal metabolic rate. Categories were: light (<3 METs), moderate (3–6 METs) or high (>6 METs; Ainsworth et al., 2000). Food diaries were coded by a trained researcher and data were entered into a computerized dietary analysis package (Dietplan 6.3, Forestfield Software, Horsham, UK) to provide estimated daily energy and nutrient intakes.

MEASUREMENT OF PHYSIOLOGICAL INDICATORS

Height, weight, and waist circumference were assessed via standardized protocols to ensure accurate measurement (Norgan, 2005). Total body adiposity was also measured under standardized conditions (Kushner, 1992) by tetra-polar bioelectrical impedance analysis using the Bodystat® 1500 single-frequency body composition analyzer.

A fasting venous blood sample was taken for the assessment of fasting glucose and insulin levels, total cholesterol, high density lipoprotein (HDL) cholesterol, triacylglycerol (TAG), C-reactive protein (CRP), non-esterified fatty acids (NEFA), and plasminogen activator inhibitor-1 were measured using the Instrumentation Laboratory 650 (Milan, Italy) automated analyzer. Low-density lipoprotein (LDL) cholesterol levels were calculated using Friedewald's equation (Friedewald et al., 1972). All hormones (free testosterone, androstenedione, insulin, cortisol, sex hormone binding globulin, dehydroepiandrosterone-sulfate, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were measured using an ELISA technique with consumables and reagents supplied by DGR Diagnostics (DRG Instruments GmbH, Germany). A 75-g glucose load for the OGTT was provided using Lucozade (GlaxoSmithKline, UK), following manufacturer's recommended method, and plasma glucose and serum insulin measurements were taken at 0 and 120 min to determine fasting insulin sensitivity and 2-h glucose tolerance. The updated non-linear homeostatic model assessment (HOMA2) was used to calculate insulin resistance, β-cell function and insulin sensitivity using the Oxford Diabetes Trials Unit calculator (www.dtu.ox.ac.uk). The HOMA2 estimates correspond well to non-steady state estimates of β-cell function and insulin sensitivity derived from the hyperinsulinemic and hyperglycemic clamp (Levy et al., 1998). Insulin resistance was defined as a HOMA2 value >1.

COGNITIVE TESTS

Three brief computerized tests were used from the CANTABeclipse (v3) suite of cognitive tests (Cambridge Cognition Ltd., Cambridge, UK), incorporating touch-screen responses. These tests were chosen because they are believed to be sensitive to dysfunction of the hippocampus and medial temporal lobe areas, which may be particularly susceptible to insult from dietary fats and effects of metabolic syndrome (Kanoski and Davidson, 2011). One is a verbal test, the other two are visuo-spatial in format.

Verbal recognition memory test (2 × 5 min)

In the verbal recognition memory (VRM) test, the participant was shown a list of 18 words on the screen, one at a time, which they were asked to remember as best they could. Then they were assessed as follows: (i) immediate recall: the number of words correctly recalled out loud immediately following the presentation (the participant faced away from the screen while their responses (correct words, new words, or repetitions) were scored on the computer by the investigator) (ii) immediate recognition: participants were presented with a randomized sequence of 36 words, half of which were on the original list to be memorized; as each word was presented, they were required to touch 'yes' or 'no' targets on the screen to indicate whether they recognized the word as being on the list to memorize (iii) delayed recognition: after a delay of 20 min, the word recognition procedure was repeated as before.

Delayed matching to sample (DMS) (10 min)

This test assessed both simultaneous and delayed recognition of novel patterns (delayed pattern matching, DMS); participants were shown a complex visual sample pattern and then (usually after a short delay) they were presented with an array of four patterns, and had to touch the pattern that was identical to the original sample pattern. Outcomes assessed response latencies, number of correctly chosen patterns, and the probability of an error after either correct or incorrect responding.

Paired associate learning task (PAL) (10 min)

This test assessed visuo-spatial memory and new learning, and can be used as a screening test for early signs of dementia or age-related memory decline. Boxes were displayed around the screen opening in randomized order, revealing either a novel pattern or no pattern. The sample patterns were then displayed in the middle of the screen, one at a time, and the participant had to touch the box where the pattern was originally located. If the participant made an error, the patterns were re-presented to remind the participant of their locations. The difficulty level increased through the test. In the clinical mode used here, the number of patterns revealed increased from 1, then 3, 6–8, with errors typically occurring once at least six pattern locations had to be recalled.

Outcomes for each level of difficulty covered the errors made by the participant, the number of trials required to locate the pattern(s) correctly, memory scores, and stages completed.

PROCEDURE

Participants from the larger study of women with PCOS and matched controls attended the clinic at the University of Roehampton in the morning, having fasted overnight, for assessment on a range of physiological measures (described above). At this time they were asked to read the participant information on this supplementary study of cognitive function. Those volunteering to take part then signed their consent ($n = 38$).

As part of their assessments, all participants underwent an OGTT (see above), with blood draws 2 h apart. One hour after the glucose drink, they were seated in a quiet cubicle in front of a computer monitor with touch-screen response capability. Their distance from the screen was standardized as recommended by Cambridge Cognition Ltd. The sequence of tests was explained to them, and careful verbal instruction was given throughout, following the recommended text.

Prior to starting the CANTABeclipse test battery, the NART (NFER Nelson) was administered: this is believed to represent a form of stable verbal IQ that can act as an index of premorbid intelligence, although it also reflects educational attainment and socioeconomic status more generally (Crawford et al., 1988). Participants were presented with a series of cards one by one, with each card having a single word printed in capital letters. The participant was asked to pronounce each word out loud in the manner they believed was the correct pronunciation. The test was scored by summing the number of words that were not pronounced correctly (i.e., NART errors).

Next, the CANTAB eclipse tests were carried out, in the following order: VRM-immediate recall; VRM-immediate recognition; delayed matching to sample (DMS); visuo-spatial learning (PAL); VRM-delayed recognition. The battery lasted approximately 30 min, with a delay of 20 min between completion of the immediate and delayed VRM tests. After this, participants were thanked and debriefed, and returned to the clinic for completion of physiological assessments.

DATA ANALYSIS

Variables were assessed for acceptably normal distribution by checking for skewness ($< \pm 1$) and extreme outliers by boxplots. The following variables were found to be skewed and were transformed by natural logarithm: fasting insulin, trans fatty acid intake, visuo-spatial learning total errors (eight shapes), VRM immediate free word recall.

Cognitive performance was significantly related to age (free word recall, DMS correct choice), activity (pedometer counts/day; visuo-spatial learning errors), and NART errors (word recall and recognition); therefore, these variables were controlled for using partial correlations or as covariates in subsequent multiple regressions.

Macronutrient intakes are, by definition, closely related to overall energy intake. Therefore, separate multiple linear regression mediation analyses with bootstrapping and covariates (Preacher and Hayes, 2008) were used to examine the possibility that macronutrient intake associations with cognition might be primarily mediated by overall energy intake. These were applied where overall energy intake was a significant predictor of cognitive performance after controlling for confounding variables by partial correlations. The outcomes of interest in these analyses were as follows: whether total energy intake had significant direct effects on cognition; whether the macronutrient had a significant direct effect on cognition after adjusting for the other variables in the model (all the above expressed as unstandardized regression coefficients (B) and their significance); and finally whether there was an indirect effect of the nutrient mediated via total energy intake (bootstrapping test; difference of mean effects from zero indicated by bias-adjusted 95% confidence intervals). The SPSS (v.19) syntax macro for these analyses was downloaded from the web (A. F. Hayes personal website, accessed 05.07.13: http://www.afhayes.com/spss-sas-and-mplus-macros-and-code.html).

RESULTS

PARTICIPANTS

All participants were female, aged between 25 and 45 years old. Four participants were current smokers, 12 were ex-smokers and 22 were non-smokers. Nine participants were non-native English speakers, but nevertheless fluent. Twenty-one participants reported taking dietary supplements regularly. Descriptive statistics for anthropometry are given in **Table 1**: 63% had BMIs (weight (kg)/height (m)^2) within the healthy range, 24% were overweight ($25 < \text{BMI} < 30$), while 13% were obese (BMI = 30–35). Mean BMI was just under 25; by contrast, mean waist circumference was 82.6, thus being in the band (80–88 cm) associated with moderate risk for type-2 diabetes (T2D) and cardiovascular disease (The InterAct Consortium, 2012). The distribution of participants in these risk categories was 50% at low risk, 21% at moderate risk, and 29% at high risk. Not surprisingly, average waist:hip ratio and % body fat are both close to the border between low and raised risk for T2D.

Table 1 | Participant characteristics ($n = 38$).

	Mean	SD	Min.	Max.
Age (years)	32.7	5.3	25	45
BMI (kg/m^2)	24.9	4.5	18.4	35.0
Waist (cm)	82.6	12.4	64	109
Waist:hip ratio	0.79	0.07	0.68	0.99
Body fat (%)	31.4	7.1	20.0	46.2

Descriptive statistics for macronutrient intake are given in **Table 2**. These participants ate on average quite a low carbohydrate diet (44% of energy; **Table 2**), with relatively high protein (16.3%) and fat intakes (39.7%) compared with the recommendations for the UK population, i.e., 50% carbohydrate, 35% total fat, and 15% protein (Food Standards Agency, 2006). Similarly, the average saturated fat intake (13.9%) was higher than the recommended maximum of 11%. As with the anthropometric measures, these dietary data indicate that a significant proportion of the sample had unhealthy dietary practices and physiological indicators, which may be helpful for examining diet–cognition relationships.

PHYSIOLOGICAL HEALTH

Both dietary fat intake and body fat % were associated with higher levels of peripheral indices related to metabolic syndrome, including total and LDL blood cholesterol, plasma C-reactive protein, blood pressure, and WHR, as indicated by significant positive correlations (**Table 3**). Waist circumference was very strongly associated with % body fat ($r = 0.83$, $p < 0.001$), but not significantly related to dietary fat intake (total fat $r = 0.28$, $p = 0.08$). Overall, the correlations support a stronger influence of saturated than unsaturated fats on these indices. However, it is notable that, whereas body fat was strongly associated with indicators of insulin

Table 2 | Average daily macronutrient and energy intakes from 7-day diet diaries ($n = 38$).

	Mean	SD	Min.	Max.
Total energy[a] (MJ)	8.0	1.85	4.9	12.3
Protein (%)	16.3	2.8	12.1	23.1
Carbohydrate (%)	44.0	5.8	32.6	56.6
Fat (%)	39.7	6.5	27.1	58.6
Saturated fat (%)	13.9	3.7	7.8	25.6
Saturated fat (g)	28.2	9.4	14.3	48.6
Unsaturated fat (g)	42.0	10.7	21.6	63.8

[a] *Including energy from alcohol.*
Macronutrient proportions are given as % total energy from food.

Table 3 | Correlations (Pearson's *r*) between % body fat, dietary fat intakes and physiological indices related to metabolic syndrome (*n* = 38).

	% Body fat	Dietary fat (g)	Saturated fat (g)	Unsaturated fat (g)	Sat:UFA ratio	Trans fat (g)
Fasting insulin	0.59***	–	–	–	–	–
HOMA IR	0.49*	–	–	–	–	–
CRP	0.37*	0.28*	–	–	–	–
LDL-Chol.	0.39*	0.29^{+}	0.38*	–	0.36*	0.32^{+}
Total blood cholesterol	–	0.38*	0.39*	0.31^{+}	–	–
BP (systolic)	0.39*	0.29*	0.29*	–	–	–
BP (diastolic)	0.46**	–	–	–	–	–
Waist:hip ratio	0.59***	0.38*	0.30^{+}	0.31^{+}	–	–

UFA, total unsaturated fatty acid intake; Sat:UFA ratio, ratio of total saturated to unsaturated fat intake; HOMA IR, homeostasis Model of assessment of insulin resistance; CRP, C-reactive protein; LDL-Chol., low-density lipoprotein blood cholesterol; BP, blood pressure.$^{+}$*p < 0.10 (two-tail), *p < 0.05, **p < 0.01, ***p < 0.001.*

resistance (fasting insulin and HOMA IR), no form of dietary fat intake was correlated to these measures. Furthermore, neither waist circumference, nor WHR was correlated with cognitive outcomes (controlling for age, activity and verbal IQ).

COGNITIVE PERFORMANCE OUTCOMES

The performance outcomes were on average typical for relatively young and cognitively intact participants (**Table 4**; Cambridge Cognition Ltd.). Most outcomes showed a reasonable spread of performance across the sample for detecting associations with dietary factors, although the numbers of errors in the word recognition tests were quite restricted, and the mean % correct choices for the DMS task was high and slightly above normative data for this age group (93.4% vs. 87.5%), indicating the possibility of ceiling effects due to low task demands. The NART errors were on average quite high, suggesting moderate verbal IQ and educational status across the sample, although this may have been adversely affected by nine participants having English as a second language.

DIET AND COGNITIVE FUNCTION

Associations with dietary fats

Associations between dietary fats and measures of cognitive function were first examined by partial correlations controlling for age (pedometer) activity and verbal IQ (NART errors), as these background measures were associated with various cognitive outcomes. Higher intakes of total, saturated and trans fats, as well as greater saturated to unsaturated fat ratio, were associated with more errors on the visuo-spatial learning task (**Table 5**). Higher blood total cholesterol, itself associated with saturated fat intake (**Table 3**), was likewise associated with more visuo-spatial learning errors; however, by contrast, total unsaturated fat intake was not significantly related to this outcome (**Table 5**).

Performance on the verbal memory task was also associated with fat intake, in that greater fat intake was predictive of more errors in both the immediate recall of words and for recognition of presented words immediately and after a 20 min delay (**Table 5**). However, in contrast to visuo-spatial learning errors, verbal memory errors were associated with greater intakes of both saturated and unsaturated fats, but not with the ratio nor with trans fats. Blood cholesterol did not predict verbal memory, but higher fasting insulin was associated with more word recognition errors.

Partial correlations were similarly conducted between macronutrient intakes, fasting insulin and outcomes for the DMS task: the only correlation found to approach significance was between the saturated to unsaturated fat ratio and mean correct latency ($r = -0.30$, $p = 0.08$), but as this was a negative correlation, implying shorter response times for more saturated fat intake vs. unsaturated fat, it may not be a reliable finding. In case the outcomes for DMS averaged over all trials reflected tasks that were insufficiently demanding, separate partial correlations were conducted for the trials with longer delays (4 and 12 s), but still no significant associations were seen (data not shown).

Table 4 | Descriptive statistics for the main cognitive performance outcomes (*n* = 38).

Outcome	Mean	SD	Range
Visuo-spatial learning errors (PAL; eight shapes)	5.2	5.9	0–26
Words forgotten (VRM recall)	9.1	2.6	2–13
Word recognition errors (immediate)	1.8	1.8	0–6
Word recognition errors (delayed)	2.0	2.1	0–6
DMS correct choice (%; all delays)	93.4	5.6	73–100
DMS correct latency (s; all delays)	3.41	0.72	2.07–5.27
NART errors	20.0	6.6	5–36

Associations with carbohydrate and protein intakes

Potential associations between intake of the other macronutrients, carbohydrate and protein, and cognitive performance, were also examined by partial correlations controlling for the same variables. There were no significant associations between carbohydrate intake and cognition (largest $r = 0.29$, $p = 0.09$, for delayed verbal recognition errors); however, protein intake was significantly positively correlated to word recognition errors (immediate, $r = 0.41$; delayed, $r = 0.40$, both $p < 0.05$) and to visuo-spatial learning

Table 5 | Partial correlations[a] between measures of memory function and dietary fat intakes or physiological indices in 25- to 45-year-old women ($n = 38$).

Test outcome	Total fat (g)	Saturated fat (g)	Unsaturated fat (g)	Sat:UFA ratio	Trans fat (g)	Blood cholesterol	Fasting insulin
Visuo-spatial learningerrors	0.38*	**0.51*** **	––	**0.48****	**0.41****	0.32*	–
Words forgotten	**0.40***	0.33*	**0.46***	–	–	–	–
Word recog. errors – immediate	0.48**	0.38**	0.47**	–	–	–	0.32*
Word recog. errors – delayed	0.51***	0.47**	0.41**	–	–	–	0.36*

[a] *Correlations were adjusted for verbal IQ (NART errors), age, and physical activity (pedometer counts). Nutrient intakes are from 7-day diet diaries. Only correlations significant at p < 0.05 or better are shown.*
Correlations for macronutrients in bold remained significant after testing for possible mediation by energy intake.
**p < 0.05, **p < 0.01, ***p < 0.001.*

errors ($r = 0.34$, $p < 0.05$), i.e., greater protein intake predicted worse verbal memory.

Mediation analyses examining the influence of total energy intake

The lack of associations with carbohydrate intake and cognition suggests that the other significant links are unlikely to be due to an effect of overall energy intake *per se*; nevertheless, this is an important concern, so to address the question whether these relationships between macronutrient intakes and cognition merely reflect a negative consequence of higher energy intake, which then mediates the apparent influence of macronutrients on cognition, multiple linear regression mediation analyses were conducted for each case where total energy was significantly correlated to performance (after controlling for age, activity, and verbal IQ), with the cognitive outcomes as dependent variables, and including age, activity, and verbal IQ scores as covariates.

The results for protein were as follows: for immediate verbal recognition errors, the mediation model did not show significant direct effects for either protein ($B = 0.026$) or total energy (as mediator; $B = 0.001$), and there was no indirect effect of protein mediated by energy intake (95% CI overlapping with 0: bootstrapping bias-corrected 95% CI = −0.027 to 0.073), despite a significant overall adjusted effect of protein on recognition errors (**Table 6**). For delayed recognition errors, total energy intake did have a significant direct effect on performance ($B = 0.002$, $p < 0.05$); however, protein did not have a significant direct effect, and although the overall model was significant (**Table 6**), the indirect effect of protein via total energy intake as mediator was not significant (bootstrapping bias-corrected 95% CI = −0.005 to 0.092). For visuo-spatial learning errors (eight shapes), the mediation model did not show significant direct effects for either protein or total energy (as mediator), and there was no indirect effect of protein mediated by energy intake (bootstrapping 95% CI = −0.010 to 0.030), despite a significant overall adjusted effect of protein on recognition errors (**Table 6**: $B = 0.018$, $p = 0.05$). In summary, it would appear that protein does not reliably predict verbal or visual memory, when covariates and energy intake are adjusted for; however, this also cannot be attributed simply to a mediating effect of energy intake.

The mediation results for total fat intake were as follows: for immediate verbal free recall, energy intake did not have a significant direct effect on number of words forgotten ($B = -0.0001$), whereas total fat intake was directly associated with words forgotten ($B = 0.006$, $p < 0.05$)). There was no evidence for a significant indirect effect of fat intake on words forgotten mediated by energy intake (bootstrapping 95% CI = −0.006 to 0.002). By contrast, for both immediate and delayed recognition errors, there were no significant direct effects of either fat (imm. $B = 0.029$; delayed $B = 0.027$) or energy intake (imm. $B = 0.0005$; delayed $B = 0.001$), and no indirect effects of fat mediated by energy intake (bootstrapping 95% CI: imm. = −0.032 to 0.046; delayed = −0.026 to 0.068). For visuo-spatial learning errors, neither total fat ($B = 0.015$) nor energy intake ($B = 0.0001$) had a significant direct effect. There was also no evidence for a significant indirect effect of fat intake on visuo-spatial learning errors mediated by energy intake (bootstrapping 95% CI = −0.007 to 0.002). In summary, for immediate word recall, but not for word recognition or visuo-spatial learning errors, higher total fat intake was significantly associated with more words forgotten independently of energy intake.

The mediation results for saturated fat intake were as follows: for immediate verbal free recall, neither energy intake nor saturated fat intake had a significant direct effect on number of words forgotten (energy $B < 0.0000$; sat. fat $B = 0.008$). There was no evidence for a significant indirect effect of saturated fat intake on words forgotten mediated by energy intake (bootstrapping 95% CI = −0.005 to 0.007). Likewise, for both immediate and delayed recognition errors, there were no significant direct effects of either saturated fat (imm. $B = 0.033$; delayed $B = 0.050$) or energy intake (imm. $B = 0.0012$; delayed B = 0.0016), and no indirect effects of saturated fat mediated by energy intake (bootstrapping 95% CI: imm. = −0.009 to 0.117; delayed = −0.020 to 0.123). By contrast, for visuo-spatial learning errors, saturated fat had a significant direct effect ($B = 0.045$, $p < 0.05$), whereas energy intake did not ($B < 0.0000$). There was no evidence for a significant indirect effect of saturated fat intake on visuo-spatial learning errors mediated by energy intake (bootstrapping 95% CI = −0.020 to 0.023). In summary, although saturated fat was overall associated with poorer performance on word recall and recognition (**Table 6**), these effects were no longer significant when energy intake was

Table 6 | Adjusted R^2 values and significance for the overall multiple regression models examining potential mediation by total energy intake of the macronutrient-cognition association, including age, verbal IQ and activity as covariates.

Test outcome	Total fat (g)	Saturated fat (g)	Unsaturated fat (g)	Trans fat (g)	Protein (g)
Visuo-spatial learning errors	0.20*	0.31**	–	0.25*	0.18*
Words forgotten	0.34**	0.29**	0.35**	–	–
Word recog. errors – immediate	0.34**	0.31**	0.34**	–	0.31**
Word recog. errors – delayed	0.33**	0.34**	0.32**	–	0.31**

Empty cells indicate that there was no partial correlation between the nutrient and the cognitive outcome, hence no mediation was tested.
**p < 0.05, **p < 0.01.*

adjusted for; however, this also could not be attributed simply to a mediating effect of energy intake. By contrast, higher saturated fat intake was significantly associated with more visuo-spatial learning errors, independently of any mediating influence of energy intake.

The mediation results for unsaturated fat intake were as follows: for immediate verbal free recall, energy intake did not have a significant direct effect on number of words forgotten ($B = -0.0001$), whereas unsaturated fat intake was directly associated with words forgotten ($B = 0.011$, $p < 0.05$). There was no evidence for a significant indirect effect of unsaturated fat intake on words forgotten mediated by energy intake (bootstrapping 95% CI = −0.007 to 0.004). However, for immediate recognition errors, there were no significant direct effects of either unsaturated fat ($B = 0.049$) or energy intake ($B = 0.0009$), and no indirect effects of fat mediated by energy intake (bootstrapping 95% CI = −0.021 to 0.077). By contrast, for delayed recognition, the direct effect of energy intake was significant ($B = 0.0018$, $p < 0.05$), whereas the direct effect of unsaturated fat was not ($B = 0.029$). Moreover, there was evidence for an indirect effect of unsaturated fat on delayed word recognition significantly mediated by energy intake (bootstrapping 95% CI = 0.002–0.129).

In summary, when possible mediation by energy intake was tested for, there were opposing associations between unsaturated fat and verbal memory: for immediate word recall, higher unsaturated fat intake was associated with more words forgotten independently of energy intake. By contrast, unsaturated fat intake was not associated with immediate word recognition, and for delayed word recognition, the association was clearly mediated by energy intake. As regards the association between the saturated to unsaturated fat ratio and visuo-spatial learning errors, it is not meaningful to test this for mediation by total energy intake, as the ratio is independent of how much energy is eaten.

Associations with mono- and polyunsaturated fat intake

Given the unexpected evidence for a detrimental effect on verbal memory (free word recall) of higher intakes of unsaturated fats, we examined whether this association was specific to MUFA vs. PUFA fat intake, by applying the mediation analyses to intake of these fat types separately. For PUFA intake, there was no significant direct effect for either energy intake ($B < 0.0000$) or PUFA intake ($B = 0.017$, $p > 0.10$) on words forgotten (overall model adjusted $R^2 = 0.29$, $p < 0.01$). However, for MUFA intake, whereas there was no significant direct effect of energy intake, the direct effect of MUFA intake on words forgotten remained significant ($B = 0.017$, $p < 0.05$; partial correlation excluding energy intake = 0.45). For both PUFA and MUFA, there was no evidence of indirect effects mediated by energy intake (95% CI: PUFA = −0.007 to 0.015; MUFA = −0.012 to 0.005). The partial correlation between MUFA intake and words forgotten remained significant and was little altered after additionally controlling for whether or not the participant was taking dietary supplements (0.42, $p < 0.05$).

The mediation results for the positive association between trans fatty acid intake and visuo-spatial learning errors were as follows: the direct effect of trans fats on visuo-spatial learning errors was marginally significant ($B = 0.900$, $p = 0.05$), whereas there was no direct effect of energy intake ($B = 0.0004$), and there was no evidence for mediation of the trans fat effect by energy intake (bootstrapping 95% CI = −0.019 to 0.787). The overall regression model was significant (**Table 6**).

DISCUSSION

This study used detailed dietary measures together with sensitive tests of cognition to test the hypothesis that higher habitual dietary fat intake may be associated with poorer memory function in women in early to middle adulthood. The findings largely supported this hypothesis: higher fat intake was associated with worse performance on tests of visuo-spatial learning and verbal (VRM) learning and memory, when adjusting for the influence of age, activity, and verbal IQ. Furthermore, for saturated fat and trans fatty acid intakes, and for the ratio of saturated to UFA intake, these associations were shown to be independent of overall energy intake. By contrast, partial correlations between higher protein intake and worse memory performance were no longer significant when adjusted for energy intake, although mediation of these effects of protein by energy intake *per se* was not shown to be significant in this relatively small sample. Carbohydrate intake was not significantly associated with any of the cognitive performance tests.

With respect to brain regions that may be most affected, this study was limited to tests thought to be sensitive to medial temporal lobe and particularly hippocampal function, i.e., tasks involving visual and verbal memory and learning associations. Whereas two of the three tests administered (visuo-spatial learning and verbal recall/recognition) revealed significant associations between dietary fats and performance, the DMS test did not. The

DMS test shares similarities with the visuo-spatial learning task (PAL), in that both are tests of short-term visual memory using pattern stimuli, and both would be expected to be sensitive to function of the medial temporal lobe areas of the cerebral hemispheres. However, the visuo-spatial learning task more explicitly involves new learning, and at the level of eight shapes for which recall location is needed the test is more challenging than DMS, which involves detecting a target stimulus among only four choices. Indeed, with correct choices made on an average of 93% of trials (somewhat higher than the normative score for this age group; Cambridge Cognition, Ltd.), DMS may not have been sensitive enough to subtle impairments of medial temporal lobe function in this relatively healthy and young sample. Nevertheless, our conclusions are limited by the lack of tests designed specifically to assess frontal lobe function, for example. In addition, test sensitivity may have been affected by administering the 75-g glucose drink prior to testing, because it is well-established that glucose loads can improve performance, particularly on tasks involving medial temporal lobe function, albeit usually in lower doses (Smith et al., 2011). Thus, whereas this manipulation served to ensure that all participants were in the same acute nutritional state, it may have somewhat reduced the variation in performance across participants. This would imply that the actual impact of habitual fat intake on cognition could be greater than that seen in this paradigm.

These findings are in line with epidemiological data in the elderly (Kalmijn et al., 2004; Morris et al., 2004; Gillette Guyonnet et al., 2007; Eskelinen et al., 2008; Okereke et al., 2012), but additionally suggest that dietary factors, e.g., high saturated fat, may lead to cognitive impairment in much younger women. This interpretation is limited by the cross-sectional nature of the data, and the small sample size, but unlike many larger population studies, we used detailed 7-day dietary diaries, and cognitive tests designed to assess hippocampal function that may be particularly sensitive to nutritional insult. Moreover, this is supported by notable effect sizes, with the overall regression models explaining 20–35% of the variance. Also, we only examined linear relationships, and it is possible the non-linear modeling would produce even greater effect sizes, i.e., there could be threshold effects. Another strength of the study was that all women were tested in the same acute nutritional state, i.e., following a glucose load after overnight fasting.

However, one finding was unexpected: higher UFA intake was associated specifically with poorer verbal recall memory, independently of energy intake. This contrasts with verbal recognition performance, for which the association with UFA intake was found to be indirect and mediated by energy intake. Separate examination of MUFA and PUFA associations with memory suggested that MUFA intake, but not PUFA intake, was linked to poorer verbal recall, and this could not be accounted for by overall energy intake. It is important to examine a possible independent effect of overall energy intake, as a recent intervention in the elderly found that energy restriction (or at least weight loss) improved verbal memory, possibly via increased insulin sensitivity or reduced inflammation (Witte et al., 2009). Of interest also in that study, raising intake of MUFA did not benefit memory, although PUFAs were not increased, and numbers were limited to 20/group. Such beneficial cognitive effects of weight loss may be specific to verbal memory, suggesting a role for hippocampal changes (Kretsch et al., 1997).

Of course, there are several observational studies, both cross-sectional and longitudinal, that support positive rather than negative associations between MUFA and cognitive function (Solfrizzi et al., 2006; Naqvi et al., 2011; Okereke et al., 2012). Moreover, enhanced MUFA intake was found to be associated with better cognition in a randomized controlled trial (Martinez-Lapiscina et al., 2013). MUFA intake in a non-Mediterranean sample such as this may differ in the influence of unadjusted confounders than that in a Mediterranean culture; nevertheless, other studies have found significant cognitive benefits from higher PUFA but not MUFA intake, in both Mediterranean (Psaltopoulou et al., 2008) and non-Mediterranean cultures (Samieri et al., 2013). Furthermore, whereas PUFA intake in a London population was protective against cardiovascular disease, MUFA intake was not (Clarke et al., 2009). Even more critically, a recent 4-year prospective epidemiological study of aging in Australians found that high MUFA (and energy) intake predicted mild cognitive impairment (Cherbuin and Anstey, 2012). These findings and those reported here suggest that associations between MUFA intake and memory function need careful examination in a variety of populations. It is possible that in certain cultures there is residual confounding between MUFA intake and another ingredient, or indeed behavior, which could adversely affect memory function. Even so, interpretation of such findings, including our own, is complicated by the lack of distinction between different forms of fatty acid in these studies: in particular, among PUFA, a higher proportion of n-6 vs. n-3 essential fatty acids may be an important predictor of adverse outcomes for brain as well as cardiovascular health (Loef and Walach, 2013), perhaps because of respective pro-inflammatory vs. anti-inflammatory actions of their eicosanoid products (Calon and Cole, 2007). This may be particularly important given the typically very high ratio of n-6 to n-3 fatty acids (e.g., at least 15:1) in modern Western diets (Loef and Walach, 2013), and increasingly so in some developing countries particularly in Asia (Misra et al., 2010).

Beyond the dietary fat associations with memory, there was limited evidence for relationships between physiological indices and cognition. Even in these younger women, body fat levels were associated with physiological indices of risk for T2D and cardiovascular disease, such as LDL cholesterol, CRP, blood pressure and insulin resistance (HOMA IR and fasting insulin), as well as WHR. Nevertheless, these physiological measures were, by and large, unrelated to cognitive function in this sample – the exception being higher fasting insulin and total cholesterol, which had moderate associations with poorer word recognition and visuo-spatial learning errors, respectively. This might reflect the relatively healthy status of this sample, as dietary fat type is known to affect indices of metabolic syndrome (Clarke et al., 2009), and other research has shown cognitive impairments associated with T2D and abdominal fat (Isaac et al., 2011), even in adolescents (Schwartz et al., 2013), although this relationship may be less clear in women (Kanaya et al., 2009).

The mechanisms by which dietary fats might impair the activity of neurons in the hippocampus are not known for certain; however, several pathways have been proposed. For example, suppression of neurogenesis may be important, i.e., preventing the formation of new neurons – which occurs only in the hippocampus and one other brain region (subventricular zone) – as high-fat diets reduce brain derived neurotrophic factor (BDNF) in rats, which is believed to be a key factor in neurogenesis (Molteni et al., 2002). There is experimental evidence in laboratory models that fatty acids could modulate adult hippocampal neurogenesis and thus affect learning and memory (Stangl and Thuret, 2009; Zainuddin and Thuret, 2012). Similarly, high levels of saturated and trans fatty acids, and low PUFA intake, have been associated with increased cholesterol and inflammatory markers that themselves are linked to impairment of neurogenesis and cognition as well as cardiovascular disease (Clarke et al., 2009; Marioni et al., 2009). By contrast, PUFAs, especially n-3 fatty acids, essential components of neuronal cell membranes as well as precursors for anti-inflammatory eicosanoids, appear to have protective effects (Vercambre et al., 2009), promoting neurogenesis in the hippocampus (Cao et al., 2009). Other mechanisms may include changes in insulin sensitivity of hippocampal neurons, and local permeability of the blood–brain barrier (McNeilly et al., 2011; Davidson et al., 2012; Pancani et al., 2013).

In summary, we found evidence that high intake of saturated and trans fats in particular, and possibly MUFA, may adversely affect memory and learning even in relatively young women. Our results are confined to women, and our sample had a high proportion diagnosed with PCOS: thus, extrapolating to the general population is not yet justified. Results are also limited to the tasks employed, and will benefit from replication and extension in a larger sample in which relevant genotyping could also be deployed (e.g., ApoA4, FADS), and including a wider range of tests, to determine their reliability and specificity. Moreover, this was an observational cross-sectional study, and we cannot be certain about causal interpretations. Nevertheless, as they stand, they have important implications for public health messages: arguably, younger adults may find messages linking diet to learning and memory to be more compelling than those aimed at promoting cardiovascular health, even if the health behavior requirements are the same. Furthermore, in elderly patients already suffering from dementia, dietary interventions have generally not been successful (Fotuhi et al., 2009). Thus, intervention studies conducted in younger participants before overt cognitive decline has become apparent, and which carefully disconfound variables such as socioeconomic status, education, and other health behaviors (Gillette Guyonnet et al., 2007) are more likely to produce effective recommendations to alter the unaffordable trajectory of diet-mediated cognitive decline.

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AUTHOR CONTRIBUTIONS

E. Leigh Gibson was primary author of this manuscript, and designed and executed the cognitive testing component, and analyzed those data. Suzanne Barr was responsible for recruitment and assessment of participants for dietary and physiological measures, as well as data analysis of dietary diaries. Yvonne M. Jeanes designed the larger observational study, initiated recruitment, and carried out data collection and analysis.

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The effects of breakfast on behavior and academic performance in children and adolescents

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Breakfast consumption is associated with positive outcomes for diet quality, micronutrient intake, weight status and lifestyle factors. Breakfast has been suggested to positively affect learning in children in terms of behavior, cognitive, and school performance. However, these assertions are largely based on evidence which demonstrates acute effects of breakfast on cognitive performance. Less research which examines the effects of breakfast on the ecologically valid outcomes of academic performance or in-class behavior is available. The literature was searched for articles published between 1950–2013 indexed in Ovid MEDLINE, Pubmed, Web of Science, the Cochrane Library, EMBASE databases, and PsychINFO. Thirty-six articles examining the effects of breakfast on in-class behavior and academic performance in children and adolescents were included. The effects of breakfast in different populations were considered, including undernourished or well-nourished children and adolescents from differing socio-economic status (SES) backgrounds. The habitual and acute effects of breakfast and the effects of school breakfast programs (SBPs) were considered. The evidence indicated a mainly positive effect of breakfast on on-task behavior in the classroom. There was suggestive evidence that habitual breakfast (frequency and quality) and SBPs have a positive effect on children's academic performance with clearest effects on mathematic and arithmetic grades in undernourished children. Increased frequency of habitual breakfast was consistently positively associated with academic performance. Some evidence suggested that quality of habitual breakfast, in terms of providing a greater variety of food groups and adequate energy, was positively related to school performance. However, these associations can be attributed, in part, to confounders such as SES and to methodological weaknesses such as the subjective nature of the observations of behavior in class.

Keywords: breakfast, behavior, academic performance, children, adolescents, learning

INTRODUCTION

Breakfast is widely acknowledged to be the most important meal of the day. Children who habitually consume breakfast are more likely to have favorable nutrient intakes including higher intake of dietary fiber, total carbohydrate and lower total fat and cholesterol (Deshmukh-Taskar et al., 2010). Breakfast also makes a large contribution to daily micronutrient intake (Balvin Frantzen et al., 2013). Iron, B vitamins (folate, thiamine, riboflavin, niacin, vitamin B_6, and vitamin B_{12}) and Vitamin D are approximately 20–60% higher in children who regularly eat breakfast compared with breakfast skippers (Gibson, 2003). Consuming breakfast can also contribute to maintaining a body mass index (BMI) within the normal range. Two systematic reviews report that children and adolescents who habitually consume breakfast [including ready-to-eat-cereal (RTEC)] have reduced likelihood of being overweight (Szajewska and Ruszczynski, 2010; de la Hunty et al., 2013). Breakfast consumption is also associated with other healthy lifestyle factors. Children who do not consume breakfast are more likely to be less physically active and have a lower cardio respiratory fitness level (Sandercock et al., 2010). Moreover, there is evidence that breakfast positively affects learning in children in terms of behavior, cognitive, and school performance (Hoyland et al., 2009).

The assumptions about the benefit of breakfast for children's learning are largely based on evidence which demonstrates acute effects of breakfast on children's cognitive performance from laboratory based experimental studies. Although the evidence is quite mixed, studies generally demonstrate that eating breakfast has a positive effect on children's cognitive performance, particularly in the domains of memory and attention (Wesnes et al., 2003, 2012; Widenhorn-Muller et al., 2008; Cooper et al., 2011; Pivik et al., 2012). Additionally, the positive effects of breakfast are more demonstrable in children who are considered undernourished, typically defined as one standard deviation below normal height or weight for age using the US National Center for Health Statistics (NCHS) reference (Pollitt et al., 1996; Cueto et al., 1998). More recent evidence compares breakfast meals that differ in Glycaemic Load (GL), Glycaemic Index (GI) or both. This evidence generally suggests that a lower postprandial glycaemic response is beneficial to children's cognitive performance (Benton and Jarvis, 2007; Ingwersen et al., 2007; Micha et al., 2011; Cooper et al., 2012) however the evidence is equivocal (Brindal et al.,

2012). Moreover, it remains unclear whether this effect is specifically due to GI or GL, or both, or to other effects unrelated to glycaemic response.

Studies rarely investigate the acute effects of breakfast on behavior in the classroom and there remains a lack of research in this area. This may be, in part, attributed to the complicated nature of the measures used to assess behavior in class and the need to develop standardized, validated, and comparable coding systems to measure behavior. Similarly, few studies examine the effects of breakfast on tangible academic outcomes such as school grades or standardized achievement tests relative to cognitive outcomes. Whilst crude measures of academic performance may not provide the most sensitive indicator of the effects of breakfast, direct measures of academic performance are ecologically valid, have most relevance to pupils, parents, teachers, and educational policy makers and as a result may produce most impact.

Cognitive, behavioral, and academic outcomes are not independent. Changes in cognitive performance are likely to be reflected by changes in behavior. An increase in attention following breakfast, compared with no breakfast, may be reflected by an increase in on-task behavior during lessons. Similarly, changes in cognitive performance may also impact school performance and academic outcomes in a cumulative manner. The beneficial effects of eating breakfast on cognitive performance are expected to be short term and specific to the morning on which breakfast is eaten and to selective cognitive functions. These immediate or acute effects might translate to benefits in academic performance with habitual or regular breakfast consumption, but this has not been evaluated in most studies. Short term changes in cognitive function during lessons (e.g., memory and attention) may therefore translate, with habitual breakfast consumption, to meaningful changes in school performance by an increased ability to attend to and remember information during lessons. In class behavior also has important implications for school performance. This is because a prerequisite for academic learning is the ability to stay on task and sustain attention in class. Greater attention in class and engagement in learning activities (referred to as on-task behavior) are likely to be associated with a more productive learning environment which may impact academic outcomes in the long term.

Children may be particularly vulnerable to the nutritional effects of breakfast on brain activity and associated cognitive, behavioral, and academic outcomes. Children have a higher brain glucose metabolism compared with adults. Positron Emission Tomography studies indicate that cerebral metabolic rate of glucose utilization is approximately twice as high in children aged 4–10 years compared with adults. This higher rate of glucose utilization gradually declines from age 10 and usually reaches adult levels by the age of 16–18 years (Chugani, 1998). Average cerebral blood flow and cerebral oxygen utilization is 1.8 and 1.3 times higher in children aged 3–11 years compared with adults, respectively (Kennedy and Sokoloff, 1957; Chiron et al., 1992). Moreover, the longer overnight fasting period, due to higher sleep demands during childhood and adolescence compared with adults, can deplete glycogen stores overnight (Thorleifsdottir et al., 2002). To maintain this higher metabolic rate, a continuous supply of energy derived from glucose is needed, hence breakfast consumption may be vital in providing adequate energy for the morning. Nevertheless, breakfast is the most frequently skipped meal. Between 20–30% of children and adolescents skip breakfast in the developed world (Deshmukh-Taskar et al., 2010; Corder et al., 2011).

Despite intense public and scientific interest and a widely promoted consensus that breakfast improves concentration and alertness, Hoyland et al. (2009) were only able to identify 45 studies on the effects of breakfast on objectively measured cognitive performance in the period of 1950–2008 in their systematic review. They concluded that breakfast consumption is more beneficial than skipping breakfast to cognitive outcomes, effects which were more apparent in children who are considered undernourished. They did not consider ecologically valid outcomes of behavior (in-class or at school) and academic performance. This article complements the Hoyland et al. (2009) review by considering the evidence on the effect of breakfast on behavior (in-class or at school) and academic performance in children and considers the methodological challenges in isolating the effects of breakfast from other factors. Findings will be discussed dependent on outcome measure and study design with effects evaluated based on breakfast manipulation where possible. The effects of breakfast in different populations will be considered, including children, adolescents who are undernourished or well-nourished and from differing socio-economic status (SES) backgrounds. The habitual and acute effects of breakfast will be considered along with the effects of school breakfast programs (SBPs).

METHODS

The literature was searched for original articles and reviews published between 1950–2013 on databases: Ovid MEDLINE, Pubmed, Web of Science, the Cochrane Library, EMBASE databases and PsychINFO. The search was conducted using the key words "breakfast" or "school breakfast" combined with "children" or "adolescents" combined with "behavio$," "on-task," "off-task," "concentration," "attention," "school performance," "academic performance," "scholastic performance," "academic achievement," "school grades," "school achievement," and "educational achievement" using the Boolean operator "and." The $ symbol was used for truncation to ensure the search included all keywords associated with behavior ("behavior," "behaviour," "behavioural," "behavioral"). Studies are limited to these outcomes in children and adolescents (<18 years). The reference lists of existing reviews and identified articles were examined individually to supplement the electronic search. The presentation of the results are organized by two main outcomes: In-class behavior/behavior at school and academic performance with corresponding summary tables which detail design, sample, breakfast intervention/dietary assessment, assessment of outcomes and reported results for each article. A total of 36 studies are included. Fourteen studies included behavior measures, seventeen studies included academic performance measures, and five studies examined both behavior and academic performance.

RESULTS

IN-CLASS BEHAVIOR AND BEHAVIOR AT SCHOOL

Nineteen studies employed behavioral measures to examine the effects of breakfast on behavior at school, either by use of

classroom observations or rating scales usually completed by teachers (**Table 1**). Four studies included both classroom observations and rating scales (Kaplan et al., 1986; Milich and Pelham, 1986; Rosen et al., 1988; Richter et al., 1997).

Observations of behavior in the classroom

Direct measures of classroom behavior were utilized in 11 studies. Although there are inconsistent findings, the evidence indicated a mainly positive effect of breakfast on on-task behavior in the classroom in children. Seven of the eleven studies demonstrated a positive effect of breakfast on on-task behavior. This was apparent in children who were either well-nourished, undernourished and/or from low SES or deprived backgrounds. Two studies carried out in undernourished samples (Chang et al., 1996; Richter et al., 1997) and three studies in children from low SES backgrounds (Bro et al., 1994, 1996; Benton et al., 2007) demonstrated positive effects on on-task behavior following breakfast. One study reported a negative effect of a SBP on behavior in undernourished children (Cueto and Chinen, 2008) and three studies in children with behavioral problems demonstrated no effect of breakfast composition on behavior (Kaplan et al., 1986; Milich and Pelham, 1986; Wender and Solanto, 1991). Most studies included small samples of the order of 10–30 children which, although limited in terms of power and generalizability to the larger population, are more feasible and appropriate given the nature of the data and extensive coding methods required.

Intervention studies. Four intervention studies demonstrated a positive effect of SBPs on on-task behavior in undernourished and low SES children. Richter et al. (1997) reported a significant positive change in behavior from pre to post intervention in undernourished children aged 8 years. Following a 6-week SBP providing approximately 267 Kcal per day at breakfast, children in the intervention group displayed significantly less off-task and out of seat behavior and significantly more class participation (Richter et al., 1997). Concomitant teacher ratings of hyperactivity also declined significantly in the intervention group, however teachers reported no change in attention. This effect has also been demonstrated in adolescents. Two studies in small samples of adolescents aged 14–19 years showed an increase in on-task behavior in the classroom following an unstandardized teacher led SBP in vocational schools in USA (Bro et al., 1994, 1996). More recent evidence failed to show the same benefit in undernourished children (≤ -2 SD height-for-age of the NCHS reference) aged 11 years. Cueto and Chinen (2008) observed a reduction in on-task behavior following a 3-year SBP measured using time per day spent in the classroom as an indirect proxy measure. The design of the intervention required teachers to dedicate time to providing the breakfast mid-morning. This unexpected negative impact on on-task behavior is unlikely to occur when breakfast is delivered before school by non-teaching staff and when direct measures of classroom behavior are employed.

Acute experimental studies. Seven studies employed a within-subjects acute experimental design to examine the effects of breakfast on classroom behavior across the morning. The findings were inconsistent, with three of the seven studies showing an advantage of breakfast on on-task behavior (Chang et al., 1996; Benton and Jarvis, 2007; Benton et al., 2007).

Benton et al. (2007) observed classroom behavior and reaction to frustration following three isocaloric breakfast meals of high, medium or low GL in a sample of young children (mean age: 6 years 10 months) from a school in an economically disadvantaged area. Children spent significantly more time on-task following a low GL breakfast meal compared with medium and high GL breakfast meals. This effect was specific to the first 10 min of the observation. Children also displayed fewer signs of frustration during a video game observation, but again, effects were short lived and specific to the initial observation period. No significant effects were found for distracted behavior. Although meals aimed to be isocaloric, actual intake across conditions was variable and the macronutrient content differed between conditions. Consequently, the difference in classroom behavior may be due to differences in macronutrient content rather than GL. Four studies failed to find a similar advantage for on-task behavior in children with Attention Deficit Disorder with hyperactivity (ADD-H) or behavioral problems (Kaplan et al., 1986; Milich and Pelham, 1986; Wender and Solanto, 1991) or in primary school children without behavioral problems (Rosen et al., 1988) following breakfast meals that differed in sugar content.

Mixed results were reported when comparing the effects of breakfast vs. no breakfast in undernourished children. Chang et al. (1996) examined the effects of breakfast on classroom behavior in 57 undernourished (< -1 SD weight-for-age of the NCHS reference) and 56 adequately nourished children in Jamaican rural schools. A significant increase in on-task behavior was observed following a 520 Kcal breakfast, which was seen only in the well-equipped school. In the three less well-equipped schools, behavior deteriorated following breakfast with an observed increase in off-task behavior (talking, movement). The well-equipped school had separate classrooms for each class and each child had their own desk, an environment probably more conducive to positive in-class behavior. The deterioration of behavior following breakfast in the less well-equipped schools could reflect greater difficulties in accurately observing whether children are on-task or off-task when they do not have their own desk or are in overcrowded classrooms. In developed high income countries where school infrastructure is more standardized and where classrooms are not overcrowded, this possibly spurious effect is less likely to occur (Murphy et al., 2011; Ni Mhurchu et al., 2013). However, negative effects on behavior have also been reported in UK primary and secondary school children within deprived areas following a SBP (Shemilt et al., 2004). Therefore, other factors, including the breakfast club environment, delivery, and staff engagement with the SBP may have also influenced the impact of breakfast on behavior, as well as school structure. For example, activities during the breakfast club and general atmosphere may promote negative and excitable behavior. Nutritional status did not influence the results of Chang et al's study, however, the degree of undernourishment was mild. It is possible that positive effects may be more demonstrable in children who are more severely undernourished. In addition, an appropriate environment in terms of classroom structure and equipment is needed to accurately observe the effects of breakfast.

Table 1 | Tabulation of studies investigating the effects of breakfast on behavior at school in children and adolescents.

Authors, year	Design	Sample	BF intervention/assessment of BF	Assessment of behavior	Reported results
Kaplan et al. (1986)	RM randomized acute experimental study. Double blind.	Behavior treatment center (USA). $n = 9$ aged 9–13 years. Behavior problems: $n = 5$ ADD-H: $n = 4$.	Behavior problems: 1. High sugar BF 2. Low sugar aspartame sweetened BF ADD-H group: 1. High sugar BF + Methylphenidate 2. Low sugar aspartame sweetened BF + Methylphenidate 3. High sugar BF + placebo 4. Low sugar aspartame sweetened BF + placebo BF of either high or low sugar, not matched for energy. Stratified by behavior problems/ADD-H	In-class observation, +30–60 min post ingestion. Behavior coded: on-task during 30 min observation. Good inter-rater reliability. Conners Teacher Rating Scale hyperactivity index.	No significant difference in behavior due to high or low sugar BF.
Milich and Pelham (1986)	RM randomized acute experimental study. Double blind.	Behavior treatment center (USA). $n = 16$, male children mean age 6–9 years, diagnosed ADD-H.	Two conditions: Drink at 0800 h 1. High sugar: 50 g sugar drink 2. Low sugar: 0 g sugar drink + 175 mg aspartame	Three observations in two settings. 1. In-class observation via one way mirror. Behavior coded: on-task, class points, questions correct, and questions attempted for set tasks. 2. Structured recreational observation (1). Behavior coded: rule adhering, positive peer interaction, noncompliance, negative verbalization. 3. Structured recreational observation (2). Behavior coded: Positive/negative/neutral interaction. Good inter-rater reliability. Conners Teacher Rating Scale inattention/over-activity and aggression scales.	No significant effects of treatment on behavior in both settings.

(Continued)

Table 1 | Continued

Authors, year	Design	Sample	BF intervention/assessment of BF	Assessment of behavior	Reported results
Rosen et al. (1988)	RM acute experimental study. Double blind.	Two schools (USA). $n = 45$. Preschool: $N = 30$, mean age: 5 years 4 months. Male: 66%, Female: 33% Primary school: $n = 15$, mean age: 7 years 2 months. Male: 40%, female: 60% Middle-High SES.	Three conditions: Standard BF and 113 g drink of differing sugar content: 1. High sugar: 50 g sugar drink + BF (489 Kcal/90.8 g CHO) 2. Low sugar: 6.25 g sugar drink + BF (314 Kcal/47 g CHO) 3. Control: 0 g sugar drink sweetened with aspartame (291 Kcal/41 g CHO) Standard BF: 198 g oats, 170 g whole milk, bread (1 slice), 1 tsp margarine, 1 tsp grape jelly (287 Kcal)	In-class and free play observation +30 min post BF. 1. Preschool: Free play observation. Behavior coded: Fidget, activity change, movement, vocalization, aggression. 2. Primary school. In-class observation. Behavior coded: Fidget, on-task. Time sampling. Good inter-rater reliability. Conners Teacher Rating Scale 10-item hyperactivity index Global rating scale completed by teachers.	No significant effects of sugar on behavior in both settings. Significant increase in Conners Teacher Rating Scale hyperactivity index in high sugar condition compared with low sugar condition.
Richter et al. (1997)	SBP evaluation. Pre-post test design. 6-week intervention.	Two primary schools (South Africa). $n = 108$. Male: 50%, Female: 50% Control: $n = 55$ well-nourished children mean age ± *SD*: 8.3 ± 0.8. Intervention: $n = 53$ undernourished children mean age ± *SD*: 10.5 ± 1.9.	Two conditions: 1. SBP: 30 g Cornflakes, 100 ml semi-skimmed milk, banana (≈267.4 Kcal/1117.8 K) 2. Control: No SBP	Video recorded in-class observation following habituation. Behavior coded: on-task, off-task, passive-active, positive, or negative peer interaction, class participation, out of seat, request attention, unclear/out of view. Time sampling. ADD-H Comprehensive Teacher's Rating Scale 24-item. Teacher completed four subscales for classroom behavior: attention, hyperactivity, social skills, and oppositional behavior.	Significant decrease in off-task and out of seat behavior in SBP group from pre- post intervention. No change in control group. Significant increase in activity and class participation in SBP group from pre-post intervention. No change in control group. Significant decline in on-task behavior in control group from pre-post test. No change in SBP group. No significant change in request attention, negative peer interaction, and passive behavior. Hyperactivity subscale scores declined significantly in intervention group from pre-post test.

(Continued)

Table 1 | Continued

Authors, year	Design	Sample	BF intervention/assessment of BF	Assessment of behavior	Reported results
Chang et al. (1996)	RM randomized acute experimental study.	Four primary schools (Jamaica). $n = 113$, Male: 50%, Female: 50% Undernourished (< −1 SD weight-for-age NCHS): $n = 57$, mean age ± *SD*: 9.68 ± 0.42. Nourished: $n = 56$, mean age ± *SD*: 9.18 ± 0.77.	Two conditions: 1. In-class BF before school: 68 g bread, 28 g cheese, 227 g chocolate milk (520 Kcal) 2. Low energy control: 68 g orange (18 Kcal)	In-class observation at ≈0900–1130 h. Two "mock" classroom situations: 1. Active teaching (2 × 30 min) 2. Set task (2 × 30 min) Behavior coded: On-task, talking to peers, gross motor, class participation. Time sampling. Acceptable-good inter-rater reliability.	Significant school × treatment interaction for active teaching on-task, talks, and gross motor behavior and for set task on-task behavior. Significant increase in on-task behavior and decrease in gross motor behavior following BF during active teaching in well-equipped school. Significant increase in talking to peers during active teaching and decrease in on-task behavior during set task in poorly equipped schools following BF. No significant effects of nutritional group and treatment.
Bro et al. (1994)	SBP evaluation. Pre-post test. 20-day intervention.	Vocational secondary school (USA) $n = 10$ males aged 14–18 years. High rate of off-task behavior at baseline. Low SES.	Two conditions: 1. Teacher led in-class SBP Nutritionally balanced 2. No SBP	In-class observation conducted by teacher. Behavior coded: on-task. Time sampling. Good inter-rater reliability.	Increase in on-task behavior post SBP compared to baseline.
Bro et al. (1996)	SBP evaluation. Pre-post test. 9-day intervention.	Vocational and learning center (USA): $n = 18$, aged 15–19 years 17 males, 1 female. Low SES.	Two conditions: 1. Teacher led in-class SBP. Fruit juice, milk, English muffins, blueberry muffins, bagels, cream cheese, eggs, toast, hot cakes 2. No SBP	In-class observation conducted by teacher in academic and vocational setting. Behavior coded: on-task. Time sampling. Acceptable Inter-rater reliability in both settings. Subjective ratings of ability to stay on task.	Increase in on-task behavior at follow up compared with baseline in both vocational and academic setting. Decrease in subjective ratings of ability to stay on-task at follow up. High rate of off-task behavior at baseline.

(Continued)

Table 1 | Continued

Authors, year	Design	Sample	BF intervention/assessment of BF	Assessment of behavior	Reported results
Benton et al. (2007)	RM randomized acute experimental study.	Primary school children (UK). $n = 19$, Mean age: 6 years, 10 months. Low SES school.	Three conditions, 4-week SBP. Isocaloric BF at 0815–0845 h of differing GL 1. High GL: Cornflakes, semi-skimmed milk, sugar, waffle, syrup (305 Kcal/39 GL) 2. Medium GL: Scrambled egg, bread, jam, spread, yoghurt (284 Kcal/14.8 GL) 3. Low GL: Ham, cheese, linseed bread, spread (299 Kcal/5.9 GL)	Two observations. 1. Video recorded in-class observation at 1030–1100 h (+135 min post BF) during independent quiet work. Time sampling. Behavior coded: on-task, looking around room, talking to peers, fidgeting, negatively interacting with peers, out of seat. 2. Reaction to frustration measured by response to difficult video game. Behavior coded: concentrating, fidgeting, physical signs of frustration, negative verbal comments.	Meal × time interaction for time on-task in first 10 min of class observation. Significantly more time spent on-task after consuming low GL BF compared with med GL BF and high GL BF. No significant effect of BF on other behavior. GL of BF negatively predicted performance on video game on first test occasion (behavior better after low GL BF).
Cueto and Chinen (2008)	SBP evaluation. 11 intervention schools, 9 control schools. Multiple and full grade schools. 3-year intervention.	Primary schools (Peru) $n = 590$. SBP: $n = 300$, mean age ± *SD*: 11.87 ± 1.77. Male: 51.7%, Female: 48.3% Control: $n = 290$ mean age ± *SD*: 11.87 ± 1.90. Male: 49.7%, Female: 50.3%. 66–69% 1st grade children ≤2 SD height-for-age NCHS reference.	Two conditions: 1. Free Mid-morning SBP: BF during school break time at 1000–1100 h. Milk-like beverage and 6 biscuits (600 Kcal/60% RDA vitamins and minerals 100% RDA for Iron) 2. Control: No BF/BF at home	Behavior coded: Average time/day spent in classroom with teacher as proxy measure for on-task behavior.	Reduction in time spent in classroom indicative of on-task behavior in intervention schools. Increased time spent in recess following SBP.
Wender and Solanto (1991)	RM randomized acute experimental study. Double blind.	Lab based (USA). $n = 26$. Controls: No ADD-H $n = 9$, mean age ± *SD*: 6.7 ± 0.7. ADD-H: $n = 17$, mean age ± *SD*: 6.9 ± 0.6.	Two conditions. Isocaloric BF and drink (226 g) at 0900 h 1. High sugar: Bread (1 slice), butter (5 g), and 35 g sugar drink (≈275 Kcals) 2. Low sugar: Bread (2 slices) butter (15 g), and 0 g sugar drink sweetened 175 mg aspartame or saccharine. (≈275 Kcals)	Video recorded playroom observation at 1000, 1100, 1200, 1300 h (+60, +120, +180 min post BF and +30 min post lunch). Behavior coded: Aggression, hitting, kicking throwing. Time sampling. Good periodic inter-rater reliability.	No effects of BF on aggression.

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Table 1 | Continued

Authors, year	Design	Sample	BF intervention/assessment of BF	Assessment of behavior	Reported results
Benton and Jarvis (2007)	RM, randomized acute experimental study.	Primary school children (UK). $n = 20$. Mean age: 9 years 4 months. Male: 50%, Female: 50%.	Mid-morning snack, 1045 h after self-reported BF: 1. Muesli bar 25 g (226 Kcal/35 g CHO) 2. No snack Children classified depending on energy content of BF: 1. <150 Kcal (Mean ± SE: 61.2 ± 18.5 Kcal) 2. 151–230 Kcal (Mean ± SE: 209.7 ± 8.3 Kcal) 3. >230 Kcal (Mean ± SE: 270.3 ± 64.8 Kcal)	In-class observation at 1115–1215 h (+30 min post mid-morning snack). Behavior coded: on-task, distracted, disruptive, interacting with teacher, out of chair. Categories collapsed into on-task or off-task behavior. Time sampling.	Size of BF × snack interaction for on-task behavior. Children who consumed <150 Kcal BF spent significantly more time on-task when a snack was eaten. BF × snack interaction for off-task behavior. Children consuming <150 Kcal BF spent significantly more time off-task when no snack consumed compared with 151–230 Kcal and >230 Kcal BF. Children who consumed <150 Kcal BF spent significantly less time off-task when a snack was eaten.
Wahlstrom and Begalle (1999)	SBP evaluation. 6 intervention schools. 3 control schools 3-year intervention.	Primary schools (USA) $n = 2901$ children age 6–14 years. Proportion of children eligible for FSM or reduced priced meals: 20.4–77.3%.	Two conditions: 1. Intervention: Free SBP Unstandardized. Average daily participation rate: 68.9–97.5% 2. Control: No SBP	Interviews with teachers and questionnaires completed by teachers. Behavior assessed: Readiness to learn and social behavior. Number of discipline referrals.	Teachers perceived positive impact of SBP on social behavior and readiness to learn compared with pre intervention. Teacher reported increase attention and concentration following SBP. Decrease in discipline referrals following SBP.
Overby and Hoigaard (2012)	Cross-sectional survey study.	Four secondary schools (Norway). $n = 475$, mean age (*SD*) 14.6 ± 0.56, Male: 49.7%, Female: 50.3%.	Questionnaire, 1 item to measure BF. BF intake classified as: 1. Often: BF >5 days/week 2. Never/seldom: BF ≤5 days/per week	Self-reported behavior. 4-item questionnaire to measure disruptive behavior in class. Score range: 4–20. Higher scores indicating poorer behavior. Total scores dichotomized into two categories: No behavioral problems: 4–11 Behavioral problems: 12–20	Frequent breakfast consumption significantly associated with decreased odds of behavior problems (AOR: 0.29 95% CI: 0.15–0.55) compared with never/seldom consumption following adjustment for gender and BMI.

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Table 1 | Continued

Authors, year	Design	Sample	BF intervention/assessment of BF	Assessment of behavior	Reported results
Murphy et al. (1998)	SBP evaluation. Pre-post test. 4-month intervention.	Three primary schools (USA) $n = 133$ mean age ± *SD*: 10.3 ± 1.6 years. Male: 44%, Female: 56%. Proportion of children eligible for FSM or reduced priced meals: >70%.	Free SBP. Considered nutritionally balanced including milk, RTEC, bread, muffin, fruit, juice. Stratified by SBP participation: 1. Often: ≥80% attendance 2. Sometimes: 20–79% attendance 3. Rarely: <20% attendance	Conners Teacher Rating Scale hyperactivity index 10-item.	Significantly greater decreases in hyperactivity scores in children who increased participation in SBP post intervention compared with children who had not changed SBP participation.
Ni Mhurchu et al. (2013)	Cluster RCT, stepped wedge (sequential roll-out of intervention over 1 year period). SBP evaluation. 14 primary schools. 1 year intervention.	Primary schools (New Zealand) $n = 424$ children aged 5–13 years. Male: 47%, Female: 53%. Low SES schools.	Two conditions: 1. Free SBP: School run. Non-standardized. School selected food: Low sugar RTEC, low-fat milk, bread, spreads (honey, jam, and margarine), chocolate flavored milk powder, and sugar 2. Control: No SBP	The Strength and Difficulties Questionnaire completed by teachers. 25 items related to five dimensions: hyperactivity/ inattention, emotional symptoms, conduct problems, peer relationship problems, and pro-social behavior. PISA Student Engagement Questionnaire to measure self-report belonging and relationships with other students.	No significant effect of SBP on behavior vs. control. Proportion of children eating BF everyday did not change. Decrease in proportion of children eating BF at home, increase in proportion of children eating BF at school.
Murphy et al. (2011)	Clustered RCT with a repeated cross-sectional design. 56 control schools, 55 intervention schools. SBP evaluation. 1 year intervention.	Primary schools (UK). $n = 4350$ baseline, $n = 4472$ follow-up aged 9–11 years. Teacher completed behavior assessment on sub-sample of 5 pupils in 2 year groups. Control: $n = 473$ Intervention: $n = 485$.	Two conditions: 1. Intervention: SBP, Non- sugar coated RTEC, milk, bread, fruit. Considered nutritionally balanced 2. Control: No SBP, wait listed control	The Strength and Difficulties Questionnaire completed by teachers. Classroom behavior rated. Hyperactivity/inattention scale used as potential relationship with on-task behavior.	No difference in classroom behavior in intervention vs. control schools.

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Table 1 | Continued

Authors, year	Design	Sample	BF intervention/assessment of BF	Assessment of behavior	Reported results
Shemilt et al. (2004)	Clustered RCT with observational analysis due to contamination between treatment arms. 3-month follow up (CT testing outcomes) and 1 year follow up (behavioral outcomes).	Primary and secondary schools (UK) $n = 6042$ Control: $n = 2369$, mean age $\pm$ *SD*: 10.13 ± 3.93. Male: 52%, Female: 48%. Intervention: $n = 3673$, mean age $\pm$ *SD*: 9.59 ± 2.96 Male: 49%, Female: 51%.	Two conditions: 1. Funding for free SBP 2. Control: No funding for SBP For analysis of behavior, children classified as: 1. Non-attendees: Never attended 2. Attendees: Attended at least once	The Strength and Difficulties Questionnaire. Teachers completed questionnaire for primary school children. Self-report version for secondary school children. 25-item related to five dimensions: hyperactivity/inattention, emotional symptoms, conduct problems, peer relationship problems, and pro-social behavior. Score dichotomized into normal or borderline/abnormal for each dimension.	Significantly higher proportion of primary school BF club attendees had borderline/abnormal conduct and total difficulties scores compared to non-attendees following adjustment for confounders. Significantly higher proportion of secondary school BF club attendees had borderline/abnormal pro-social scores compared with non-attendees following adjustment for confounders. Adjusted for school type, gender, FSM status.
O'Sullivan et al. (2009)	Cross-sectional survey study. The Western Australian Pregnancy cohort study.	School children (Australia) $n = 836$, aged 13–15 years, Male: 50.7% Female: 49.3% Majority well-nourished, 5.7% underweight.	Three-day food diary. BF intake classified based on 5 core food groups defined by AGHE: Bread and cereals, vegetables, fruit, dairy, and dairy alternatives, meat, and meat alternatives. 1. No food or drink/water only 2. Non nutritious food and drink 3. Food from 1 AGHE core food group 4. Food from 2 AGHE core food group 5. Food from $\geq$3 AGHE core food group	Child Behavior Checklist completed by parents (higher score indicates poor behavior), 118-item. Internalizing behavior: Somatic complaints, withdrawal, anxious/depressed Externalizing behavior: Aggression, delinquency Total behavior: Internalizing subscale, externalizing subscale, social thought, and attention problems.	Increase in BF quality associated with decrease in internalizing behavior score and a decrease in externalizing behavior scores. Increase in BF quality associated with decrease in total child behavior score. Stepwise decrease in total score with increasing breakfast quality. Adjusted for: PA, sedentary behavior, weight status, family income, maternal education, maternal age of conception, family structure, family functioning.

(Continued)

Table 1 | Continued

Authors, year	Design	Sample	BF intervention/assessment of BF	Assessment of behavior	Reported results
Miller et al. (2012)	Prospective cohort study. Part of ECLS-K national study. Data collection in five waves: 1999 (preschool), 2000 (grade 1), 2002 (grade 3), 2004 (grade 5), 2007 (grade 8).	Preschool- primary school children (USA) $n = 21400$ at baseline, $n = 9700$ at final follow up, aged 5–15 years (mean 6.09 years) Male: 51%, Female: 49%.	Parental questionnaire, 1 item to assess family BF frequency. BF classified as frequency/week (0–7)	Internalizing and externalizing subscales of the Social Rating Scale adapted from Social Skills Rating System. Externalizing subscale behavior coded: arguing, fighting, angry, impulsivity, disturbed activities, talked during quiet study. Internalizing subscale behavior coded: anxious, lonely, sad, low self-esteem. Teachers rated behavior until grade 5. Children completed scales at grade 8. Acceptable to good reliability on both scales.	No significant association between frequency of family BF and behavior. Fixed effects model results used as provides most unbiased estimates: account for all controls and eliminates between-subject variation. Extensive controls: Gender, ethnicity, family SES, parental education, family income, parental job prestige, family structure, area of residence, language, maternal employment during preschool, birth weight, teaching quality, school quality, region of residence, parental working hours, single parent family.

ADD-H, attention deficit disorder-hyperactivity; AGHE, australian guide to health eating; BMI, body mass index; BF, breakfast; CHO, carbohydrate; CT, cognitive testing; ECLS-K, early childhood longitudinal study kindergarten cohort; FSM, free school meals; GI, glycaemic index; GL, glycaemic load; IG, independent groups; Kcal, kilocalorie; NCHS, national center for health statistics; PA, physical activity; PISA, programme for international student assessment; RCT, randomized control trial; RDA, recommended daily allowance; RM, repeated measures; RTEC, ready to eat cereal; SBP, school breakfast program; SD, standard deviation; SES, socio-economic status.

One study examined the effects of breakfast size with or without a mid-morning snack (Benton and Jarvis, 2007). The results indicated that children who consumed a small breakfast (<150 Kcal) spent significantly more time on-task when a mid-morning snack was also eaten. This effect was not evident in children who consumed more energy at breakfast (151–230 Kcal and >230 Kcal). Correspondingly, children who consumed <150 Kcal at breakfast spent significantly more time off-task when no snack was eaten compared with children who consumed more energy at breakfast. This suggests a mid-morning snack is only beneficial for children who have skipped or eaten very little for breakfast and corrects the energy deficiency.

Rating scales and questionnaires

Twelve studies utilized teacher completed rating scales to assess children's behavior at school following breakfast. These studies usually employed global scales to assess a range of behavioral domains including: attention, disruptive behavior, hyperactivity, pro-social behavior, and aggression. The majority used standardized, established measures of behavior comparable across studies. Measures included the Strength and Difficulties Questionnaire (SDQ), Social Skills Rating System (SSRS), Child Behavior Checklist (CBCL) Conners Teacher Rating Scale (CTRS), and The Attention Deficit Disorder—Hyperactivity Comprehensive Teacher's Rating Scale (ACTeRS). Of the 12 studies that utilized rating scales and questionnaires, only two studies used unstandardized questionnaires and interviews with teachers to measure behavior (Wahlstrom and Begalle, 1999; Overby and Hoigaard, 2012). Six of the twelve studies demonstrated a positive effect of breakfast on behavior at school, which was mainly hyperactivity and disruptive behavior.

Intervention studies. Six intervention studies reported mixed evidence for the effects of SBPs on behavior at school. Two studies in low SES and undernourished children aged 8–10 years reported beneficial effects on hyperactivity (Richter et al., 1997; Murphy et al., 1998). In a longitudinal analysis of a 4-month SBP, Murphy et al. (1998) found significantly greater decreases in CTRS hyperactivity scores in children who increased participation in the SBP compared with children whose participation was unchanged. Similarly, results from a 6-week SBP in undernourished children indicated a significant decline in ACTeRS hyperactivity scores following the SBP, but no change in attention, social skills and oppositional behavior during lessons (Richter et al., 1997). Wahlstrom and Begalle (1999) reported an increase in social behavior and readiness to learn from interviews with teachers following a 3-year SBP. Their results also indicated a decrease in overall discipline referrals following the SBP. Whilst this evidence indicates an apparent benefit of SBPs on school behavior, methodological shortcomings, including a lack of randomization and the inclusion of an appropriate control group, cannot preclude the effects of confounding factors.

Three recent robust randomized control trials (RCT) that address the above inadequacies failed to find a similar benefit for school behavior measured by the SDQ following a 1 year intervention. Both Ni Mhurchu et al. (2013) and Murphy et al. (2011) reported no significant effects of a 1 year SBP on hyperactivity, inattention, emotional symptoms, conduct and peer relationship problems, and pro-social behavior in children. However, in both trials, SBP attendance was low and variable, limiting the potential impact on behavior. The barriers to participation in SBPs include a lack of parental support, a lack of teaching support, social stigma, busy morning schedules, transport issues preventing children from getting to school early and breakfast clubs causing children to arrive late to the first lesson (Reddan et al., 2002; McDonnell et al., 2004; Greves et al., 2007; Lambert et al., 2007).Furthermore, the proportion of children eating breakfast everyday remained unchanged whilst the proportion of children eating breakfast at home decreased, suggestive of a shift in consumption from at-home to at-school, rather than a change/increase in consumption. This may account for the lack of observed effects on behavior. Shemilt et al. (2004) indicated a negative impact of a SBP on behavior in both primary and secondary school children within deprived areas. Although this study aimed to employ a RCT design, contamination between treatment arms necessitated a longitudinal observational analysis of behavioral outcomes and SBP attendance, rather than the planned intention to treat analysis. Results at 1 year follow up indicated that children who attended the breakfast club had a higher incidence of borderline or abnormal conduct, pro-social, and total difficulties compared to children who did not attend the breakfast club (Shemilt et al., 2004). Teachers also indicated that children were more energetic, less well-behaved and were difficult to control in the classroom as a result of attending the breakfast club. Parallel qualitative data from teachers, breakfast club staff and researchers who observed the breakfast club suggested that children's behavior deteriorated during the breakfast club as a result of inadequate supervision and training, and a lack of teaching staff who seemed to be regarded with more authority by children. Observations of the breakfast club indicated behavior was often boisterous or disruptive and there was a general lively atmosphere. This suggests that factors associated with the delivery of the SBP had more impact on behavioral outcomes than the subtle nutritional effects of breakfast in this study. In addition, this study epitomizes the difficulties in isolating the independent effects of breakfast.

Acute experimental studies. Three acute experimental studies examined the effects of breakfast meals that differed in sugar content on CTRS hyperactivity, inattention/over-activity and aggression subscales. Both Milich and Pelham (1986) and Kaplan et al. (1986) showed no effect of the sugar content of breakfast and behavior in children with ADD-H or behavioral problems. However, Rosen et al. (1988) observed a small significant increase in hyperactivity scores following a breakfast with high sugar content compared with low sugar in children without behavior problems (Rosen et al., 1988).

Cross-sectional studies. Two cross-sectional studies in well-nourished adolescent populations reported a significant association between habitual breakfast consumption and behavior. Overby and Hoigaard (2012) found that frequency of breakfast was significantly associated with less self-reported disruptive behavior during lessons in adolescents (mean age 14.6 years). Adolescents who habitually consumed breakfast (>5 days/per

week) had significantly reduced likelihood of disruptive behavior [Odds Ratio (OR): 0.29, 95% CI: 0.15–0.55] compared with those who ate breakfast less frequently (≤5 times per week). A similar association was also evident between breakfast quality based on the number of food groups within the breakfast meal and CBCL scores (higher score indicates poor behavior) in adolescents (O'Sullivan et al., 2009). Higher breakfast quality scores were most strongly associated with lower CBLC externalizing behavior scores (which indicates aggression and delinquency). The results indicated a stepwise decrease in total scores on the CBCL with increasing breakfast quality, indicative of a possible dose-response relationship.

Prospective cohort studies. Although there is some associative evidence of a relationship between habitual breakfast consumption and behavior in adolescents, the same relationship was not apparent in a well-controlled prospective cohort study. Miller et al. (2012) reported no association between frequency of breakfast and negative behavior (e.g., arguing, fighting, angry, and disruptive) in 21,400 school children aged 5–15 years following a 10 years follow up and adjustment for extensive confounders.

ACADEMIC PERFORMANCE

Twenty-two studies employed academic performance measures to investigate the effects of breakfast on academic outcomes (**Table 2**). The academic performance outcomes employed by studies included either school grades or standardized achievement tests. Twenty-one studies demonstrated that habitual breakfast (frequency and quality) and SBPs have a positive effect on children and adolescents' academic performance.

Average school grades

Ten studies examined the effects of breakfast on average school grades. The majority produced a composite score from school reported grades across a range of subjects, usually considered "core" subjects. Two studies relied on self-reported school grades (Lien, 2007) or self-reported subjective ratings of school performance (So, 2013). Seven of the ten studies were in 12–18 year olds, reflecting the schooling system in which grading is more common in older pupils. Only three studies were carried out in primary school children aged 7–11 years (Murphy et al., 1998; Kleinman et al., 2002; Rahmani et al., 2011). One study included children of low SES (Murphy et al., 1998) and two studies included undernourished children (Kleinman et al., 2002; Gajre et al., 2008). All 10 studies identified demonstrated that habitual breakfast (frequency and quality) and SBPs have a positive effect on children and adolescents' school performance, with three studies observing clearest effects on mathematics grades (Murphy et al., 1998; Kleinman et al., 2002; Morales et al., 2008).

Intervention studies. Three intervention studies demonstrated positive effects of SBPs on school grades, particularly mathematics grades in both well-nourished, undernourished and low SES children aged 7–10 years. Effects were demonstrable after an intervention period of 3–6 months. A significant increase in school grades was apparent following an intervention providing 250 ml 2.5% fat milk at breakfast, which was apparent in girls only (Rahmani et al., 2011). Although it was not clear if the sample included undernourished children, the effect coincided with a significant increase in weight of the girls following the intervention in schools which received the intervention compared to control schools. Supportive evidence from Kleinman et al. (2002) found that following a 6-month SBP, children who had improved their nutritional status from at risk (energy and/or >2 nutrients <50% RDA) to adequate significantly increased their mathematics grades. Murphy et al. (1998) reported that following a 4-month SBP, children who increased participation were significantly more likely to increase their mathematics grades compared to those who had decreased or maintained participation.

Cross-sectional studies. Seven cross-sectional studies demonstrated a consistent positive association between habitual breakfast and school grades in adolescents.

Frequency of breakfast consumption was associated with school performance in five studies. Breakfast skipping (eating breakfast <5 days/week) was associated with lower average annual school grades in a sample of 605 Dutch adolescents aged 11–18 years who were in higher educational streams (Boschloo et al., 2012). This association was evident in both sexes and independent of age. Additionally, breakfast skipping was associated with more self-reported attention problems, which partially mediated this relationship. A larger cohort of nearly 6500 Korean adolescents of similar age range (10–17 years) demonstrated a similar association across all ages. However, the association was stronger in younger children (10–11 and 13–14 years) than older children (16–17 years) (Kim et al., 2003). Effects were seen in both genders, except for in 10–11 year olds, where the significant association between regular breakfast intake and school performance was only apparent in boys.

This association is also evident in undernourished adolescents (Gajre et al., 2008). Gajre et al. (2008) demonstrated that eating breakfast >4 days/week significantly predicted total average grades in a sample of children aged 11–13 years, a third of whom were undernourished. Analysis of individual subject domains indicated that regular breakfast eaters had significantly higher grades for science and English, but not mathematics compared to children who never ate breakfast (Gajre et al., 2008).

Lien (2007) demonstrated, in a large sample of adolescents aged 15–16 years, that those who never ate breakfast were twice as likely to have lower self-reported school grades compared with those who consumed breakfast every day (7 days/week). This finding was consistent in boys and girls. Moreover, the odds of having lower self-reported school grades decreased with successive quintiles of breakfast eating frequency suggestive of a dose-response relationship. Recent evidence from an internet based study demonstrated a similar relationship between habitual breakfast and self-rated academic performance in over 75,500 adolescents aged 12–18 years (So, 2013). Regular breakfast eaters (7 days/week) had increased likelihood of rating their school performance as higher compared with breakfast skippers (0 day/week).

Two studies demonstrated a consistent association between breakfast composition derived from energy and food groups provided and school grades in adolescents aged 12–17 years. Morales

Table 2 | Tabulation of studies investigating the effects of breakfast on academic performance in children and adolescents.

Authors, year	Design	Sample	BF intervention/assessment of BF	Assessment of school performance	Reported results
Lien (2007)	Cross-sectional survey study.	School children (Norway) $n = 7305$ aged 15–16 years. Male: 49.4%, Female: 50.6%.	Questionnaire, 1-item to assess BF frequency. BF intake classified as: 1. Seldom/never 2. 1–2 days/week 3. 3–4 days/week 4. 5–6 days/week 5. Everyday	Self-reported most recent grade for: 1. Mathematics 2. Norwegian 3. English 4. Social Science Grade scale: 1 (lowest) to 6 (highest). Total average grade calculated and dichotomized as: ≤ 3 or >3.	Increased odds of having low school grades (≤ 3) in children who seldom/never ate BF compared with everyday consumption in boys and girls (AOR: 2.0, 95% CI: 1.3–3.1 and AOR: 2.0 95% CI: 1.3–3.01, respectively). Adjusted for: parental education, family structure, immigrant status, smoking, dieting, soft drink consumption.
So (2013)	Cross-sectional survey study. Korea Youth Risk Behavior Web-based survey.	School children (Korea) $n = 75643$ mean age $\pm$ *SD*: 15.10 ± 1.75. Male: 51%, Female: 49%.	Internet questionnaire, 1-item to assess BF frequency. BF classified as frequency/week (0–7)	Self-reported academic performance rating for previous 12 months: 1. Very high 2. High 3. Average 4. Low 5. Very low Dichotomized into two groups: 1. <Average academic performance 2. $\geq$Average academic performance	BF eaters (7 days/week) had increased likelihood of rating higher school performance compared with BF skippers (0 day/week). AOR males: 1.7, 95% CI: 1.57–1.83; AOR females: 1.92, 95% CI: 1.76–2.97. Adjusted for: age, BMI, smoking, alcohol, parental education, family SES, PA (vigorous and moderate), muscular strength, mental stress.
Murphy et al. (1998)	SBP evaluation. Pre-post test. 4-month intervention.	Three primary schools (USA) $n = 133$ mean age $\pm$ *SD* 10.3 ± 1.6 years. Male: 44%, Female: 56%. Proportion of children eligible for FSM or reduced priced meals: >70%.	Free SBP. Considered nutritionally balanced including milk, RTEC, bread, muffin, fruit, juice. Stratified by SBP participation: 1. Often: $\leq$80% attendance 2. Sometimes: 20–79% attendance 3. Rarely: <20% attendance	School reported grades for: 1. Mathematics 2. Reading 3. Science 4. Social studies Letter grade converted into numeric value: $A = 4$, $B = 3$, $C = 2$, $D = 1$, $F = 0$.	Higher mathematics grades post intervention in children who regularly participate in SBP compared to those who rarely or sometimes participate. Children who increased their SBP participation were significantly more likely to increase mathematics grades compared to those who had decreased or unchanged participation. No effects of SBP on other grades.

(Continued)

Table 2 | Continued

Authors, year	Design	Sample	BF intervention/assessment of BF	Assessment of school performance	Reported results
Kleinman et al. (2002)	SBP evaluation. Pre-post test. 6-month intervention.	Primary schools (USA) $n = 97$ aged 9–12 years. Nutritionally at risk (energy and/or >2 nutrients <50% RDA): $n = 29$. Adequate: $n = 68$.	Two conditions, SBP. 1. Free SBP for 6 months 2. No SBP	School grades obtained from school records: 1. Mathematics 2. Reading 3. Science 4. Social Studies Letter grade converted into numeric value: $A = 4$, $B = 3$, $C = 2$, $D = 1$, $F = 0$.	Significant increase in mathematics grades in children who improved nutritionally status from at risk to adequate post intervention.
Rahmani et al. (2011)	SBP outcome evaluation, IG. 2 intervention schools, 2 control schools. 3-month intervention.	Four primary schools (Iran) $n = 469$ Male: 49% mean age ± *SD*: 7.9 ± 0.8 years. Female: 51%, mean age ± *SD*: 7.5 ± 0.9 years. Medium SES.	Two conditions: 1. School feeding program: 250 ml 2.5% fat milk at 0930 h 2. Control: No milk	Average grade point.	Girls had significantly higher average grade point following intervention compared with control. Girls were significantly higher in weight following intervention compared with control.
Gajre et al. (2008)	Cross-sectional survey study.	School children (India) $n = 379$ aged 11–13 years. Male: ≈55% Female: ≈45% Underweight: 20.8% Stunted: 38.5% NCHS reference.	Questionnaire to assess BF eating frequency and type. BF defined as first eating occasion during the morning before school. BF intake classified as: 1. Regular: >4 days/week 2. Irregular: Skipping BF 2–3 days/week 3. Never Composition of breakfast not reported	End of year grades for: 1. Mathematics 2. Sciences 3. English Total average grade and individual subject grades used in analysis.	Regular BF group had significantly higher marks for science, English and total grade compared to no BF group. Regular BF significantly predicted total average grade. Regular BF and education of mother predicted English grades. Regular breakfast, type of family and height for age significantly predicted science grades. No association between BF and mathematics grades.

(Continued)

Table 2 | Continued

Authors, year	Design	Sample	BF intervention/assessment of BF	Assessment of school performance	Reported results
Morales et al. (2008)	Cross-sectional survey study.	School children (Spain) $n = 467$ aged 12–17 years. Male: 42%, Female:58%.	Seven-day food diary (Mon-Sun) and FFQ. BF intake classified as: 1. Full BF: >25% of TE, includes ≥4 foods groups of dairy, cereals, fruit, fat 2. Good quality: 3 food groups of dairy, cereals, and fruit 3. Better options: Missing one food group 4. Poor quality: Missing two food groups 5. No BF	Average end of course grades: 1. Language 2. Mathematics 3. Chemistry 4. Biology 5. Social Sciences 6. Physical education Total average grade calculated.	Full and good quality BF groups associated with higher total, mathematics, chemistry, and social science grades compared with no BF. Physical education, biology, and languages grades were highest in no BF group compared with full and food quality BF groups.
Boschloo et al. (2012)	Cross-sectional survey study.	School children (Netherlands) $n = 605$ aged 11–18 years. Male: 44%, Female: 56%. All children in advanced educational tracks in secondary schools.	Questionnaire, 1-item to assess BF frequency on school days. BF classified as: 1. BF eaters: 5 days/week 2. BF skippers: <5 days/week	Average end of year school grades: 1. Dutch 2. Mathematics 3. English as a foreign language Grade range: 1(very bad) to 10 (outstanding) Attention problems: Attention Problems Scale from the Dutch Youth Self Report.	BF skipping significantly associated with lower school performance and more self-reported attention problems. Attention problems partially mediated the relationship between BF skipping and school performance. Adjusted for: age, sex, educational track, parental education.
Kim et al. (2003)	Cross-sectional survey study.	School children (Korea) $n = 6463$ aged 10–11, 13–14, 16–17 years. Male: 53%, Female: 47%.	FFQ and dietary behavior questionnaire. BF intake classified as: 1. Regular BF 2. No regular BF	Average grade from last school semester. Scores range from 1–5 obtained from school records 1. Korean 2. Mathematics 3. Social Studies 4. Science 5. Physical education 6. Music 7. Art 8. Practical course 9. Ethics 10. English (grade 8 and 11)	Regular BF associated with higher average grade in 10–11 years old boys, higher average grade in 13–14 years old boys and girls and higher average grade 16–17 years old boys and girls. Adjusted for: parental education, physical fitness, physical status.

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Table 2 | Continued

Authors, year	Design	Sample	BF intervention/assessment of BF	Assessment of school performance	Reported results
Herrero Lozano and Fillat Ballesteros (2006)	Cross-sectional survey study.	School children (Spain) $n = 141$ aged 12–13 years. Male: 49.6%, Female: 50.4%.	Recall BF of previous day (1 day only). BF intake classified as: 1. Good quality: 3 food groups of dairy, cereals and fruit 2. Improvable quality: Missing one of the food groups 3. Insufficient quality: Missing two food groups 4. Poor quality: No BF Contribution of a mid-morning snack to BF considered	Average end of year grade.	Significantly higher average grades obtained in good quality BF groups compared with poor quality. Average grade increased when good quality snack was eaten in poor and insufficient BF quality groups.
Cueto and Chinen (2008)	SBP evaluation. 11 intervention schools, 9 control schools. Multiple and full grade schools. 3-year intervention.	Primary schools (Peru) $n = 590$. SBP: $n = 300$, mean age $\pm$ *SD*: 11.87 $\pm$ 1.77. Male: 51.7%, Female: 48.3% Control: $n = 290$ mean age $\pm$ *SD*: 11.87 $\pm$ 1.90. Male: 49.7%, Female: 50.3% 66–69% 1st grade children $\leq$2 SD height-for-age NCHS.	Two conditions: 1. Free Mid-morning SBP: BF during school break time at 1000–1100 h. Milk-like beverage and 6 biscuits (600 Kcal/60% RDA vitamins and minerals 100% RDA for iron) 2. Control: No BF/BF at home	Unstandardized tests developed to account for variability in curriculum: 1. Arithmetic 2. Reading comprehension	Higher arithmetic and reading scores in multiple grade intervention schools compared to control post intervention. No significant effect of SBP in full grade schools.
Acham et al. (2012)	Cross-sectional survey study.	School children (Uganda) $n = 645$ aged 9–15 years. Male: 46%, Female: 54% Underweight: 13% Stunted: 8.7%.	Questionnaire, 1-item to assess BF frequency. BF intake classified as: 1. BF 2. BF and/or mid-day meal 3. No BF or mid-day meal	Unstandardized tests: Developed to account for variability in school environment. 1. English 2. Mathematics 3. Life Skills 4. Oral comprehension Maximum score of 400. Cut-off of $<$120 used to define poor performance. 68.4% scored $<$120.	Boys who had consumed BF and mid-day meal were significantly more likely to score $\geq$120 than those who only had one meal (OR: 1.99 95% CI: 1.0–3.9). No association between BF alone and test scores. Adjusted for household size, mothers education, land quantity owned, school attendance, gender head of household, feeding habits, age, household wealth.

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Table 2 | Continued

Authors, year	Design	Sample	BF intervention/assessment of BF	Assessment of school performance	Reported results
Powell et al. (1998)	SBP evaluation. RCT. 1 school year intervention.	16 Primary schools. (Jamaica) $n = 810$ children aged 7–11 years. Undernourished (< −1 SD weight-for-age NCHS): 405 Nourished: 405.	Two conditions: 1. Intervention: Free SBP. Cheese sandwich/spiced bun and cheese, flavored milk (576–703 Kcal/27.1 g PRO). Served before school 2. Control: ¼ orange (18 Kcal/0.4 g PRO)	The Wide Range Achievement Test: 1. Reading 2. Spelling 3. Arithmetic	Significant positive effect of BF on Arithmetic. Grade × Treatment interaction indicated the positive effect on arithmetic scores was mainly demonstrated in younger children. No effects of BF on spelling and reading. No differential effects by nutritional group.
Simeon (1998) Study 1	SBP evaluation. 1 school semester intervention.	School based (Jamaica) $n = 115$.12–13 years Rural schools, low ability children, low attendance at school Undernourished: ≈50%.	Three condition. BF at 0900 h. 1 school semester intervention. 1. School BF: 100 ml milk (130 Kcal), cake (250 Kcal), or meat filled pasty (599 Kcal) 2. Syrup drink (31 Kcal) 3. No BF	The Wide Range Achievement Test: 1. Spelling 2. Arithmetic 3. Reading (not used in analysis)	Syrup drink and no BF groups combined to form one control group as no significant differences found on all outcomes. Children receiving school BF performed better on arithmetic test relative to control group post intervention.
Wahlstrom and Begalle (1999)	SBP evaluation. 6 intervention schools, 3 control schools. 3-year intervention.	Primary schools (USA) $n = 2901$ children age 6–14 years. Proportion of children eligible for FSM or reduced priced meals: 20.4–77.3%	Two conditions: 1. Intervention: Free SBP Unstandardized. Average daily participation rate: 68.9–97.5% 2. Control: No SBP	School achievement tests, Incomparable across schools. 1. Mathematics 2. Reading	Within school effects (pre-post intervention) show general increase in scores for reading and mathematics.
Jacoby et al. (1996)	SBP evaluation. RCT. 1 month intervention.	10 Primary school (Peru) $n = 352$. Intervention: $n = 201$, mean age ± *SD*: 136.2 ± 18 months Male: 46%, Female: 54% Control: $n = 151$, mean age ± *SD*: 138.9 ± 20 months. Male:53%, Female 47% Normal weight and underweight and stunted children.	Two conditions, SBP. 1. Intervention: SBP: 600 Kcal, 60% RDA various vitamins and minerals and 100% RDA iron 2. Control: No SBP	Achievement test for: 1. Reading comprehension 2. Vocabulary 3. Mathematics	No effects of SBP on any achievement tests. Significant weight × treatment interaction children in intervention schools of higher weight increase vocabulary scores.

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Table 2 | Continued

Authors, year	Design	Sample	BF intervention/assessment of BF	Assessment of school performance	Reported results
Meyers et al. (1989)	SBP evaluation. pre-post test. 3-month intervention.	16 Primary schools (USA) $n = 1023$ children aged 8–12 (grades 3–6) Male: 51%, Female: 49% Low income.	SBP. Stratified by SBP participation 1. Non attendees: <60% attendance 2. Attendees: ≥60% attendance	The Comprehension Test of Basic Skills. 1. Language 2. Reading 3. Mathematics	Lower total scores at baseline in non-attendees. Greater increase in total and language scores in attendees compared with non-attendees. SBP attendance positively associated with total scores at follow up.
Ni Mhurchu et al. (2013)	Cluster RCT, stepped wedge (sequential roll-out of intervention over 1 year period). SBP evaluation. 14 primary schools. 1 year intervention.	Primary schools (New Zealand) $n = 424$ school children aged 5–13 years. Male: 47%, Female: 53%. Low SES schools.	Two conditions: 1. Free SBP: Non-standardized. School selected food: Low sugar RTEC, low-fat milk, bread, spreads (honey, jam, margarine), chocolate flavored milk powder, and sugar 2. Control: No SBP	Standardized school achievement tests: 1. Literacy 2. Numeracy Self-report assessment of reading ability using questionnaire. Scores from 1 (not very well) to 5 (very well).	No significant effects on achievement tests, self-report reading ability and attendance. Proportion of children eating BF everyday did not change. Decrease in proportion of children eating BF at home, increase in proportion of children eating BF at school.
Edwards et al. (2011)	Cross-sectional survey study.	School children (USA) $n = 800$ aged 11–13 years. $n = 694$ complete data on gender Male: 48%, Female: 52% 13.5% eligible for FSM.	Adapted questions from Youth Risk Behavior Surveillance survey. BF intake classified as 1. BF ≥ 5 days/week 2. BF < 5 days/week	MAP tests. Standardized computer tests for 1. Mathematics 2. Reading	Higher mean mathematics MAP scores associated with eating BF ≥ 5 days/week compared with <5 days/week. Regression analysis indicated BF intake was significantly associated with mean MAP mathematics scores. No association between BF and MAP reading scores: Adjusted for: FSM status.

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Table 2 | Continued

Authors, year	Design	Sample	BF intervention/assessment of BF	Assessment of school performance	Reported results
Lopez-Sobaler et al. (2003)	Cross-sectional survey study.	School children (Spain) $n = 180$ aged 9–13 years. Male: 57%, Female: 43%.	Weighed 7-day food diary. Definition of BF: Cut-off of ≥20% of daily energy requirement. BF intake classified as: 1. AB: ≥20% of daily energy requirement 2. IB: <20% of daily energy requirement	Spanish SAT-1 test. Three sub-batteries: 1. Verbal 2. Reasoning 3. Calculation Direct scores, centile scores, and IQ score obtained.	Higher reasoning SAT-1 scores obtained by AB group compared with IB group. Higher total SAT-1 scores obtained by AB group compared with IB group. Better quality breakfast significantly predicated better reasoning and total scores.
O'Dea and Mugridge (2012)	Cross-sectional survey study.	School Children (Australia) $n = 824$ grades 3–7 (aged 8–13 years). Male: 49%, Female: 51% $n = 755$ parents.	Questionnaire and interview with dietitian. BF defined as solid or liquid eaten before 1000 h on day of testing. BF intake classified as: 0. No food/drink 1. Non-nutrient liquid 2. Confectionary/snack food 3. Grain/cereal or fruit/vegetable 4. Grain/cereal + vitamin C 5. Protein + vitamin C 6. Grain/cereal + protein or Grain/cereal + calcium 7. Grain/cereal + protein + vitamin C or Protein + calcium + vitamin C 8. Grain/cereal + protein + calcium 9. Grain/cereal + protein + calcium + vitamin C 10. Grain/cereal + protein + Vitamin C + calcium including low-fat option	Standardized school achievement tests, NAPLAN test scores for: 1. Literacy 2. Numeracy	Nutritional quality of BF significantly predicted literacy scores. Non-significant association between BF and numeracy scores. Few children skipped BF. Adjusted for: age, gender, SES, maternal education.

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Table 2 | Continued

Authors, year	Design	Sample	BF intervention/assessment of BF	Assessment of school performance	Reported results
Miller et al. (2012)	Prospective cohort study. Part of ECLS-K national study. Data collection in five waves: 1999 (preschool), 2000 (grade 1), 2002 (grade 3), 2004 (grade 5), 2007 (grade 8).	Preschool-primary school children (USA) $n = 21400$ at baseline, $n = 9700$ at final follow up, aged 5–15 years (mean 6.09 years) Male: 51%, Female: 49%.	Parental questionnaire, 1 item to assess family BF frequency. BF classified as frequency/week (0–7)	Standardized achievement tests 1. Reading 2. Mathematics 3. Science (grades 3, 5, 6)	No significant association between frequency of family BF and test scores. Fixed effects model results used as provides most unbiased estimates: accounts for all controls and eliminates between subject variations. Extensive controls. Adjusted for: Gender, ethnicity, family SES, parental education, family income, parental job prestige, family structure, area of residence, language, maternal employment during preschool, birth weight, teaching quality, school quality, region of residence, parental working hours, single parent family.

AD, adequate breakfast; AOR, adjusted odds ratio; BF, breakfast; BMI, body mass index; CI, confidence intervals; CT, cognitive testing; ECLS-K, early childhood longitudinal study–kindergarten cohort; FFQ, food frequency questionnaire; FSM, free school meals; GI, glycaemic index; GL, glycaemic load; IB, inadequate breakfast; IG, independent groups; IQ, intelligence quotient; Kcal, kilocalorie; KJ, kilo joules; MAP, measure of academic progress; NAPLAN, the national assessment program literacy and numeracy; NCHS, national center for health statistics; OR, odds ratios; PRO, protein; PA, physical activity; RCT, randomized control trial; RDA, recommended daily allowance; RM, repeated measures; RTEC, ready to eat cereal; SAT, scholastic aptitude test; SBP, school breakfast program; SD, standard deviation; SES, socio-economic status.

et al. (2008) found that adolescents who habitually ate breakfast that provided >25% of total estimated energy needs and included four or more foods groups from dairy, cereals, fruit, and fat were more likely to achieve higher grades than those consuming no breakfast or breakfast lacking the specified food groups. Analysis of individual subject domains indicated that mathematics, chemistry and social science grades were highest in full (>25% of total energy needs and ≥4 food groups) and good (<25% energy and three food groups) quality breakfast groups compared with no breakfast. Physical education, biology and languages grades were highest in the no breakfast group compared with full and good quality breakfast groups. Supportive findings from Herrero Lozano and Fillat Ballesteros (2006) indicated that higher average grades were obtained in adolescents who habitually consumed a breakfast containing three food groups from dairy, cereals and fruit compared with those consuming no breakfast or breakfast providing one of the specified food groups. The contribution of a mid-morning snack to breakfast quality was also considered in the analysis, which indicated a positive association between a mid-morning snack and school grades specific to children who had consumed no breakfast.

Standardized achievement tests

Age specific standardized achievement tests are routinely administered by schools in developed countries for monitoring and provide an overall indication of intellectual level. Various subtests are included, usually literacy/reading, numeracy/arithmetic and reasoning. Standardized achievement tests employed by studies include the Wide Range Achievement test (WRAT), the National Assessment Program—Literacy and Numeracy (NAPLAN), Measure of Academic Progress (MAP), Scholastic Aptitude Test (SAT), and Assessment Tool for Teaching and Learning (asTTle). Twelve studies used standardized achievement tests to measure school performance. Two studies conducted in developing countries used unstandardized achievement tests developed for the purpose of the research to account for variability in curriculum and school environment (Cueto and Chinen, 2008; Acham et al., 2012). Studies were generally conducted in children aged 6–13 years with 10 of the 12 studies in children younger than 13 years. Evidence indicated a positive effect of SBPs on test scores, with clearest effects on arithmetic scores in both well-nourished and undernourished samples. Evidence also indicated a positive association between habitual breakfast frequency and quality, and test scores.

Intervention studies. Six of the seven intervention studies demonstrated positive effects of SBPs on standardized achievement tests in children aged 4–14 years, with clearest effects on arithmetic scores in undernourished children. Four of the seven studies demonstrated a benefit of breakfast on arithmetic scores (Powell et al., 1998; Simeon, 1998; Wahlstrom and Begalle, 1999; Cueto and Chinen, 2008). Four of the studies were carried out in samples which included undernourished children (Jacoby et al., 1996; Powell et al., 1998; Simeon, 1998; Cueto and Chinen, 2008) and two studies included low SES samples (Meyers et al., 1989; Ni Mhurchu et al., 2013). Effects were demonstrable after an intervention period of at least 1 month and up to 3 years.

Two studies found positive effects on arithmetic test scores from the WRAT following a relatively large breakfast meal (>500 Kcal) compared with a low energy control in undernourished and well-nourished children (Powell et al., 1998; Simeon, 1998). Cueto and Chinen (2008) examined the effects of a mid-morning SBP providing 600 Kcal and 60% of the daily requirements for several vitamins and minerals and 100% of the daily requirement for iron in a large sample of children, two thirds of whom were undernourished (≤ −2 SD height-for-age of the NCHS reference). Higher arithmetic and reading scores were demonstrated following the SBP in intervention schools compared to control schools, particularly in schools which tended to have higher levels of poverty, undernourished children and lower achievement. Comparable results were reported by Jacoby et al. (1996) following the same breakfast intervention for 1 month in children where the majority were below height-for-age but relatively overweight (due to increased body water and weight-for-height classification). Children in intervention schools of higher weight (and therefore likely to be undernourished) increased vocabulary scores post intervention. No effects were observed in normal weight children who were therefore likely to be well nourished.

In children aged 8–12 years from low SES backgrounds, Meyers et al. (1989) reported greater increases in language and total test scores in SBP attendees compared with non-attendees. Wahlstrom and Begalle (1999) also demonstrated an increase in scores for reading and mathematics from pre to post intervention. However, both studies were not well-controlled. A recent large RCT in pupils from low SES schools in New Zealand failed to show any benefit of a 1 year SBP on school achievement tests for literacy and numeracy and self-reported reading ability (Ni Mhurchu et al., 2013).

Cross-sectional studies. Four cross-sectional studies demonstrated a consistent positive association between habitual breakfast consumption and achievement test scores in children, including undernourished children.

Frequency of breakfast consumption was associated with achievement scores in two studies. Acham et al. (2012) demonstrated in well-nourished and undernourished 9–15 year olds predominantly considered low ability, that those who had consumed breakfast and a mid-day meal were almost twice as likely to score highly on achievement tests compared to those who only had one meal. This association was specific to boys, and consuming breakfast alone was not associated with school performance (Acham et al., 2012). This gender difference is not consistent across studies with evidence demonstrating increased odds of having lower self-reported school grades when skipping breakfast compared with habitually consuming breakfast in both genders (Lien, 2007). Edwards et al. (2011) indicated that higher mean mathematics MAP scores were associated with habitually eating breakfast (≥5 days/week) compared with less frequent consumption (<5 days/week). No association was found between breakfast frequency and reading MAP scores.

Two studies demonstrated an association between breakfast composition (energy, food group, and micronutrient content) and achievement scores in children aged 8–13 years. Habitually consuming a breakfast providing ≤20% of total energy needs

was associated with poorer total SAT performance, particularly logical reasoning in 9–11 year olds (Lopez-Sobaler et al., 2003). However, SES was not controlled. O'Dea and Mugridge (2012) demonstrated a significant association between habitual breakfast quality according to food groups (carbohydrate and protein) and micronutrients (vitamin C and calcium) and NAPLAN literacy scores in children aged 8–13 years. No significant association was found between breakfast quality and numeracy scores.

Prospective cohort studies. Miller et al. (2012) demonstrated, in a large cohort of 21,400 school children aged 5–15 years, a non-significant association between breakfast eating frequency and scores on standardized achievement tests for reading, mathematics and science following adjustment for an extensive set of confounders. This was specific to breakfast that was eaten with the family rather than total breakfast intake.

DISCUSSION

THE EFFECTS OF BREAKFAST ON BEHAVIOR

Overview of findings

This review identified 19 studies that examined the effects of breakfast on behavior in children and adolescents of which 11 studies demonstrated a positive effect of breakfast on behavior. The evidence suggests a mainly positive effect of breakfast on on-task behavior in the classroom. This effect was apparent in children irrespective of whether they were well-nourished and undernourished or from low SES or deprived backgrounds. However, most of the research on the impact of breakfast on behavior has taken the form of SBP evaluations, which lack scientific rigor. Three RCTs have not found similar benefits for behavior using standardized measures following a 1 year SBP, although, participation in the SBP was consistently low in some trials, which is likely to account for the lack of effects. In order for SBPs to impact on behavioral outcomes, the barriers to participation need to be addressed. Studies in children with pre-existing behavior problems (e.g., ADD-H) demonstrated no benefit of breakfast of differing sugar content. Findings for other behavioral outcomes including off-task behavior, distractibility, hyperactivity, and disruptive behavior are inconsistent. The frequent null findings reported suggest the effects of breakfast may be specific to selective behavioral domains.

The increase in on-task behavior following breakfast may indicate that children who eat breakfast are more able to concentrate, pay attention and are more alert at school. This is supported by evidence that demonstrates positive effects of breakfast on cognitive performance including attention and memory (Hoyland et al., 2009). Similarly, more on-task behavior in the classroom may be associated with improvements in academic performance supported by the positive association between habitual breakfast intake and academic performance (Boschloo et al., 2012; So, 2013). Moreover, an improvement in classroom behavior has the potential to reduce disruption and produce a more productive learning environment.

Methodological issues

Behavioral measures. Classroom behavior was typically measured by coding observed behavior into predefined domains. Most of the studies focus primarily on on-task and off-task behavior within the classroom. Other behavioral domains measured less frequently include: being distracted, disruptive behavior, positively, or negatively interacting with peers, interacting with teacher, and reaction to frustration. One study did not directly observe classroom behavior and measured overall time spent in the classroom as a proxy measure for on-task behavior, which is an inadequate assessment of behavior (Cueto and Chinen, 2008). The measures used to code classroom behavior are often non-validated, unstandardized coding methods developed for the purpose of the research, and often inter-rater reliability is unspecified or merely recorded as acceptable. Overall, the general theme is the subjective nature of these studies and reliance on interpretation of behavior. There is a lack of studies that use systematic, validated, and reliable coding systems to measure classroom behavior. Two recent studies have demonstrated effects on on-task behavior following school lunch manipulations using a validated observation protocol (Golley et al., 2010; Storey et al., 2011). Future studies investigating the effects of breakfast on behavior should adopt validated and reliable, focused coding schemes to measure classroom behavior. Given the subjective nature of the methods to assess behavior, observers should also be blind to treatment condition.

Observational methods: Real-time vs. Recorded observations. Several issues concern the observational methods used to assess behavior. Real-time classroom observations carried out by teachers or researchers were common. Only four studies utilized video recorded classroom observations likely to produce more accurate and ecologically valid behavioral measures and offer the possibility of post hoc verification by independent observers (Milich and Pelham, 1986; Wender and Solanto, 1991; Richter et al., 1997; Benton et al., 2007). Video recorded classroom observations are therefore a more accurate and reliable behavioral measure. During real-time classroom observations, the researcher is required to observe multiple pupils within the lesson. The dual processing of watching and recording in the classroom is a complex task. The use of a video recorded classroom observation may have the advantage of increased accuracy via the ability to replay, review, and control observer fatigue (Haidet et al., 2009). Secondly, due to the reactive nature of the observation process, the Hawthorne effect may be present, such that children and teachers change their behavior because they are under observation (Roethlisberger and Lombard, 1977). Not having observers present during the observation or utilizing video recorded observation methods may limit this anticipated behavior change. Finally, the habituation period, where cameras/observers are introduced, is often not reported. This habituation period may allow children to become familiar to the presence of observers/cameras in order to reduce reactive behavior change. Future studies should consider, when possible, a video recorded observation to yield a more accurate, reliable observation whilst maintaining ethical safeguards.

Design. Various breakfast manipulations are employed. There are few direct comparisons of breakfasts varying in composition precluding conclusions about the effects of breakfast composition on behavior. Additionally, many studies lack randomization

and the inclusion of an appropriate comparable control group. Most studies are based on small samples and limited to children aged <13 years, with fewer studies in adolescents. Metabolic and behavioral effects of breakfast may be different in older children aged >13 years. Classroom behavior is dynamic and can be different across year groups and ages. Previous research has found differences in behavior between older and younger children in the classroom following school lunch manipulations, where younger children tend to be more distracted when working alone with the reverse true for older children and adolescents (Golley et al., 2010; Storey et al., 2011). The influence of gender on behavior is also not considered by most studies. For example, Chang et al. (1996) demonstrated that girls talked and displayed more movement compared with boys in a set task classroom situation. Further research in this field should include larger samples providing sufficient power and also include older children >13 years and consider the effects of gender on behavior.

THE EFFECT OF BREAKFAST ON ACADEMIC PERFORMANCE

Overview of findings

This review identified 21 studies that demonstrated suggestive evidence that habitual breakfast (frequency and quality) and SBPs are associated with children and adolescents' academic performance. This effect was apparent in both well-nourished or undernourished samples and/or children from low SES backgrounds. Increased frequency of habitual breakfast was consistently positively associated with improved school performance. Some evidence suggested that increased quality of habitual breakfast in terms of providing a greater variety of food groups (3–4) and adequate energy (>20–25% of total estimated energy needs) is positively related to school performance.

Evidence suggested a positive effect of SBPs on arithmetic test scores and mathematic grades. Three studies demonstrated clearest effects on mathematic grades (Murphy et al., 1998; Kleinman et al., 2002; Morales et al., 2008) and four studies demonstrated a benefit of breakfast on arithmetic scores (Powell et al., 1998; Simeon, 1998; Wahlstrom and Begalle, 1999; Cueto and Chinen, 2008; Edwards et al., 2011). However, some of the evidence was inconsistent (Gajre et al., 2008; O'Dea and Mugridge, 2012). Gajre et al. (2008) found that regular breakfast eaters (>4 days per week) had significantly higher marks for science and English compared to those who never eat breakfast, but there was no difference in mathematics marks. However, total marks, which included mathematics, were significantly higher in the regular breakfast group compared with the no breakfast group. Similarly, the majority of studies employing composite measures of school grades across subject domains show a positive association which, may be related to increased power afforded by composite measures.

Some evidence suggested that effects may be more apparent in undernourished children who improved their nutritional status from at risk to adequate following a SBP (Kleinman et al., 2002). Cueto and Chinen (2008) reported that positive effects on achievement test scores following a SBP, particularly in schools which tended to have more undernourished children and lower achievement. In support, studies that were carried out in samples including undernourished children demonstrated consistent positive effects of breakfast on school performance (Jacoby et al., 1996; Powell et al., 1998; Simeon, 1998; Cueto and Chinen, 2008). This is suggestive of a possible mechanism by which breakfast may improve school performance. The observed increase in school performance may be facilitated by correction of nutritional deficiencies due to the fortification of many breakfast products, particularly with iron and iodine which have largely been implicated in improving cognitive function which may influence school performance (Tiwari et al., 1996; Grantham-McGregor and Ani, 2001; Falkingham et al., 2010). Whilst nutritional influences may have contributed toward the improved school performance, school attendance also increased in many studies following which may account for most of the improvement in school grades (Hoyland et al., 2009; Defeyter et al., 2010).

Methodological issues

Influence of confounders. Research on breakfast and educational outcomes is a particularly difficult area given the potential for confounding. The majority of studies that employ academic outcomes are cross-sectional, so adjustment of potential confounders is critical. Adequate control for confounders varied within the studies identified. An important potential confound is SES. It is likely that children and adolescents who eat breakfast differ from those who do not eat breakfast in ways that also influence educational outcomes. There is a consistent evidence that SES is associated with breakfast eating, with children from higher SES backgrounds more likely to regularly eat breakfast than children from lower SES backgrounds, an effect which is consistent across gender and age (Delva et al., 2006; Moore et al., 2007; Doku et al., 2011; Hallström et al., 2011, 2012; Overby et al., 2011). Similarly, there is well established consistent evidence that SES is a central determinant of academic performance and cognitive ability (Brooks-Gunn and Duncan, 1997; McLoyd, 1998; McCulloch and Joshi, 2001; Machin and Vignoles, 2004). However, some studies failed to adequately adjust for SES in their analysis or used various proxy measures of SES which may be inadequate. If SES is not accounted for in the analysis, it is likely associations observed are because children select into both high breakfast consumption frequency and higher school grades as a result of SES. Further work investigating the effects of breakfast on school performance should carefully consider the role of confounding, and apply adequate controls in the analysis, particularly for SES.

Academic performance measures. Studies employed a wide range of outcomes as academic performance indicators, either by use of average school grades or standardized achievement tests. Two studies relied on self-reported school grades (Lien, 2007) or self-reported subjective ratings of school performance (So, 2013) which are open to socially desirable and inaccurate reporting. Moreover, direct measures of academic performance, although ecologically valid are however, crude measures that may be insensitive to the effects of breakfast. Although many confounders are controlled for in the studies reviewed, it may be inappropriate to use broad measures of scholastic achievement such as end of year grades since many other factors interplay to determine grades.

There are multiple, modifiable, and unmodifiable, determinants of academic performance that may act over and above the subtle nutritional effects of breakfast.

Design. The evidence is based on studies investigating the effects of either habitual breakfast consumption or SBPs on academic performance. The majority of studies on habitual breakfast intake are cross-sectional. The dominance of cross-sectional evidence, although offering a unique opportunity to establish the effects of habitual breakfast on academic performance, provides no indication of causality or temporality. Only one well controlled prospective cohort study has been published to date (Miller et al., 2012). This study focused on breakfast that was eaten with the family rather than total breakfast intake, however this may still be reflective of habitual breakfast consumption particularly in younger children who are more likely to have family meals (Fulkerson et al., 2006) and since most regular breakfast eaters have breakfast at home (Hoyland et al., 2012).

SBP intervention studies also present difficulties in attributing the direct effects of the breakfast meal or the regime of providing a free school breakfast in a breakfast club environment to academic outcomes (Defeyter et al., 2010). Many studies lack details of the composition and amount of food provided and consumed, precluding conclusions regarding breakfast type. SBPs are often associated with increased attendance (Jacoby et al., 1996; Simeon, 1998; Kleinman et al., 2002) punctuality (Murphy et al., 1998), readiness to learn (Wahlstrom and Begalle, 1999), decreased dropout rates (Cueto and Chinen, 2008) better behavior in the classroom (Bro et al., 1994; Richter et al., 1997) and increased pro-social behavior (Shemilt et al., 2004), all of which are likely to impact school performance concurrently. The positive effects of SBPs on other outcomes that will also influence academic performance make it difficult to attribute the effects either to the breakfast meal or as an artifact of increased attendance and punctuality. Furthermore, the intervention duration is particularly important in relation to academic performance because it is likely that a stable period of operation is needed to impact both breakfast eating behavior and academic outcomes. Two studies following a 1 year SBP reported no increase in the total number of children eating breakfast (Murphy et al., 2011; Ni Mhurchu et al., 2013). Clearly, the increase in school performance reported in studies that do not impact breakfast eating behavior is likely to be an artifact of other outcomes.

Dietary assessment. Studies that examine the effects of habitual breakfast consumption on scholastic outcomes also have limitations in terms of how breakfast is measured and defined. Varying definitions of breakfast and classifications of habitual consumption are used. Often dichotomous classifications using different cut-offs (e.g., $\geq$5 days/week, $<$5 days/week) to define habitual breakfast consumption are employed precluding comparisons between these categories. This crude indication of habitual consumption is unlikely to reflect true intake of breakfast.

Measurements of habitual breakfast intake are normally brief dietary assessments, given their use in situations for to measure specific aspects of diet. One item questionnaires (e.g., breakfast yes/no) are often used which may yield an inadequate assessment of habitual intake. Additionally there is a lack of validation studies examining the accuracy of brief dietary assessment or measures of specific meals compared with other methods which tend to examine total diet. Different measurement periods are used to define habitual breakfast and studies do not differentiate between weekday and weekend breakfast consumption, despite the importance for school performance where weekday (school-days) breakfast meals may be more important. Measures focus on either frequency or composition and it is rare both to be considered. Self-report measures also have limitations because breakfast is often subjectively defined and interpreted by the respondent, allowing for bias, inaccurate recall, and misreporting. Furthermore, all food and drink consumed as part of breakfast may not be considered. For example, food consumed on the way to school or food that is not traditionally consumed for breakfast may be excluded.

The majority of studies on habitual breakfast intake are based on adolescent samples aged 12–18 years. Accurate nutritional assessment in adolescents is problematic and challenging compared with younger children, who are more likely to eat breakfast at home (Hoyland et al., 2012). There is an overall trend of increased inaccuracy and underreporting of food intake with age (Livingstone et al., 2004). Validation studies show dietary records provide unbiased and accurate estimates of diet in normal weight children up until the age of 9 years whereas adolescents and older children are more likely to underreport dietary energy intake by approximately 20% (Livingstone et al., 1992; Bandini et al., 1997). Adolescence is a period of rapid growth, increasing body image concerns, changing eating habits, increased independence over diet, greater peer influence and decreased cooperation with authority, all of which may decrease compliance and reporting accuracy in this population (Livingstone et al., 2004).

Further work should consider, both frequency and composition of breakfast as well as differentiating between weekday and weekend breakfast when measuring habitual breakfast intake. A longer measurement period to define habitual breakfast (e.g., at least 7 days) is needed to adequately measure breakfast intake and a dichotomous classification system to define habitual breakfast is insufficient.

SUMMARY OF THE EFFECT OF BREAKFAST ON BEHAVIOR AND ACADEMIC PERFORMANCE

Overall, the evidence suggests beneficial effects of breakfast for on-task behavior in the classroom, mainly in younger children $<$13 years. This effect was apparent in children who were well-nourished, undernourished and/or from deprived or low SES backgrounds. For school performance outcomes, evidence suggests a positive association between habitual breakfast frequency and quality on school grades or achievement test scores. Similarly, evidence from SBPs suggest a positive effect on school performance, particularly mathematics grades and arithmetic scores and in undernourished children and/or children from deprived or low SES backgrounds. The positive effects of breakfast on academic performance appear clearer than those on behavior, probably due to the difficulties surrounding accurate measures

of behavior which are inherently subjective in nature. These outcomes are ecologically valid, have more relevance to pupils, parents, teachers, and educational policy makers and as a result may produce most impact.

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The effect of breakfast cereal consumption on adolescents' cognitive performance and mood

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The aim of the current study was to investigate the effect of breakfast consumption on cognitive performance and mood in adolescents, and any interaction that breakfast consumption might have with cognitive load. The rationale for this approach was that the beneficial effects of any intervention with regard to cognitive function may be more readily apparent when more demands are placed on the system. Furthermore, as skipping breakfast is particularly prevalent within this age group, thus, we focused on adolescents who habitually skip breakfast. Cognitive load was modulated by varying the level of difficulty of a series of cognitive tasks tapping memory, attention, and executive functions. Mood measured with Bond–Lader scales (1974) as well as measures of thirst, hunger, and satiety were recorded at each test session both at baseline and after the completion of each test battery. Forty adolescents (mean age = 14:2) participated in this within-subjects design study. According to treatment, all participants were tested before and after the intake of a low Glycaemic index breakfast (i.e., a 35 g portion of AllBran and 125 ml semi-skimmed milk) and before and after no breakfast consumption. Assessment time had two levels: 8.00 am (baseline) and 10.45 am. The orders of cognitive load tasks were counterbalanced. Overall it appeared that following breakfast participants felt more alert, satiated, and content. Following breakfast consumption, there was evidence for improved cognitive performance across the school morning compared to breakfast omission in some tasks (e.g., Hard Word Recall, Serial 3's and Serial 7's). However, whilst participants performance on the hard version of each cognitive task was significantly poorer compared to the corresponding easy version, there was limited evidence to support the hypothesis that the effect of breakfast was greater in the more demanding versions of the tasks.

Keywords: adolescent, cognitive performance, breakfast, mood, cognitive load

INTRODUCTION

The importance of breakfast consumption in terms of nutritional benefits has been well documented (Smith et al., 1999). Conversely, skipping breakfast has been associated with increased levels of snack food consumption (Billion et al., 2002), and increased likelihood of being overweight or obese (Dubois et al., 2006; Timlin et al., 2008; Croezen et al., 2009). Skipping breakfast during adolescence has also been associated with unhealthy lifestyles such as alcohol, tobacco, and substance use (Revicki et al., 1991; Isralowitz and Trostler, 1996).

In addition to physical health and nutritional benefits, cognitive scientists have investigated the effects of breakfast consumption on cognitive function and the specific cognitive processes that are affected. The majority of these studies have examined the effects of breakfast skipping in adults and children (typically 8–11 year olds).

Several experimental studies have suggested that, in both adults and children, behavior and cognitive performance is improved after consumption of breakfast compared to omission of breakfast. For example, research has shown that breakfast is associated with short-term improvements to memory (Smith et al., 1994; Vaisman et al., 1996; Benton and Parker, 1998; Wesnes et al., 2003); long-term memory (Chandler et al., 1995); attention (Wesnes et al., 2003; Ingwersen et al., 2007); mood (Smith et al., 1999; though see Benton et al., 2001); arithmetic (Powell et al., 1998); creativity (Wyon et al., 1997); and behavior (Bro et al., 1994). Despite this wealth of evidence supporting a link between breakfast consumption and cognitive benefit some studies have reported no benefit of breakfast consumption over breakfast omission (e.g., Dickie and Bender, 1982; Cromer et al., 1990; Lopez et al., 1993; for a review see Rampersaud et al., 2005) and some studies have produced rather mixed results (e.g., Smith et al., 1994). Furthermore, in general, the data are less supportive for the effects of breakfast on cognitive functions such as attention, problem-solving, and reading compared to memory (Rampersaud et al., 2005). Also, there appears to be no consensus on the specific cognitive processes that are affected by breakfast consumption (e.g., Dye et al., 2000).

A number of nutritional mechanisms have been proposed in order to explain the effects of breakfast consumption and composition on cognitive function. For example, Widenhorn-Müller et al. (2008) suggested that alleviating hunger improves mood and subsequently cognitive performance. Other authors have focused on the key role of glucose as a mediator for cognitive function,

primarily as glucose is the only fuel that can be used directly by the brain. Whilst breakfast consumption/omission may not have a significant effect on low cognitive loads tasks, involving mostly information processing; high cognitive load tasks require an increase in the metabolic resources to successfully complete the task (Cooper et al., 2011). Hence the beneficial effects of breakfast consumption compared to breakfast omission may be elucidated under condition of high cognitive load. However, many of the studies, cited above, have used cognitive batteries in which cognitive load has not been investigated. Hence, conflicting findings may, in part, result from studies employing tasks of varying levels of difficulty. Previous studies have also varied in terms of research design and cognitive measures; breakfasts served; timing of post-consumption tests; and socio-economic status and the age of the participants.

Although numerous studies have examined the effect of breakfast vs. breakfast omission in adults and middle childhood there is a paucity of studies conducted with adolescent populations. There are a number of reasons to look specifically at the adolescent populations, and the four main reasons (rapid period of growth, complexity of academic work, tendency to skip breakfast, and ratio of brain size to body weight) are further discussed in Cooper et al. (2011). We conducted a review of the literature and found only two papers that directly examined breakfast consumption vs. breakfast omission in adolescents (Widenhorn-Müller et al., 2008; Cooper et al., 2011); although other studies have manipulated glycaemic index of breakfast (e.g., Smith and Foster, 2008). Widenhorn-Müller et al. (2008) employed a crossover design involving 104 pupils between 13 and 20 years of age. Pupils were tested both after a standardized breakfast consisting of 60 g of whole wheat bread, 20 g of butter, 20 g of nougat spread, and 30 g of strawberry jam and tested without breakfast. Water and unsweetened peppermint tea were offered "*ad libitum*." Pupils completed pen and pencil standardized tests of attention and concentration, and tests of verbal and spatial memory. Mood measures were assessed by a self-administered questionnaire. The results showed breakfast had a significant effect on self-reported alertness and male pupils reported feeling more positive. Breakfast also had a significant effect on accuracy scores on a test of visuospatial memory in males, but no effect on sustained attention or verbal memory. However, due to the cognitive tests being conducted using pen and paper, only the accuracy scores of the cognitive tests were reported so it is not possible to tell whether breakfast had an effect on the speed of responses or whether there was any speed-accuracy trade-off. Moreover, the authors did not control for habitual breakfast consumption. This may be an important confounding variable in studies that pit a standard breakfast condition against a no breakfast condition. Furthermore, cognitive measures were made immediately post-breakfast, thus, potentially masking the beneficial effects of breakfast that are not apparent until later in the morning (Hoyland et al., 2009).

Cooper et al. (2011) also examined the effects of breakfast consumption vs. breakfast omission on adolescent's cognitive function and mood. Their study used a randomized crossover design with trials scheduled 7 days apart. Participants were provided with a range of breakfast foods from which they could choose "*ad libitum*." Participants were asked to complete a mood questionnaire and a range of cognitive tasks; including a visual search task, the Stroop test, and the Sternberg paradigm. Each cognitive task had two levels of difficulty, apart from the Sternberg paradigm that had three levels. Results showed that accuracy on the more complex level of the visual search task was higher following breakfast consumption compared to breakfast omission. Across the morning, participants showed better performance on the Stroop test and responses on the Sternberg paradigm following breakfast consumption compared to breakfast omission. Breakfast consumption also had a beneficial effect on a number of the self-report measures.

Overall, across studies there is emerging evidence that breakfast is beneficial in terms of self-report measures; although the effects of breakfast consumption on cognitive function in adolescents appear to be rather mixed. One possibility is that differences in the findings relating to cognitive function are a result of the different breakfasts provided. For example, Cooper et al. (2011) used an "*ad libitum*" breakfast to allow participants to consume a breakfast similar to their habitual breakfast intake, whilst Widenhorn-Müller et al. (2008) used a standardized breakfast popular in Germany. For the current study it was decided to serve a low Glycaemic Index (GI) breakfast for two main reasons. First, given that little is known about the effect of breakfast composition on adolescents' cognitive function, providing a standardized low GI breakfast would allow the researchers to exert exact control over participants' nutritional intake at breakfast. Second, consumption of a low GI breakfast, compared to a high GI breakfast, has been shown to benefit cognitive function in both adults (Benton et al., 2003), children (Mahoney et al., 2005; Ingwersen et al., 2007), and adolescents (Cooper et al., 2012).

Unlike the aforementioned studies, the present study focused on adolescents who habitually skip breakfast as it has been found that skipping breakfast is particularly prevalent within this age group (Videon and Manning, 2003). Thus, rather than having a sample comprised of adolescents who habitually consume breakfast, the current study sampled only from adolescents who routinely skip breakfast. The skipping of breakfast within this age group has often been attributed to the switch to independent, hurried lifestyle and the reliance on fast-food sources of food and the consumption of snacks (Larson et al., 2009). In addition to the nutritional impact of this lifestyle, young adults are often under high levels of stress as a result of lifestyle changes (Croft et al., 1986). Furthermore, there have been relatively few studies that have examined the effect of breakfast in adolescents [for a review see Hoyland et al. (2009)], and none that have specifically focused on breakfast skippers.

The aim of the current study was to investigate the effect of breakfast consumption on cognitive performance and mood and any interaction that breakfast consumption might have with differing levels of cognitive load in 13–15 years old using a randomized crossover design. The underlying rationale for this approach was that the beneficial effects of any intervention with regard to cognitive function may be more readily apparent when more demands are placed on the system. Following the recommendation of Schmitt et al. (2005) and Westenhoefer et al. (2004) this study employed a cognitive test battery that encompassed

a range of cognitive tests that have been shown to be sensitive to dietary manipulation. Cognitive load was modulated by manipulating task difficulty. In this instance lower vs. higher cognitive load was included as a within subjects factor in the design, with half of the participants undertaking the less demanding version of all of the tasks first and the other half the more demanding versions of the tasks first.

METHODS

PARTICIPANTS

Forty adolescents (mean age = 14:2, range 13:2–15:6 years; 21 females and 19 males) whom did not habitually consume breakfast participated. We intentionally sampled from a small age range as neuroanatomical studies have shown that young, middle, and late adolescents differ in brain maturation (Giedd, 2008), and vary in glucose metabolism (Chugani, 1998). Participants were recruited from students studying at an inner-city high school in the North East of England. The school served predominantly lower-middle class children. We further controlled for socioeconomic status by looking at the Level of Parental Education (LPE) (Lien, 2006). LPE can be used as a reliable estimate of socio economic status (Hauser, 2008) and is associated to educational performance and attainment, and breakfast behaviors. We specifically targeted adolescents of lower-socio economic status as research has suggested that these participants are more likely to skip breakfast (e.g., Affenito et al., 2005; Utter et al., 2007); suggesting that future interventions may need to specifically target people in this demographic. In order to be included in the current study all adolescents had to have parents that had not undertaken any higher educational studies. Habitual breakfast consumption was measured by asking adolescents to complete online food diaries across 5 school days prior to commencing the test phase of the study. Although, there is no clear universal definition of breakfast (e.g., Rampersaud et al., 2005), qualitative research suggests that adolescents have a well-defined idea of the types of food that constitute breakfast as well as the time breakfast is consumed (Chapman et al., 1998; Mullan and Singh, 2010). For the purpose of the present study, breakfast was defined as any food consumed between waking and school lunchtime. Only young people who met the above criteria and had skipped breakfast on 5 consecutive school days in the week prior to commencement of testing were invited to participate in the main test phase of the study. Prior to testing all pupils completed a health screen questionnaire. All participants were reported to be healthy and BMI [calculated by dividing body mass (kg) by the square of the height (m^2)] was used to recruit a sample that fell within the normal BMI (Cole et al., 2000) (see **Table 1**). All participants were free from any food allergy or use of prescription drugs, and all participants spoke English as a first language and no participants had any special educational needs.

DESIGN

The study was approved the Life Sciences Ethics Committee at Northumbria University. Participants were recruited through one school and in accordance with the British Psychological Society Code of Ethics. Written consent was obtained from the head teacher, parents or guardians, and pupils. The short-term effects of cereal consumption on cognition were investigated using a crossover design in which 40 adolescents were given a ready-to-eat breakfast cereal or no breakfast cereal. According to treatment, all participants were provided with 35 g of Allbran (low GI breakfast cereal selected from an international table of glycaemic index; Foster-Powell et al., 2002) and 125 ml of skimmed milk or no breakfast. Adolescents were tested prior to consumption of breakfast (baseline) and then 120 min post start of breakfast consumption. The order of breakfast consumption and breakfast omission was fully counterbalanced, so that half of the children consumed breakfast on the first test day and omitted breakfast on the second test day while the remaining children were presented with the same conditions but in the reverse order. Half of the participants completed the low cognitive load tests followed by the high cognitive load tests, and the remaining participants completed the high cognitive load tests followed by the low cognitive load tests, thus, participants acted as their own controls.

A sample size of 40 was selected to obtain a statistical power of 0.80 on the assumption of a small to medium size, i.e., partial-$\eta^2 = 0.05$, of the effect of the primary variables of interest (i.e., the differential effect of breakfast, and the breakfast by task load interaction, between baseline and 120 min post start of breakfast consumption) and of a correlation $r = 0.5$ between repeated measures.

MEASURES

The test battery comprised a series of computerized tasks derived from standard psychometric measures. All tasks were programmed in JAVA language and the timing of the test battery and reaction times were made independently of the computer's internal timing. The presentations of high and low cognitive load tasks were counterbalanced across participants. The tasks utilized in the current study comprised: Delayed Word recall; Choice reaction time; Rapid Visual Information Processing; Stroop; and Serial subtractions. In addition to the test battery, participants were asked to complete the Bond–Lader mood scale, and visual analog scales for thirst, hunger, and satiety.

Table 1 | Anthropometric characteristics of participants.

	n	Age (years)	Height (cm)	Body mass (Kg)	BMI (kg/m^2)	Waist circumference
Male	19	14.1 ± 0.5	169.1 ± 8.3	60.3 ± 2.3	21.3 ± 2.4	74.7 ± 6.0
Female	21	14.3 ± 0.5	162.5 ± 5.1	56.8 ± 6.2	21.6 ± 2.8	66.5 ± 6.4
Total	40	14.2 ± 0.5	165.2 ± 6.4	58.6 ± 4.7	21.4 ± 2.6	69.6 ± 6.1

All values are mean ± standard deviation.

Delayed word recall

Participants were presented with lists of 15 words taken from Snodgrass and Vanderwart (1980). Words were matched for familiarity and word length. Stimulus duration was one second, as was the inter-stimulus duration. The cognitive load of this task was modulated by presenting words that can be categorized (low cognitive load) and words that cannot be categorized (high cognitive load). At the end of the entire test battery participants were asked to write down as many words as they remembered from both of the original lists and these items were scored according to whether they appeared on the "categorized" or "uncategorized" list.

Choice reaction time

Choice reaction time tasks is a widely used test of attention and has previously demonstrated sensitivity to the improvements and decrements seen in cognitive performance following a number of food components and dietary supplements. Fifty stimuli were presented with an inter-stimulus interval that varied randomly between 1 and 3.5 s. Accuracy and reaction times (ms) were recorded. The cognitive load of this task was modulated by presenting either two choices of response (low load) or four choices of response (high load). In the low cognitive load version of this task participants were required to press the "x" key on a computer keyboard as soon as they saw the letter "N" and the "?" key each time they saw the letter "M." In the high load version of this task participants were required to also press the "c" key as soon as they saw the letter "B" and the ">" key as soon as they saw the letter "V" presented on a computer screen.

Rapid visual information processing task (RVIP)

Participants were instructed to monitor a continuous series of digits for targets of three consecutive odd or three consecutive even digits. The participant responded to the detection of a target string by pressing a response key as quickly as possible. The task was continuous and lasted for 5 min, with 8 correct target strings being presented in each minute. Dependent variables include the number of target strings correctly detected (hits), number of false alarms, and reaction time for hits. The cognitive load of this task was modulated by altering the rate at which the digits were presented: 80 per min (low load) or 100 per min (high load).

Stroop color-word test (Stroop, 1935)

Participants were presented with words describing one of four colors ("RED," "YELLOW," "GREEN," "BLUE") on a computer screen. The cognitive load of this task was modulated by presenting either congruent (low load) or incongruent stimuli (high load). The participant was instructed to press the corresponding button as quickly as possible in order to identify the font color (e.g., if the word "Red" is presented in a blue font, the correct response would be to press the "blue" button).

Serial subtractions

A modified, 2 min, computerized version of the serial subtraction tests was utilized. In this task, participants were asked to count backwards in threes or sevens from the given randomly generated number, as quickly and accurately as possible, using the numeric keypad to enter each response. Participants were also instructed verbally that if they make a mistake they should carry on subtracting from the new incorrect number. Each three-digit response was entered via the numeric keypad with each digit being represented on screen by an asterisk. Pressing the "enter" key signals the end of each response and clears the three asterisks from the screen. The task was scored for total number of subtractions and number of errors. In the case of incorrect responses, subsequent responses were scored as positive if they were correct in relation to the new number. The cognitive load of this task was modulated by instructing participants to either subtract "threes" (low load) or "sevens" (high load).

Mood (Bond and Lader, 1974)

Mood was assessed with Bond–Lader scales following completion of the cognitive test battery. Scores from the 16 Bond–Lader visual analog scales were combined as recommended by the authors to form three mood factors: "alert," "calm" and "contentment." The scales were completed by participants placing a cross with the mouse and cursor on a 100 mm line displayed on a computer screen between the description "not at all" and "extremely" for each of the listed mood states (i.e., alert, content, and calm). Each mood factor was scored as a percentage along the line demoting more of the relevant adjective.

Visual analog scales

Hunger, Thirst and Satiety were assessed using visual analog scales (1–100; with 1 indicating the lowest levels). As in the Bond–Lader Mood Scales, participants completed the VAS by placing a cross with the mouse and cursor on a 100 mm line displayed on a computer screen. VAS were scored as percentage along the line denoting more of the relevant adjective.

PROCEDURE

All participants were tested in a quiet room within their High School. The researcher visited the school on three separate occasions and participants were tested individually on laptops. Each participant undertook a familiarization session which preceded the start of the main test phase of the study by 1 week. The purpose of each cognitive test was explained to participants and a demonstration given. Participants then completed the full battery of cognitive tests which lasted about 30 min. Throughout the familiarization phase, researchers were available to answer any questions. Participants also completed the Bond–Lader Mood Scales and VAS measuring hunger, thirst, and satiety. This enabled participants to become familiar with the test protocol. In this visit the researcher obtained participant's informed consent; and collected parental consent forms. Participants were also given a health screening questionnaire to be completed and signed both by the participant and their parents/guardian. Participant's height and body mass were also measured. These measures allowed the determination of BMI. In order to ensure confidentiality participants were provided with a stamped addressed envelope in which to return the questionnaire directly to the research team. Demographic data were collected and participants were randomly allocated to treatment conditions. Participants were reminded that, for the testing session,

they would need to arrive at their school for 8.00 am, having consumed no caffeine for at least 12 h previously and no food from midnight.

Following standard protocol, the two testing visits took place 1 week apart (Widenhorn-Müller et al., 2008), and participants reported to school at 8 am following an overnight fast from midnight the evening before the trial. Upon arrival at school participants were asked to complete a computerized cognitive test battery and the mood questionnaire and rate satiety, thirst, and hunger. Each cognitive test was preceded by 6 practice stimuli in order to re-familiarize participants with each individual cognitive test. Participants were then provided with a breakfast (breakfast trial) at 8.30 am or no breakfast (no breakfast trial). No additional help or feedback was provided on any of the test trials. They were given 15 min to consume breakfast or 15 min of resting in the no breakfast trial. Following testing, participants started their normal lessons and then returned for testing again at 10.45 am. Participants in both groups were allowed to drink water across the school morning, if desired. The experimental protocol is shown in **Figure 1**.

DATA ANALYSIS

Primary analyses consisted of repeated-measures analysis of variance (ANOVA) with two within-subjects variables: breakfast trial (breakfast vs. no breakfast) and time (pre- vs. post-breakfast consumption). This type of analysis was applied to each dependent variable used in the study. Moreover, in the case of cognitive tasks, this analysis was applied first to the low cognitive load and then to the high cognitive load condition. Further analyses to assess any differential effect of the cognitive load variable were conducted only if there was at least a significant breakfast by time interaction either at the low or at the high level of load version of the task being considered. A significance level of 0.05 was used through the study and effect sizes (*partial*-η^2—indicated in the text simply as η^2) were reported for F ratios larger than one. As a rule of thumb *partial*-η^2 of the following magnitudes: 0.01, 0.06, 0.14, correspond to small, medium, and large effect sizes, respectively. For each ANOVA, the outcome of the interaction between breakfast trial by time is reported first, followed by the main effects.

For each measure of cognitive function, mood, thirst, hunger and satiety, preliminary analyses had been conducted to ascertain whether there was any significant effect of either gender or trial order. Since these factors did not have any significant effect, data had been collapsed across gender and trial order in all subsequent analysis. Factorial repeated within-subjects and mixed models ANOVAs were conducted using SPSS version 18.

Further preliminary analyses were conducted to test if there was any significant difference at baseline (i.e., the first measurement of the morning) between breakfast and no breakfast conditions for each dependent variable used. The only significant difference emerged in the Serial 3's task. However, given the large amount of pair-wise comparisons being performed this significant difference may simply reflect a Type 1 statistical error.

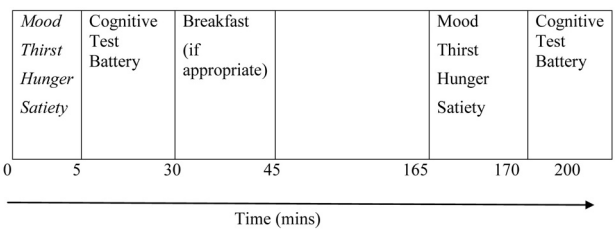

FIGURE 1 | Experimental Protocol.

RESULTS

SELF REPORT MEASURES

Alertness

Analysis revealed a significant and a rather large effect of the breakfast by time interaction [$F_{(1, 39)} = 12.89$, $p < 0.05$, $\eta^2 = 0.249$] with alertness increasing following breakfast consumption (41.08 vs. 50.12) compared to a decrease in alertness in the no breakfast trials [40.37 vs. 36.44 (see also **Table 2** for this and the other self report measures)]. There was also a significant main effect of breakfast [$F_{(1, 39)} = 11.32$, $p < 0.05$, $\eta^2 = 0.225$] with participants reporting feeling more alert on the breakfast trial compared to the no breakfast trial (45.60 vs. 38.40, respectively). There was no significant main effect of time [$F_{(1, 39)} = 2.13$, $p > 0.05$, $\eta^2 = 0.05$].

Calm

The analysis on self-report measure of calmness showed a significant breakfast by time interaction [$F_{(1, 39)} = 5.96$, $p < 0.05$, $\eta^2 = 0.133$]. All participants reported feeling less calm across time, although this effect was far more pronounced in the no breakfast trials (63.10 vs. 55.16) compared to the breakfast trials (64.21 vs. 63.12).

There were also significant main effect of breakfast [$F_{(1, 39)} = 11.21$, $p < 0.05$, $\eta^2 = 0.223$; 63.7 vs. 59.1 for breakfast and no breakfast conditions, respectively]; and a significant main effect of time [$F_{(1, 39)} = 6.96$, $p < 0.05$, $\eta^2 = 0.152$; 63.7 vs. 59.1 for pre and post breakfast conditions, respectively].

Contentment

The analysis revealed a significant and a rather large effect of the interaction between breakfast and time [$F_{(1, 39)} = 9.53$, $p < 0.05$, $\eta^2 = 0.196$], with participants in the breakfast trials reporting a greater level of contentment later in the morning (55.4 vs. 61.6), whilst participants in the no breakfast trials reported lower level of contentment later in the morning (53.9 vs. 51.4).

There was no significant main effect of time [$F_{(1, 39)} = 1.54$, $p > 0.05$, $\eta^2 = 0.038$], but there was a significant main effect of breakfast [$F_{(1, 39)} = 14.34$, $p < 0.05$, $\eta^2 = 0.269$; 58.5 vs. 52.7 for breakfast and no breakfast conditions, respectively].

Hunger

There was a significant breakfast by time interaction [$F_{(1, 39)} = 6.73$, $p < 0.05$, $\eta^2 = 0.147$], with participants in the breakfast trials showing a larger reduction in self-reported hunger across the morning (66.0 vs. 49.2) compared to participants in the no

breakfast trials (61.3 vs. 56.4). Moreover, there was a significant and rather large effect of time [$F_{(1, 39)} = 23.45$, $p < 0.05$, $\eta^2 = 0.375$; 63.7 vs. 52.8 for pre and post breakfast conditions, respectively), while the main effect of breakfast was not significant ($F < 1$).

Satiety

Analysis showed that there was a significant and large effect of the breakfast by time interaction [$F_{(1, 39)} = 11.06, p < 0.05, \eta^2 = 0.221$] with participants in the breakfast trials reporting feeling fuller across the school morning (28.4 vs. 48.1) than participants in the no breakfast trials (31.9 vs. 38.0). There was a significant and very large effect of time [$F_{(1, 39)} = 36.21$, $p < 0.05$, $\eta^2 = 0.481$; 30.2 vs. 43.1, for pre and post breakfast conditions, respectively], but no significant effect of breakfast [$F_{(1, 39)} = 2.89, p > 0.05, \eta^2 = 0.069$].

Thirst

There was no significant breakfast by time interaction [$F_{(1, 39)} = 2.81, p > 0.05, \eta^2 = 0.067$], with reported thirst in the breakfast trials (58.2 vs. 50.3) and in the no breakfast trials (55.1 vs. 54.6), neither significant main effects of time ($F < 1$) or of breakfast [$F_{(1, 39)} = 3.66, p > 0.05, \eta^2 = 0.075$].

DELAYED WORD RECALL

Easy word recall

Participants performance on all of the cognitive tasks are reported in **Table 2**. There was no significant breakfast by time interaction [$F_{(1, 39)} = 1.03$, $p > 0.05$]. Participants in the breakfast trials across the school morning recalled (66.00 vs. 64.67) while in the no breakfast trials the means were (60.5 vs. 63.17). There was a significant main effect of breakfast trial [$F_{(1, 39)} = 4.09$, $p < 0.05$, $\eta^2 = 0.095$], with significantly more correct words recalled in the breakfast trials compared to the no breakfast trials (65.33 vs. 61.83). However, there was no main effect of time ($F < 1$).

Hard word recall

There was a significant and substantially large effect of the breakfast by time interaction [$F_{(1, 39)} = 13.96$, $p < 0.05$, $\eta^2 = 0.264$]. Participants recalled more correct words in the breakfast trials across the school morning (50.33 vs. 54.08) compared to the no breakfast trials under which performance decreased (51.7 vs. 44.0). There was a significant effect of breakfast [$F_{(1, 39)} = 5.73$, $p < 0.05$, $\eta^2 = 0.128$] and no significant main effect of time ($F < 1$).

When easy and hard tasks were compared it appeared that there was, as expected, a significant and very large effect of difficulty, [$F_{(1, 39)} = 70.83$, $p < 0.05$, $\eta^2 = 0.645$], indicating that more words were recalled in the easy (63.58) than in the hard condition (50.04). More interestingly the three-way interaction was significant and rather large in terms of the magnitude of the size of its effect, [$F_{(1, 39)} = 12.68$, $p < 0.05$, $\eta^2 = 0.245$], indicating that differences in performance in favor of the breakfast condition emerged only when the recall task was made harder.

Table 2 | Performance on Mood dimensions, Hunger, Satiety, Thirst and on Cognitive Tasks as a factor of Breakfast and Test Time.

Task	Breakfast Time 1	Breakfast Time 2	No breakfast Time 1	No breakfast Time 2
Alertness	41.08 ± 12.48	50.12 ± 12.48	40.37 ± 15.42	36.44 ± 14.38
Calm	64.21 ± 12.28	63.12 ± 12.86	63.10 ± 14.58	55.16 ± 8.72
Contentment	55.40 ± 13.85	61.60 ± 9.14	53.90 ± 14.37	51.44 ± 10.56
Hunger	66.02 ± 11.83	49.18 ± 13.78	61.30 ± 16.00	56.35 ± 21.11
Satiety	28.37 ± 13.92	48.10 ± 14.51	31.93 ± 15.48	38.03 ± 19.74
Thirst	58.15 ± 17.85	50.27 ± 12.03	55.13 ± 16.61	54.60 ± 17.07
Easy word recall (% correct)	66.00 ± 14.46	64.67 ± 12.09	60.50 ± 17.56	63.17 ± 11.09
Hard word recall (% correct)	50.33 ± 14.34	54.08 ± 13.51	51.75 ± 15.36	44.00 ± 12.32
Easy 2 choice (% correct)	97.15 ± 3.35	96.45 ± 2.92	96.65 ± 3.18	96.70 ± 2.62
Easy 2 choice reaction time (ms)	427.78 ± 63.76	422.47 ± 63.76	421.43 ± 59.86	423.01 ± 61.27
Hard 4 choice	98.08 ± 2.92	98.13 ± 2.56	98.75 ± 2.04	98.38 ± 2.13
Hard 4 choice reaction time (ms)	475.75 ± 61.76	466.26 ± 60.85	481.64 ± 60.70	477.52 ± 84.31
Easy stroop (% correct)	98.12 ± 2.44	97.62 ± 2.44	97.51 ± 2.65	97.73 ± 2.60
Easy stroop reaction time (ms)	857.73 ± 91.89	822.90 ± 97.05	872.63 ± 124.46	833.05 ± 152.57
Hard stroop (% correct)	98.86 ± 2.38	99.24 ± 1.24	97.14 ± 10.35	98.68 ± 2.35
Hard stroop reaction time (ms)	904.90 ± 117.04	872.05 ± 111.79	915.66 ± 228.49	869.05 ± 161.24
Easy RVIP (% correct)	58.69 ± 18.48	55.65 ± 21.88	58.55 ± 21.84	55.37 ± 23.02
Easy RVIP reaction time (ms)	506.08 ± 37.87	494.61 ± 42.31	505.99 ± 45.79	491.34 ± 41.29
Hard RVIP (% correct)	45.99 ± 15.41	49.13 ± 17.84	49.17 ± 12.99	46.50 ± 17.56
Hard RVIP reaction time (ms)	502.13 ± 38.62	502.57 ± 44.09	500.59 ± 37.08	493.15 ± 45.05
Easy serial 3's	31.13 ± 12.28	32.30 ± 13.03	35.35 ± 12.51	31.85 ± 11.47
Hard serial 7's	20.58 ± 9.69	21.80 ± 9.26	21.60 ± 8.55	19.20 ± 8.65

REACTION TIME

Easy choice reaction time

Analysis of accuracy and reaction time data showed no significant interaction or significant main effects ($Fs < 1$).

Hard choice reaction time

Analysis of accuracy and reaction time data showed no significant interaction or significant main effects ($Fs < 1.83$, $ps > 0.05$, largest $\eta^2 < 0.045$).

STROOP TASK

Easy stroop

In looking at accuracy data no significant interaction or significant main effects emerged ($Fs < 1.95$, $ps > 0.05$, largest $\eta^2 < 0.048$). The analysis of the reaction time data showed that neither the interaction nor the main effect of breakfast were significant ($Fs < 1$). However, there was a significant main effect of time [$F_{(1,\ 39)} = 5.99$, $p < 0.05$, $\eta^2 = 0.133$] indicating that faster reaction times occurred in the second administration of the test.

Hard stroop

There were no significant results on accuracy data ($Fs < 1.75$, $ps > 0.05$, largest $\eta^2 < 0.044$), The analysis of the reaction time data showed that neither the interaction nor the main effect of breakfast were significant ($Fs < 1$). However, there was a significant main effect of time [$F_{(1,\ 39)} = 4.34$, $p < 0.05$, $\eta^2 = 0.10$] indicating that faster reaction times occurred in the second administration of the test.

RVIP

Easy RVIP

Analysis of accuracy and reaction time data showed no significant interaction or significant main effects ($Fs < 3.6$, $ps > 0.05$, largest $\eta^2 < 0.084$).

Hard RVIP

Analysis of accuracy and reaction time data showed no significant interaction or significant main effects ($Fs < 3.46$, $ps > 0.05$, largest $\eta^2 < 0.081$).

SERIAL 3'S AND SERIAL 7'S

Serial 3's

Analysis revealed a significant interaction between breakfast and time [$F_{(1,\ 39)} = 6.23$, $p < 0.05$, $\eta^2 = 0.138$], with performance decreasing across the school morning in the no breakfast trials compared to the breakfast trials. The main effects of breakfast and of time were not significant ($Fs < 2.64$, $p > 0.05$, largest $\eta^2 < 0.064$].

Serial 7's

There was a significant breakfast by time interaction [$F_{(1,\ 39)} = 5.25$, $p < 0.05$, $\eta^2 = 0.119$], with increased performance across the school morning in the breakfast trials and reduced performance in the no breakfast trials. None of the main effects was significant ($F < 1$).

When easy and hard tasks were compared it appeared that there was, as expected, a significant and very large effect of difficulty, [$F_{(1,\ 39)} = 105.91$, $p < 0.05$, $\eta^2 = 0.731$], indicating that more correct responses were given in the serial 3's (32.66) than in the serial 7's condition (20.79). The effect of the breakfast by time interaction was significant and large in size, [$F_{(1,\ 39)} = 9.17$, $p < 0.05$, $\eta^2 = 0.19$], indicating increased performance across the school morning in the breakfast trials (31.8 vs. 33.6) and reduced performance in the no breakfast trials (21.2 vs. 20.4). However, the three-way interaction was not significant, ($F < 1$), indicating that difficulty of the task did not qualify the breakfast by time interaction.

DISCUSSION

The number of adolescents skipping breakfast appears to be increasing (Rampersaud et al., 2005) and there is thus, a need for studies to investigate the effects of breakfast omission on cognitive function and mood. This study is important as it contributes to a limited number of studies investigating the effects of breakfast consumption in this age group. Furthermore, unlike a number of prior studies, all of the adolescents in the present study were habitual breakfast skippers from low socioeconomic backgrounds. To our knowledge this is the first study to specifically target this group. This is important, given the prevalence of adolescents who habitually skip breakfast (Dwyer et al., 2001); especially those from low socioeconomic backgrounds (Rampersaud et al., 2005).

The overall findings produced a rather mixed pattern of results. The findings of the present study clearly demonstrate that following breakfast consumption self-report measures of alertness, and contentment were higher when compared to breakfast omission. These findings replicate a number of studies that have shown breakfast consumption to have a positive effect on mood (Wesnes et al., 2003; Widenhorn-Müller et al., 2008). For example, Widenhorn-Müller et al. (2008) found an increase in alertness and information uptake following breakfast consumption compared to breakfast omission. However, unlike the present study in which alertness significantly increased both for girls and boys following breakfast consumption, Widenhorn-Müller et al., only found this effect in girls. The results of the present study are also in accordance with Wesnes et al. (2003) who also found a positive effect on self-rated alertness and contentment following breakfast consumption compared to breakfast omission in 9–16-year-olds. All participants, in the current study, reported feeling less calm across the school morning; although this effect was more pronounced following breakfast omission. These results contradict those of Cooper et al. (2011) who found no difference in self-reported calmness across breakfast conditions. Cooper and colleagues draw attention to the fact that many previous studies have used mood questionnaires specifically designed for use with adult populations and that adolescents may have difficulty in completing the scales. However, the current study successfully used a computerized version of the Bond–Lader Mood Scale and found no evidence of adolescents experiencing any difficulty in completing the scale.

As anticipated, following breakfast consumption there was a significant reduction on self-reported levels of hunger. Similarly, participants reported feeling more satiated following breakfast compared to no breakfast. There was no significant effect of breakfast consumption on thirst, but it is worthwhile

remembering that participants were free to drink water across the trial period.

In looking at the findings from the cognitive function tasks the pattern of findings is not so straightforward.

WORD RECALL

In the easy version of this task, participants' performance in the breakfast trials outperformed participants in the breakfast omission trials. However, neither the main effect of time nor the time by breakfast interaction were significant. In the more cognitively demanding version of this task the results showed a significant time by breakfast interaction with participants recalling more correct words in the breakfast trials vs. the no breakfast trials. Moreover, when easy and hard tasks were compared more words were correctly recalled in the easy version of the task than the hard version. A three-way interaction indicated that performance in favor of the breakfast trials only emerged in the harder version of the task. These findings support the suggestion that tasks with higher cognitive demands are more sensitive to nutritional manipulations (Scholey et al., 2001; Cooper et al., 2011). Cooper et al. (2011) reported that adolescents response times, on a high load working memory test, improved 120 min post consumption of a low GI breakfast compared to breakfast omission; although there was no significant effect of breakfast on accuracy data. By contrast, adolescents in the current study showed superior accuracy, in the high cognitive load task, later in the school morning following breakfast consumption compared to breakfast omission.

CHOICE REACTION TIME

Analysis of the Choice Reaction time data found no significant main effects or interactions on either the easy or hard version of the task. These results contrast with Conners and Blouin (1983) findings that showed that adolescent's performance on attention span and vigilance improved following breakfast consumption.

STROOP TASK

Analysis of the both versions of the Stroop Task data revealed no significant main effects or interactions. The results of the present study failed to replicate those of Cooper et al. (2011) who reported that while performance declined across the school morning the reduction in performance was not as noticeable following breakfast consumption. Given the role of glucose for cognitive activity (Pollitt and Matthews, 1998) and the role of the frontal lobe in determining performance in the Stroop Task it is rather surprising that we did not find an effect of breakfast consumption. A number of researchers have proposed that a high GI (high GL) breakfast results in higher blood glucose concentration and this results, in turn, in greater activation of the hypothalamic pituitary-adrenal axis, and increased frontal lobe functioning which is crucial at inhibiting the response to incongruent stimuli (e.g., Dye et al., 2000; Micha et al., 2006). However, previous studies have revealed a rather mixed pattern of findings with some studies showing that a high GI and high Glycaemic Load (GL) breakfast tends to produce better performance on this test compared to either a low GI or a low GL breakfast (e.g., Micha et al., 2008); while other reporting that GI of breakfast has no effect on adolescents performance (Micha et al., 2010); and yet another study showing that a low GI breakfast is more beneficial to adolescents performance compared to both a high GI breakfast and breakfast omission (Cooper et al., 2012).

In the current study, participants' speed of response on the Stroop test improved across time. One may argue that these findings may be due to practice effects. However, adolescents were provided with extensive training and practice during the training phase and took part in practice trials prior to each test phase, thus, reducing the likelihood of practice effects. Second, Cooper et al. (2011) also reported that adolescents' responses on the Stroop test were faster later in the morning (120 min following breakfast consumption) compared to immediately following breakfast consumption.

RVIP

For both RVIP versions of the task there were no significant main effects and no significant interactions.

SERIAL 3'S AND SERIAL 7'S

In both Serial 3's and Serial 7's tasks performance decreased across the school morning in the no breakfast trials compared to the breakfast trials where performance increased numerically. As anticipated there was a significant effect of task difficulty. Moreover, independently of the level of cognitive load of the task, the breakfast by time interaction was significant, indicating increased performance across the school morning in the breakfast trials and reduced performance in the no breakfast trials. However, the three-way interaction (breakfast by time by cognitive load) was not significant, indicating that difficulty of the task did not qualify the breakfast by time interaction.

SUMMARY AND FUTURE RESEARCH

Overall, the findings clearly demonstrate that task difficulty (cognitive load) mattered in general but only in some aspects of cognition. It was only in the recall task that performance appeared to be significantly modulated by the interactive combination of the effect of breakfast consumption and task difficulty; with improved performance at time two when the task was harder. Although our results partially replicate other studies in demonstrating an effect of breakfast consumption on memory (e.g., Smith et al., 1999) and attention (Wesnes et al., 2003), the findings of the current study only partially replicates those of Cooper et al. (2011) who report that breakfast consumption was particularly beneficial on more cognitively demanding tasks. Moreover, the current findings lend little support to Cooper et al.'s (2012) findings that showed improved performance in 12–14 year-olds on the Stroop test and Flanker task. Whilst the present study presents clear evidence that the hard versions of cognitive tasks were more demanding than the easy versions, the threshold at which cognitively demanding tasks become sensitive to various nutritional manipulations is currently unclear.

The findings of the current study warrant further investigation. The nutritional manipulation in the present study was the comparison of a low GI breakfast compared to no breakfast. It is possible that consumption of a low GI breakfast did not result in significantly greater glucose availability which may be

required to fuel the brain during tasks of high cognitive demand (Smith and Foster, 2008). In order to address the role of glycaemic index further studies employing adolescents need to be conducted including measurements of biomarkers (e.g., blood glucose levels). In addition, unlike the study by Cooper et al. (2011) in which adolescents were provided with breakfast *ad libitum*, the current study provided a fixed amount of breakfast cereal and this may account for some of the different findings between the two studies; [although this explanation cannot account for the different findings to those of Cooper et al. (2012), where breakfast was not provided on at *ad libitum* basis].

Unlike prior studies, the current study specifically targeted adolescents from low SES who habitually skipped breakfast. Although this allowed us to focus on a specific group of participants, it also limits the generalizability of the results and as such may potentially account for differences in findings to those of Cooper et al. (2012). However, given the general rather poor health habits of this population (e.g., Benton and Nabb, 2004) it is important for researchers to examine the effects of nutritional interventions in this population.

Further studies are also required to explore the optimal timing of breakfast (see Hoyland et al., 2009). Both the current study and that of Cooper et al. (2012) assessed performance 120 min following breakfast consumption, but found quite different results. However, it should be noted that these studies tested adolescents from different SES groups and some of the cognitive tasks differed. Given the inconsistent results observed in the current literature, an important task for future studies is to carefully investigate which cognitive tasks, and associated high and low load versions of these tasks, are sensitive to nutritional manipulations in different groups. Finally, further studies need to explore why adolescents often skip breakfast in order to develop a successful intervention to tackle this unhealthy behavior.

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Breakfast and cognition: sixteen effects in nine populations, no single recipe

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There is a consensus regarding the universal significance of breakfast (BF) for health, wellbeing, and cognition. The success of free school BF programs (e.g., Hasz and Lamport, 2012), which reportedly improve academic performance, fortifies this belief. However, studies showing cognitive effects of BF *vs.* skipping BF in large mixed cohorts of children (e.g., Wesnes et al., 2012) are often lacking metabolic and nutritional specifics. This creates uncertainty regarding the metabolic consequences of BF. Another uncertainty exists regarding skipping BF, which has been argued to have universally negative cognitive consequences—a claim that was recently announced a "*presumption*" unsupported by scientific evidence (Casazza et al., 2013, p.1). Surprisingly, in discussions regarding skipping BF, the neuroprotective and cognitively beneficial effects of intermittent fasting (IF) (featuring skipping BF every other day), although well-documented, are never mentioned.

Furthermore, the positive effect of free school BF on academic performance (previously unquestionable) was not supported in a randomized controlled trial (Mhurchu et al., 2013). In a meta-analytical review (Adolphus et al., 2013), behavioral results of school BF programs were considered lacking "*scientific rigor*." Importantly, although it has been shown that the macronutrient content of BF "*can exert small but reliable effects independent of energy value and oro-sensory qualities*" (Lloyd et al., 1996, p. 1), this aspect is also unspecified in many of BF studies.

In this opinion article, we argue against the prevalent viewpoint of the universal benefits of BF by selectively highlighting issues demonstrating the complexity of the cognitive effects:

- The differences in cognitive effects of BF depending on age and baseline metabolic characteristics
- The specificity of BF composition
- The potential relationship of data on prolonged overnight fast (due to skipped BF) with data on the cognitive effects of IF and ketogenic diets (KD).

CHILDREN

MALNOURISHED CHILDREN

It is routinely stated that BF improves cognitive performance *especially* in malnourished children (e.g., Adolphus et al., 2013). This implies that other groups of children also benefit from BF, which does not seem to be the case. Omitting BF once in malnourished children worsened such cognitive outcomes as computational skills, problem solving, visual and auditory short-term memory, comprehension, and generation of ideas (Simeon and Grantham-McGregor, 1989; Hoyland et al., 2009). Noteworthy, cognitive ability and mental processing in malnourished (underweight) children was poorer, compared to controls, independently of BF (e.g., Bisset et al., 2012).

WELL-NOURISHED CHILDREN

Among 8–10 years old well-nourished children who regularly consumed BF, skipping it once did not affect any of the following cognitive performance tasks: visual motor function, executive function/spatial problem solving, psychomotor function/speed of processing, visual attention/vigilance, visual learning and memory, and attention/working memory (Kral et al., 2012). Pollitt et al. (1981, 1983) showed that in 9–11 years old well-nourished children, skipping BF actually decreased the number of errors in memory recall.

OBESE CHILDREN

Obesity *per se* in 4–7 year olds did not impair cognitive abilities (Bisset et al., 2012). Skipping BF, however, resulted in a reduction of carbohydrate (CHO) utilization parallel to a decrease in attention (Maffeis et al., 2012). Improving the metabolic profiles of obese children (via therapy with Leptin) improved their verbal, non-verbal, and short-term memory (Paz-Filho et al., 2008).

CHILDREN WITH DIFFERENT IQs

Not only nourishment but also children's intelligence influences the cognitive outcomes of skipping BF occasionally. Those with IQ above average (>100) increased the speed of information processing, which negatively correlated with blood glucose levels. Children with IQ below average had impaired cognitive performance as a result of skipping BF, with no correlation between glucose levels and performance (Pollitt et al., 1981).

BRAIN STRUCTURE AND BF STAPLE FOODS

The way a meal affects blood glucose (assessed by glycemic index, GI) influences cognitive consequences of BF. In children, 2 h after intake, low-GI BF has either less deteriorative effects (compared to high-GI BF) on accuracy of attention and secondary memory (Ingwersen et al., 2007) or improved declarative-verbal memory. On the other hand, high-GI BF resulted in better vigilance (Micha et al., 2012). Contrary to the effects of GI, glycemic load had

no effects on cognition in 10–12-years-old children (Brindal et al., 2012).

Meticulous work by Taki et al. (2010) demonstrated amazing long-term effects of two nutritionally close BF staples (rice vs. bread) on children's brain morphology and one of the IQ components, the Perceptual Organization Index. This index was higher in the group regularly eating rice for BF, after adjusting for age, gender, socioeconomic family status, regularity of eating BF, and the variety of foods complementary to rice. Importantly, children in the rice-eating group had a significantly greater volume of gray matter. The Japanese variety of rice produces two times smaller disturbance in blood glucose compared to bread: GI of the Koshikari rice is 48, whereas the GI of bread is 100.

The gray matter volume correlation with cognition is further shown by Taki et al. (2012), where gray matter volume in the temporoparietal and prefrontal cortices positively correlated with full-scale (all components) IQ independently of meal composition, age, sex, and socioeconomic status. Similarly, reduction in volumes of gray matter (e.g., in the temporoparietal cortex) in adults is associated with mild cognitive impairment (Baron et al., 2001).

ADULTS

BREAKFAST COMPOSITION

Generally speaking, "stable metabolic conditions seem to stabilize cognitive performance" (Fischer et al., 2002, p. 411) while "deviation from habitual meal composition can produce a relative decline in mood state" (Lloyd et al., 1996, p.1).

Studying short-term effects of BF in healthy young adults, Fischer et al. (2001) showed that the best cognitive performance occurred in habitual BF eaters after a morning meal of pure fat (butter), as opposed to isocaloric protein-rich or high-CHO meals. The fat meal provided the most constant metabolic condition, judged by the ratio of glucagon to insulin concentrations in the blood. On the other hand, a decreased tolerance to glucose has repeatedly shown to result in cognitive impairment (Grodstein et al., 2001; Hiltunen et al., 2001; Elias et al., 2005). (De Feo et al., 1988) showed that even a modest but consistent decrement in glucose stability caused an early impairment in cognitive function. Low-GI BF improved both memory test performance in humans and operant conditioning tasks in rats (Benton et al., 2003) but did not influence performance in an intelligence test (Benton and Parker, 1998).

GLUCOSE TOLERANCE

Nabb and Benton (2006) showed that in glucose-tolerant adults, a single high-CHO BF resulted in improvement in the Immediate Recall Memory Test. Glucose-intolerant adults, however, did not show any cognitive improvement. In both glucose-tolerant and intolerant subjects memory scores negatively correlated with BF calorie content. High-fat/high-CHO BF in this experiment caused information processing enhancement in those with high glucose tolerance while high-fat/low-CHO BF improved vigilance in those with low glucose tolerance.

SKIPPING BREAKFAST PROLONGS THE OVERNIGHT FAST

Sleeping energy expenditure was higher when BF was habitually skipped indicating a prolongation of overnight ketosis (Kobayashi et al., in press). As mentioned above, the best cognitive performance was observed in habitual adult BF eaters after a BF of pure fat (Fischer et al., 2001), which may metabolically mimic the effects of skipping BF altogether by the same token as the KD mimics the effects of starvation (e.g., Beckett et al., 2013). Long-term effects of KD are known to be strongly neuroprotective (e.g., Zilberter, 2011) and cognitively beneficial, for instance in children (Hallböök et al., 2012) and in studies of Alzheimer's disease (Stafstrom and Rho, 2012; Beckett et al., 2013).

It is becoming evident that long-term effects of IF can be as efficient as continuous caloric restriction, which is well known for its beneficial metabolic and cognitive effects. Prolonging the overnight fast habitually happens every other day during standard IF or on a daily basis during time-restricted feeding (tRF). The standard version of IF prescribes a 24-h period of unrestricted eating followed by 24 h of caloric restriction (Johnson et al., 2006) or by complete fasting. In animal studies, tRF protocols restrict food availability to 4–8 h every day (e.g., Hatori et al., 2012). In humans, tRF is achieved by consistently reducing daily meal count and is considered more feasible than IF (Berardi et al., 2011). Animal studies have shown that metabolic consequences of tRF are similar to IF and are favorable independently of caloric intakes (Eshghinia and Mohammadzadeh, 2013). Even a short-term IF intervention in adult rats slowed age-associated decline in learning and improved cognitive functions (Singh et al., 2012). Anson et al. (2003) showed more pronounced effects lyof IF on glucose tolerance and insulin sensitivity compared to caloric restriction. Similarly, tRF has been shown to be as metabolically favorable in humans (Stote et al., 2007). In humans, IF showed long-term neuroprotective effects, e.g., in the prevention of neurodegenerative diseases (Love, 2005; Patel et al., 2005; Jadiya et al., 2011; Srivastava and Haigis, 2011), supposedly *via* improving synaptic plasticity and cognitive function (Araya et al., 2008; Fontán-Lozano et al., 2008; Liu et al., 2013).

It should be mentioned that in humans, during long-term as well short-term protocols, both IF and caloric restriction are hard to comply with due to persistent hunger (e.g., Stote et al., 2007). This difficulty is purely psychological in nature. In a within-subject experiment where two meals similar in taste and texture were administered, one containing calories and the other not (Lieberman et al., 2008), the authors concluded: "*Cognitive performance, activity, sleep, and mood are not adversely affected in healthy humans by 2 days of calorie deprivation when the subjects and investigators are unaware of the calorie content of the treatments*" (p. 667). Similar results were shown in sports medicine research: merely rinsing the mouth with CHO-containing drink without actually swallowing immediately enhanced exercise performance (Jeukendrup and Chambers, 2010).

INTERMITTENT KETOSIS

CHO restriction in high-fat diets induces chronic ketosis and mimics the metabolic consequences of fasting (Barañano and Hartman, 2008; Zilberter, 2011; Stafstrom and Rho, 2012). By the same token, a high-fat/low-CHO BF mimics the metabolic

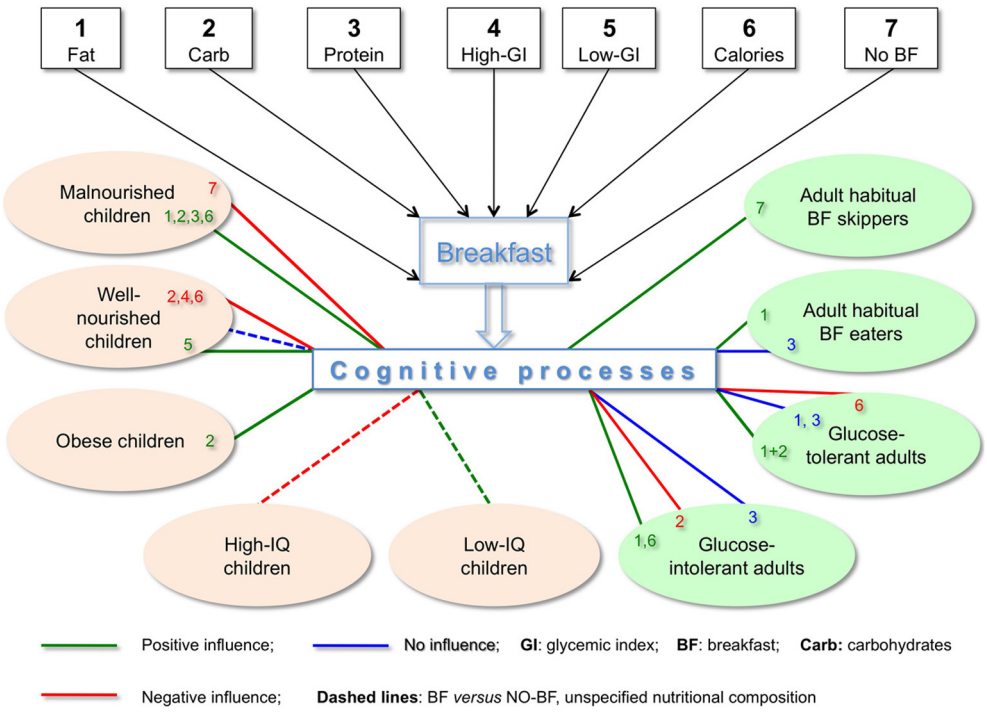

FIGURE 1 | Cognitive effects depend on nutritional composition of breakfast and characteristics of subjects. Effects on cognition in children depend on BF composition (macronutrients, GI, calories), nourishment (malnourished, well-nourished, obese) and IQ. In adults, cognitive effects of BF also depend on BF composition as well as on glucose tolerance and whether or not they eat BF habitually. Contrary to the broadly accepted belief, high-CHO BF may negatively influence cognition in well-nourished children. Also quite contrary to the common view of healthy eating, high-fat BF improved cognitive performance in adult habitual BF eaters. Chronic and/or acute conditions, wherever the information is available, are specified in the text.

features of IF or tRF. Eating a very high-fat BF, as mentioned above, improved cognition (Fischer et al., 2001), which may be due to prolongation of the overnight fast. (Freemantle et al., 2009) showed that a ketogenic BF does not interrupt the overnight ketosis. Consequently, both the "ham and egg" style BF (Smith et al., 1994) and skipping BF result in the metabolic condition that can be defined as intermittent ketosis (IK). IK occurs, for example, in followers of the Carbohydrate Addict Diet (Heller and Heller, 1994) allowing CHO intake only once a day, along with any amount of additional meals containing little or no CHO (essentially ketogenic). When this diet is mentioned in peer-reviewed publications (very seldom), it is never distinguished from other low-CHO diets despite having a significant advantage due to it's potential of combining the benefits from both low-CHO diets and IF/tRF.

Although Heller and Heller (1994) did not mention BF as a ketogenic meal, it is logical to suppose that prolonging overnight ketosis by high-fat/low-CHO BF supports IK during CAD. The matter is, CHO cravings (an element of CHO addiction) are thought to correspond to afternoon/evening drops in brain serotonin levels causing dysphoria as well as other cognitive effects of serotonin depletion (Spring et al., 2008). This can explain why successful CAD dieters prefer to have their CHO-rich meals in the evening although the diet does not prescribe an exact time for it.

CONCLUSION

The complexity of the results described in this opinion article is depicted in **Figure 1**, where we see that seven metabolically distinct BF types have 16 different effects on nine populations of children and adults, including direct data on positive cognitive effects of skipping BF (e.g., on immediate recall in short-term memory, Pollitt et al., 1981, 1983) as well as positive metabolic and/or cognitive effects shown in IF and tRF protocols featuring skipping BF (Love, 2005; Patel et al., 2005; Jadiya et al., 2011; Srivastava and Haigis, 2011; Singh et al., 2012). As succinctly summarized by Bellisle (2004, p. S230), skipping BF "*has deleterious effects, has no effect or even has beneficial effects depending on what the task is, when it is performed after breakfast, the child's IQ, the child's age and nutritional status.*" Clearly there is no single recipe for BF, and the statement "*Breakfast is the most important meal of the day*" is not as unequivocal as it is widely thought to be.

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Effects of individual glucose levels on the neuronal correlates of emotions

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This study aimed to directly assess the effect of changes in blood glucose levels on the psychological processing of emotionally charged material. We used functional magnetic resonance imaging (fMRI) to evaluate the effect of blood glucose levels on three categories of visually presented emotional stimuli. Seventeen healthy young subjects participated in this study (eight females; nine males; body weight, 69.3 ± 14.9 kg; BMI, 22 ± 2.7; age, 24 ± 3 years), consisting of two functional MRI sessions: (1) after an overnight fast under resting conditions (before glucose administration); (2) after reaching the hyperglycemic state (after glucose administration). During each session, subjects were presented with visual stimuli featuring funny, neutral, and sad content. Single-subject ratings of the stimuli were used to verify the selection of stimuli for each category and were covariates for the fMRI analysis. Analysis of the interaction effect of the two sessions (eu- and hyperglycemia), and the emotional categories accounting for the single-subject glucose differences, revealed a single activation cluster in the hypothalamus. Analysis of the activation profile of the left amygdala corresponded to the three emotional conditions, and this profile was obtained for both sessions regardless of glucose level. Our results indicate that, in a hyperglycemic state, the hypothalamus can no longer respond to emotions. This study offers novel insight for the understanding of disease-related behavior associated with dysregulation of glucose and glucose availability, potentially offering improved diagnostic and novel therapeutic strategies in the future.

Keywords: functional MRI, hypothalamus, hyperglycemia, hypoglycemia, emotion processing

INTRODUCTION

The hypothalamus is no longer regarded solely as an organizing center for the integration of somatic and autonomic responses, but as a key organ in the processing of human emotions (Karlsson et al., 2010). Recent studies using event-related functional magnetic resonance imaging (fMRI) have demonstrated that the processing of valence-laden stimuli results in hypothalamic activity patterns comparable to those of the amygdala (Mobbs et al., 2003; Wild et al., 2003; Habel et al., 2007; Watson et al., 2007; Reiss et al., 2008; Schwartz et al., 2008; Derntl et al., 2009), a structure critical for the processing of emotion (Fossati, 2012). Furthermore, the rich reciprocal neural connections between the amygdala and hypothalamus strongly suggest support a role for the hypothalamus in emotion (Herman and Cullinan, 1997; Price, 2003; Hikosaka et al., 2008). For example, the symptoms of the sleep disorder, narcolepsy (Thannickal et al., 2000), strongly indicate hypothalamic involvement in the processing of emotion. Specifically, cataleptic attacks that include a complete loss of muscle tone are the cardinal symptom of narcolepsy and occur predominantly following strong and sudden emotional arousal (Guilleminolt and Fromherz, 2005; Siegel and Boehmer, 2006). This phenomenon is solely contained within the hypothalamus, as cataplexy is fully explained by the loss of hypocretinergic neurons (Siegel, 1999).

Because of its role in sleep and sleep disorders, hypocretin has been the target of numerous research efforts (Van den Pol, 2012). Rodent studies have revealed that hypocretin cells have the highest discharge rates during active wakefulness and exploration and the lowest during REM sleep (Mileykovskiy et al., 2005). Importantly, hypocretin activation seems to be related to positively, as opposed to negatively, valenced arousal states. For example, studies looking at Fos expression show that hypocretin levels are not increased with footshock, a situation of strong negative valence (Furlong et al., 2009). Similarly, hypocretin unit activity decreases in novel situations eliciting withdrawal, but increases with novel situations eliciting exploration (Borgland et al., 2009; Sharf et al., 2010; McGregor et al., 2011). In addition, in humans, low cerebrospinal hypocretin levels are related to depression (Brundin et al., 2009).

In an fMRI study, it was shown that there is increased neural activity within the amygdala and the hypothalamus during the processing of both positive and negative stimuli; interestingly, the hypothalamic activation was at the precicse anatomical location of the hypocretin cells (Karlsson et al., 2010). Consistent with a role for hypocretin in the processing of emotion, recent human microdialysis studies have revealed that hypocretin levels are not affected by general arousal; they are elevated with feelings of excitement or laughter, but not with feelings of frustration or sadness (Blouin et al., 2013).

Intriguingly, the *in vitro* activity of hypocretin cells reveals strong inhibition after the administration of physiological levels of glucose (Burdakov et al., 2005, 2006). Over the past few decades, there have been a number of studies suggesting a role for glucose in the modulation of cognitive processes. The beneficial effects of glucose have been observed for a wide range of experimental settings and cognitive tasks across different medical populations and species. In humans, an enhancement effect following glucose administration has been shown for: cognitive performance resulting in a reduction of reaction times (Adan and Serra-Grabulosa, 2010); selective and sustained attention and control (Gagnon et al., 2010; Serra-Grabulosa et al., 2010); continuous performance tests of attention (Flint, 2004); cognitively demanding tasks (Scholey et al., 2001); and learning and memory (for an extensive review see Smith et al., 2011). In clinical populations with severe cognitive deficits, the administration of glucose has been shown to improve cognitive function, for example, memory performance in Alzheimer's disease (Manning et al., 1993; Messier et al., 1997), although there are also negative reports (Craft et al., 1999). Furthermore, in schizophrenia, higher blood glucose levels have been shown to improve verbal memory and declarative learning (Newcomer et al., 1999; Stone and Seidman, 2008).

Based on the evidence of glucose effects on cognitive function, it seems plausible to predict that glucose may also alter mood and arousal, and specifically, emotions. Under stressful conditions, induced by a foot shock, rats exhibit a significant elevation in blood glucose levels (Verago et al., 2001; Farias-Silva et al., 2002; Eguchi et al., 2011) that, according to one study, is comparable to an injection of 100 mg/kg of glucose (Hall and Gold, 1986). In humans, emotionally arousing pictures (Blake et al., 2001) are not only better remembered, but also lead to a higher blood glucose levels compared to neutral pictures, whereas emotional words are better recalled and recognized than neutral words, without a direct link to glucose levels (Ford et al., 2002).

In contrast to the extensive literature about the behavioral effects of glucose, little is known about the neural mechanisms underlying these observations in humans. Most studies investigating the neural correlates of emotions look solely at the role glucose may exert in facilitating memory. Emerging evidence suggests that this cognitive enhancement is mediated by a glucose-induced effect on the hippocampus, since the enhancement is only observed when this cortical structure is critically involved (Parent et al., 2011; Smith et al., 2011). Yet, glucose may also enhance performance by altering amygdala function, as clearly shown by direct glucose administration to the amygdala (Schroeder and Packard, 2003). Studies investigating the role of glucose on memory enhancement using emotional stimuli also found improved memory recall as well as activation differences not only within the hippocampus but also in the amygdala and frontal regions, all related to glucose levels (Brandt et al., 2006, 2010; Parent et al., 2011).

It is, therefore, tempting to speculate that hypothalamic cells are not only responsive to emotional stimuli, but are also both modulated by stimulus valence, as well as glucose levels, and the interaction thereof. In order to address this issue, we used fMRI to investigate the effect of blood glucose levels on the processing of three different categories of visually presented emotional stimuli (funny, neutral, and sad) in seventeen healthy subjects, comparing two different glucose levels. We hypothesized that, while the first level (euglycemia) would yield only modulations induced by emotions, the second level (hyperglycemia) would show the interaction.

METHODS

SUBJECTS AND DATA ACQUISITION

Seventeen young, healthy volunteers (eight women/nine men; average body weight, 69.3 ± 14.9 kg; BMI, 22 ± 2.7; age, 24 ± 3 years) underwent two fMRI sessions on a 3T TIM Trio scanner (Siemens Medical, Erlangen, Germany), using a 32-channel head coil (25 axial slices; slice thickness 1.9 mm; 128×128 matrix; $TR/TE = 2000/40$ ms). All experiments were performed at the MR Center of Excellence, Medical University of Vienna, Vienna, Austria, in accordance with the 1975 Helsinki declaration and local ethics regulations.

Subjects were instructed to fast overnight from 8:00 p.m. until scanning the next day, which started between 11:00 a.m. and 2:00 p.m. (no intake of food or beverages, except water). Blood glucose levels were measured before the start of the experiment using an Accu-Check GO (Roche Diagnostics, Vienna, Austria). A venous catheter was used to draw blood for assessing glucose levels during the experiment. Two fMRI sessions were acquired each: (1) in the euglycemic state (before glucose administration); and (2) after reaching the hyperglycemic state (after glucose administration). Each session lasted about 15 min.

PARADIGM

In each of the two sessions, subjects were presented with a set of 30 pictures taken from the complete set comprising 60 pictures. Stimuli featured funny, neutral, and sad content. More specifically, pictures depicted a wide range of scenes, such as car crashes, nature settings, empty office buildings, electrical appliances, humans and animals in comic situations, were presented for 4 s each in randomized order. Two sets were chosen to exclude novelty effects and were presented in randomized order. Stimulus material and stimulus presentation was identical to that of Karlsson et al. (2010). Subjects were asked to passively attend the stimuli without being instructed to fixate on a specific part of the image.

Between the two sessions, a 10% glucose solution (Fresenius Kabi, Graz, Austria) was infused intravenously until a blood glucose level of 160–180 mg/dl was reached. Stimulus presentation order was randomized across subjects. After the fMRI measurements, and when the glucose level had leveled off to a

euglycemic state, subjects were asked to rate all presented stimuli using a modified SAM scale comprising the dimensions valence and arousal (Bradley and Lang, 1994). These data were used to verify the selection of the stimuli categories and to exclude systematic differences in individual assessment across the two sessions, using repeated measurement ANOVAs separately per dimension.

DATA ANALYSIS

Image preprocessing for all subjects was performed with SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/), including slice-timing (Sladky et al., 2011) and motion correction, normalization to an anatomical image template, and spatial smoothing using a Gaussian kernel (FWHM = 8 mm). In addition, realignment parameters were added as nuisance regressors to model for residual motion effects. Statistical analysis was performed at the individual and group levels using SPM8. Single-subject data analysis included calculation of statistical parametric maps using the general linear model with regressors corresponding to the different emotional conditions (funny/neutral/sad).

For second-level analysis, analyses of variances were performed as implemented in SPM8 using the two different fMRI sessions (before/after glucose administration) as one factor, and the emotional categories as the second factor with three levels (funny, neutral, and sad).

In an initial evaluation, the individual glucose level differences between the two sessions were added as a covariate of no interest to obtain neuronal difference effects of sessions and runs, i.e., main effects for emotional categories and sessions.

To account for possible regulation effects due to glucose levels on the session-specific activation patterns and the perception of emotional stimuli, individual session-specific glucose values were included. Using these covariates, a second model was calculated. Both models were thresholded at $p < 0.001$, uncorrected.

RESULTS

GLUCOSE LEVELS

Mean glucose levels were 84.35 mg/dl (±7.3 *SD*) for the first session and 177.96 mg/dl (±14.5 *SD*) for the second session. The mean latency for the subjects to reach the predefined glucose levels was 1 h and 16 min (±25 min *SD*).

BEHAVIORAL DATA

On average, valence measures for the euglycemic sessions were 5.10 (±1.76 *SD*), and 5.22 (±1.85 *SD*) for the hyperglycemic sessions. Arousal levels reached 5.60 (±1.81 *SD*) for the euglycemic sessions, compared to 5.88 (±1.93 *SD*) for the hyperglycemic sessions. There was no significant difference between the measures. Global analysis pooling across the two sessions yielded a significant difference between the three stimulus conditions of funny, neutral, and sad [$F_{(2, 32)} = 215.075$, $p < 0.000$], as well as for arousal [$F_{(2, 32)} = 40.052$, $p < 0.000$]. A detailed analysis, including session analysis, returned a significant result for the factor summarizing the three conditions, but there was no significant effect for session [valence: $F_{(1, 16)} = 2.232$, $p = 0.155$; arousal: $F_{(1, 16)} = 0.012$, $p = 0.915$] nor any interaction effects between session and emotional category [valence: $F_{(2, 32)} = 2.547$, $p = 0.094$; arousal: $F_{(2, 32)} = 0.940$, $p = 0.401$].

fMRI DATA

Results of the first 2 × 3 ANCOVA, using the glucose level differences as a covariate, revealed bilateral activation differences in the primary visual cortex and amygdala, depending on the emotional category (see **Figure 1** and **Table 1** for detailed results). More specifically, the left amygdala activation profile corresponded to the three emotional conditions and was obtained for both sessions, regardless of glucose level. Within this region, positive and negative emotional categories (i.e., funny and sad) showed

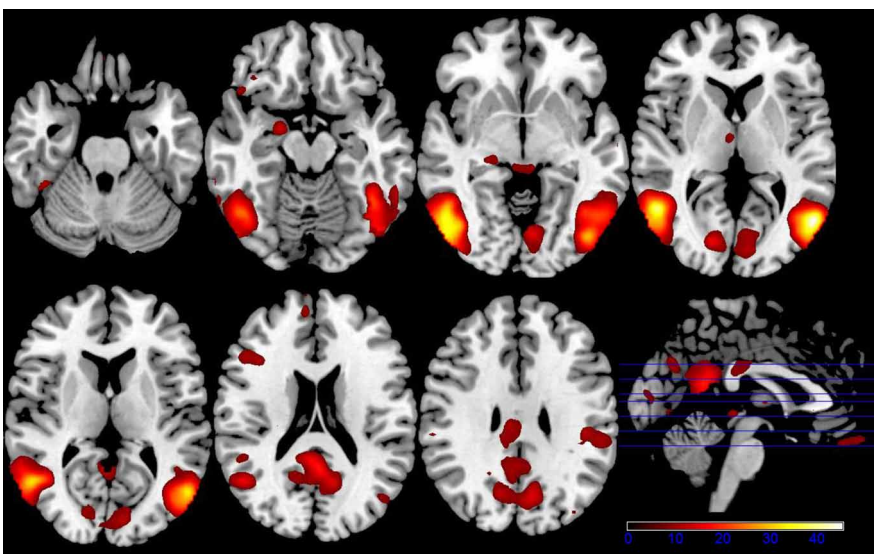

FIGURE 1 | Axial slices depicting the main effect of emotional categories resulting from a 2 × 3 ANCOVA using the individual glucose level difference as a covariate ($p < 0.001$ uncorrected for whole-brain volume analysis; for a more detailed description of activated brain regions, please see Table 1).

Table 1 | Listing for corresponding regions shown in Figures 1, 3, 5, and 6 ($p < 0.001$ uncorrected for whole-brain volume analysis).

	Region	Voxels	*Z*-values
Figure 1	l. middle temporal gyrus	2969	7.57
	r. middle temporal gyrus	3267	7.29
	l. precuneus	1999	5.16
	r. calcarine gyrus	868	4.88
	r. hippocampus	130	4.36
Figure 3	r. hippocampus	74	4.01
	r. fusiform gyrus	89	3.99
	l. calcarine gyrus	98	3.98
	r. insula	64	3.94
	l. thalamus	49	3.77
Figure 5	l. hypothalamus	3	3.25
Figure 6	r. supramarginal gyrus	45	4.39
	l. cuneus	80	3.94
	r. superior temporal gyrus	37	3.93
	r. caudate nucleus	70	3.84
	r. anterior cingulate gyrus	35	3.75

Reported are the largest five significantly activated clusters. Clusters were automatically labeled using the AAL toolbox (Eickhoff et al., 2005).

increased activation compared to baseline, while no alteration from baseline was found for the neutral emotional condition (see **Figure 2**). A *post-hoc* ROI analysis within the amygdala revealed no significant difference between funny versus sad stimuli across both glucose sessions.

For the main effect sessions, i.e., contrasting the two sessions regardless of emotional category, higher activation was observed unilaterally in the right temporal cortex, within the hippocampus (see **Figure 3** as an activation overview and **Figure 4** for contrast estimates in the hippocampus) and the fusiform gyrus, as well as in the thalamus (see **Table 1** for detailed results). The first session was the euglycemic state, compared to the hyperglycemic state, the second session.

While neutral stimuli did not show any change related to the two sessions, increased activation levels were found for the first session, diminishing in the second session for the emotional categories funny and sad. Finally, summarizing activation changes related to the two sessions and the emotional category differences corresponding to the interaction, effects were found only within the hypothalamus (see **Figure 5** and **Table 1** for detailed results). A *post-hoc* linear contrast within the interaction showed that the hypothalamus, although differentiating between the emotions in the first session, was not modulated by emotions in the second session.

The second model using the individual session-specific glucose values as a covariate showed significant activation in the left and right intra-parietal lobules and the right medial cingulate gyrus (see **Figure 6**) when comparing both sessions with respect to the two glucose levels. **Table 1** summarizes all the findings of glucose-related brain activity enhancement in the processing of emotions in young, healthy subjects.

DISCUSSION

In this study, an analysis of the interaction effect of hypo- and hyperglycemia and stimulus category (funny, neutral, sad) revealed a single activation cluster in the hypothalamus. This finding is in accordance with prior reports on hypothalamic activity during the processing of emotional stimuli (Reiss et al., 2008; Schwartz et al., 2008; Karlsson et al., 2010). Furthermore, as previously reported, the activation cluster correlates precisely with the anatomical location of the hypothalamic hypocretin cells (Karlsson et al., 2010). Karlsson et al. (2010) investigated the effects of funny and sad stimuli compared to neutral stimuli, and demonstrated modulation effects for both the amygdala and hypothalamus. Their observation of a U-shaped activation profile in the hypothalamus concurs with our findings of positive and negative emotional categories revealing high mean beta values, which indicate increased activation in the euglycemic state. However, this modulation vanishes with increased glucose levels. In this hyperglycemic state, the hypothalamus no longer responds to emotions. We, therefore, conclude that at this anatomical site of the hypothalamus, there is a small cluster of cells whose activity is simultaneously modulated by glucose levels and stimulus valence.

fMRI is a non-invasive imaging method that detects transient hemodynamic and functional changes in the brain in response to a variety of stimuli (Bandettini, 2012). The small size of the hypothalamus and its nuclei, combined with low signal changes in fMRI, require specifically optimized protocols (Robinson et al., 2008, 2009) to enable imaging of responses to food-related stimuli in this part of the brain. The use of fMRI to study hypothalamic function in humans has been reported previously (Karlsson et al., 2010). To date, only a few fMRI studies have investigated the involvement of hypothalamic neuronal activity after glucose ingestion in humans (Matsuda et al., 1999; Liu et al., 2000; Smeets et al., 2005, 2007; Vidarsdottir et al., 2007; Purnell et al., 2011).

The hypothalamus is involved in the regulation of food intake and is also responsible for integrating a wide array of hormonal and neural information (Levin et al., 2004), as well as for the evaluation of reward quality and related emotions (Lénárd and Karádi, 2012). This coupling of hypothalamic functioning and glucose ingestion was first shown by Matsuda et al. (1999), who reported on differences in hypothalamic function in lean subjects, *in vivo*, using fMRI to monitor hypothalamic function after oral glucose intake (Matsuda et al., 1999). After glucose ingestion, an increased signal was obtained in the paraventricular and ventromedial nuclei in lean subjects, whereas this inhibitory response was attenuated and delayed in obese subjects. A prolonged dose-dependent decrease in fMRI signal in the hypothalamus after glucose ingestion was confirmed by Smeets et al. (2005), who suggested a possible function for the observed hypothalamic response to changes in blood insulin levels. In addition to fMRI-related findings, other research has revealed increased slow diffusion parameters in the hypothalamus during hypoglycemia induced by fasting (Lizarbe et al., 2013).

The results of Smeets et al. (2005) suggest that the hypothalamus acts as a driving mechanism in areas involved in the

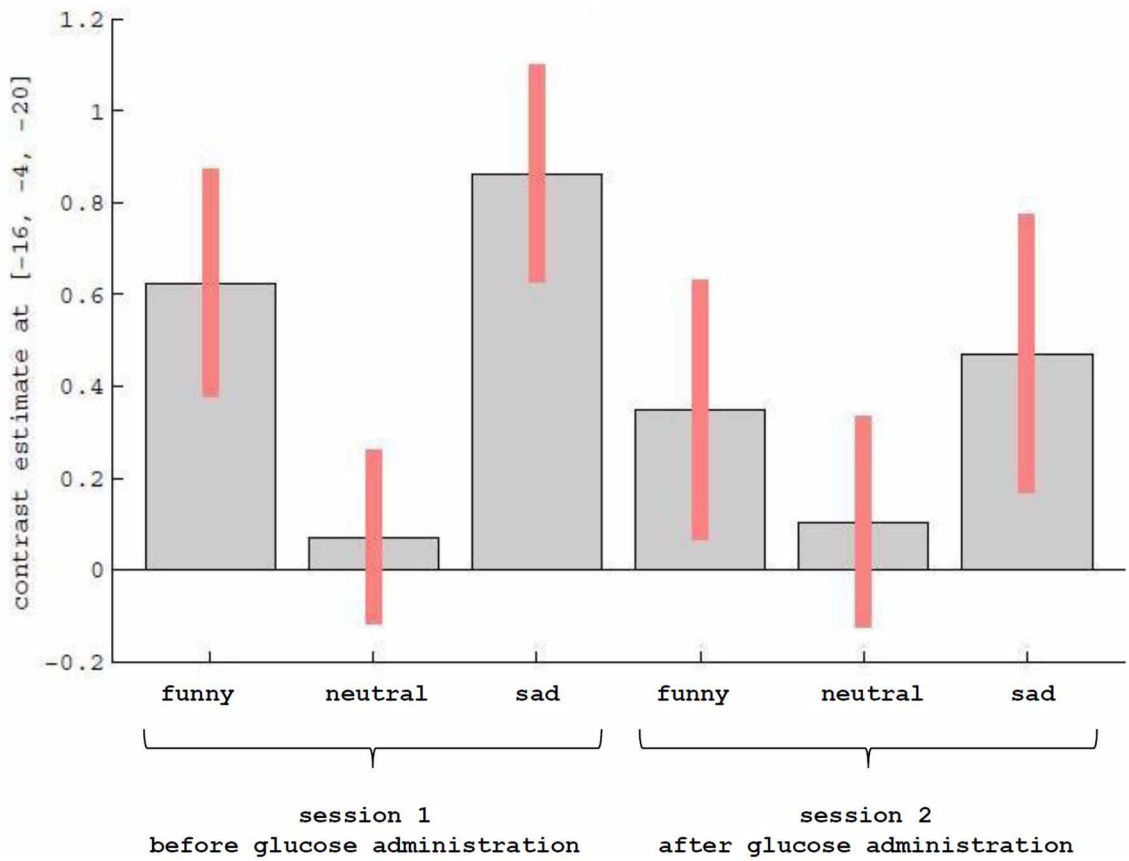

FIGURE 2 | Description of contrast estimates for the results of the main effect of emotional categories, as shown in Figure 1 in the left amygdala.

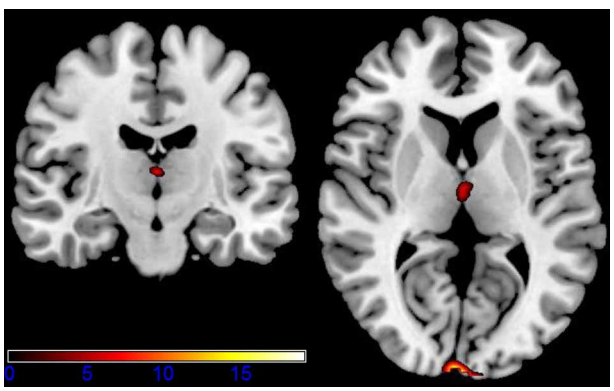

FIGURE 3 | Axial and coronal slices showing significant activation for the main effect runs (for a detailed description of the analysis, please see text; $p < 0.001$ uncorrected for whole-brain volume analysis; for a more detailed description of activated brain regions, please see Table 1).

processing of emotional stimuli, much like the amygdala, after glucose administration. This finding is also in accordance with experimental reports of hypothalamic functions being altered by glucose intake (Matsuda et al., 1999), and in line with the U-shaped activation curve exhibited within the hypothalamus in response to negative, neutral, and positively valenced stimuli during euglycemic states (Karlsson et al., 2010). Furthermore, this interaction effect of glucose and emotion control was recently recognized in a behavioral study (Niven et al., 2013), which uncovered a correlation of blood glucose levels with poor emotional regulation; the authors hypothesized that glucose provides a limited energy resource upon which self-control relies. The combined demonstration of behavioral data and functional imaging, as realized in our study, enables the investigation of emotional modulation effects related to glucose intake.

A limiting factor in our study is that there was no randomization of glucose level sessions. As euglycemia as a second session would involve the administrations of large doses of insulin to reach a euglycemic state after the hyperglycemic state, we abstained from randomization for safety reasons and unpredictable side effects.

Our findings can pave the way for a more detailed understanding of diseases associated with dysregulation of glucose and glucose availability in the brain, including early metabolic changes starting in childhood (McCrimmon et al., 2012; Reagan, 2012). It has also been shown that glycemic variability significantly impacts

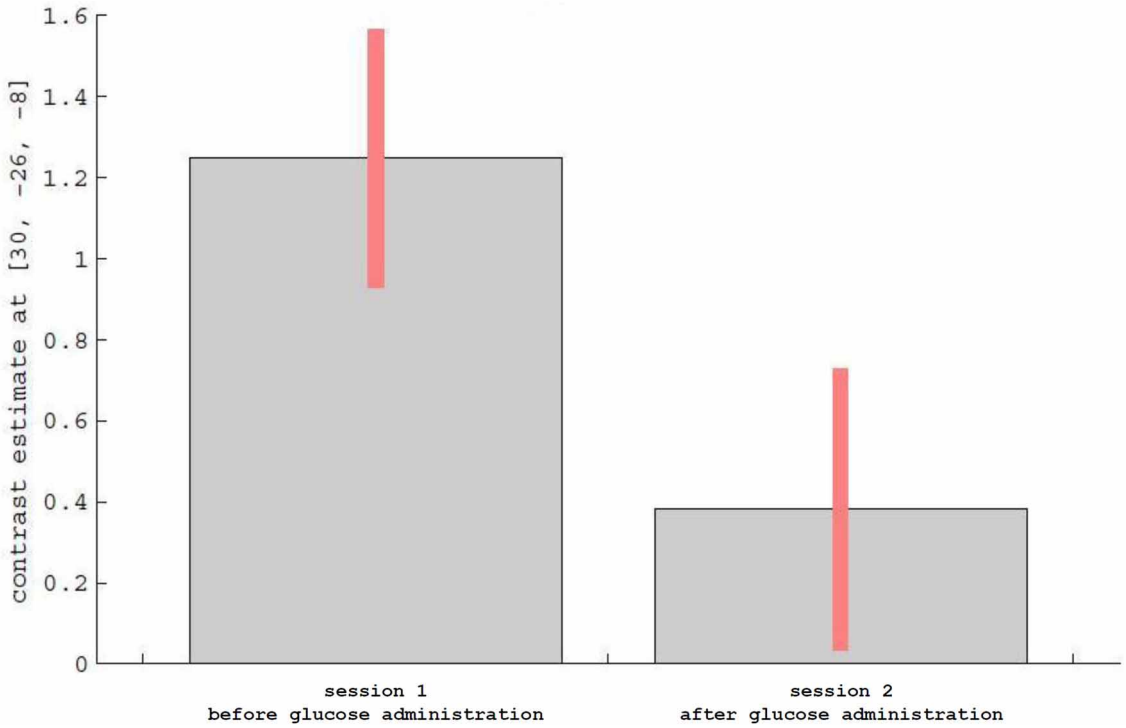

FIGURE 4 | Description of contrast estimates for the results of the main effect runs in the hippocampus, as shown in Figure 3.

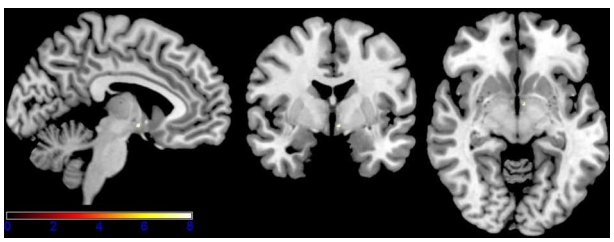

FIGURE 5 | Shown are sagittal, coronal, and axial slices overlaid with activity clusters corresponding to the interaction effect related to the two sessions and emotional categories ($p < 0.001$ uncorrected for whole-brain volume analysis; for a more detailed description of activated brain regions, please see Table 1).

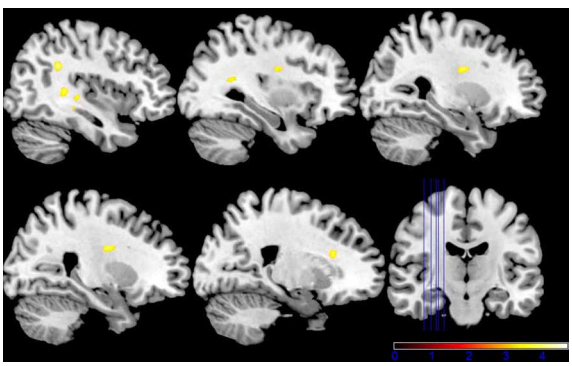

FIGURE 6 | Shown are sagittal and coronal slices overlaid with activity clusters using individual run-specific glucose levels as covariates ($p < 0.001$ uncorrected for whole-brain volume analysis; for a more detailed description of activated brain regions please see Table 1).

mood and quality of life in diabetes (Penckofer et al., 2012), and emotional disorders can negatively affect the course of diabetes (Dziemidok et al., 2011). Moreover, obesity, suggested recently to be a brain-related dysfunction in which reward-driven impulses for food take over response selection systems, was associated with elevations in emotionally driven impulsivity and cognitive inflexibility (Strüber et al., 2008; Delgado-Rico et al., 2012), as well as with emotions that trigger overeating and night-eating (Birketvedt et al., 2002; Koenders and Van Strien, 2011). Those findings might be strongly related to the neural correlates of the processing of emotions. It is reasonable to suggest that the cluster of cells revealed in the current experiment, possibly comprising hypocretin cells, predominantly mediate those effects. Our results offer novel insights into the understanding of disease-related behavior, which could potentially offer improved diagnostic and novel therapeutic strategies in the future.

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The application of near infrared spectroscopy in nutritional intervention studies

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Functional near infrared spectroscopy (NIRS) is a non-invasive optical imaging technique used to monitor cerebral blood flow (CBF) and by proxy neuronal activation. The use of NIRS in nutritional intervention studies is a relatively novel application of this technique, with only a small, but growing, number of trials published to date. These trials—in which the effects on CBF following administration of dietary components such as caffeine, polyphenols and omega-3 polyunsaturated fatty acids are assessed—have successfully demonstrated NIRS as a sensitive measure of change in hemodynamic response during cognitive tasks in both acute and chronic treatment intervention paradigms. The existent research in this area has been limited by the constraints of the technique itself however advancements in the measurement technology, paired with studies endeavoring increased sophistication in number and locations of channels over the head should render the use of NIRS in nutritional interventions particularly valuable in advancing our understanding of the effects of nutrients and dietary components on the brain.

Keywords: NIRS, nutrition, cognition, neuroimaging, intervention studies

INTRODUCTION

Near infrared spectroscopy (NIRS) is a non-invasive optical imaging technique used to monitor tissue oxygen status. In the brain, NIRS can be used to examine cerebral blood flow (CBF) and the local hemodynamic response during neural activity. The technique was originally described by Jobsis (1977) although it is only in the last two decades that NIRS has been successfully used to assess *in vivo* functional activation, following the first demonstrations by Tamura and colleagues in Japan (Hoshi and Tamura, 1993a,b). During this time the technique has been widely applied in clinical settings. However, only recently has the technology been employed in the field of nutritional neuroscience. Given that this technique has the advantage of being low cost and user friendly in comparison to other neuroimaging methodologies it is ideally suited for use in this area.

This review will cover the basics of NIRS examinations, the validity of NIRS in the assessment of brain function, the utility of NIRS in nutrition intervention studies and the challenges and future directions of NIRS in the field.

PRINCIPLES OF NEAR INFRARED SPECTROSCOPY AND NIRS METHODS

Light easily passes through biological tissue in the near infrared spectrum (650–1000 nm), allowing for easy and non-invasive illumination of brain tissue via transmission of light through the intact scalp, skull and subarachnoid space to the top layers of the cortex. As oxygenated hemoglobin (oxy-Hb) and deoxygenated hemoglobin (deoxy-Hb) are light absorbing pigments, or chromophores, which absorb light at slightly different wavelengths (800–940 nm and 600–750 nm, respectively), it is possible to measure their concentration by quantifying the amount of light absorbed in these wavelengths during the transit of light through the brain tissue (Chance et al., 1988; Obrig and Villringer, 2003; Okui and Okada, 2005). In the brain, cerebral vessels, glia and neurons work intimately to ensure the constant supply of blood borne energy substrates (i.e., oxygen and glucose) to active areas via a complex series of mechanisms controlled by numerous mediators (reviewed in Girouard and Iadecola, 2006). NIRS, like fMRI, exploits the so-called "neurovascular coupling" of neuronal activity and blood-flow to indirectly assess neuronal activity by measuring changes in oxy-Hb and deoxy-Hb. These chromophores provide proxy measures of blood flow and the consumption of oxygen by neuronal cells in the interrogated tissue. NIRS outcomes can therefore be taken to infer local neural activation or, alternatively, can be interpreted more directly as simple changes in blood flow/volume in underlying cortical tissue. The latter is particularly pertinent as both CBF and the magnitude of the hemodynamic response to neural activity decrease with normal ageing and in neurological disease (Girouard and Iadecola, 2006). A typical response during local neural activity will be seen as an increase in oxy-Hb as blood flow increases in the active tissue, coupled with a concurrent decrease in deoxy-Hb as its concentration reduces in the face of the sudden influx of oxy-Hb (Obrig and Villringer, 2003). Delivery of oxygenated blood to active areas typically exceeds local oxygen utilization and therefore increased oxy-Hb is observed throughout the period of sustained activation. The total concentration of hemoglobin (total Hb) is the sum of oxy-Hb and deoxy-Hb and typically follows the same pattern as oxy-Hb given the aforementioned "overshoot" in cerebral oxygenation (Obrig et al., 1997). In some instances

it is possible to observe a transient initial "dip" in oxy-Hb as pre-existing oxygen is extracted by the active neurons prior to the influx of oxygenated blood to the area (Buxton, 2001). In general, changes in oxy-Hb and deoxy-Hb are therefore related to changes in cerebral metabolic rates (Tamura et al., 1997) and total Hb is closely related to CBF/cerebral blood volume (CBV) (Steinbrink et al., 2006).

In terms of practical apparatus, light is introduced to the brain via laser emitting diodes that are placed directly onto the scalp and held in place via a headband. Light then passes from this source to photodetectors, or optodes, which are placed at a set distance away (usually >3 cm), passing through the scalp, skull and underlying brain tissue in a predictable banana-shaped pattern (Okada and Delpy, 2003; Mansouri et al., 2010). Brain tissue is assumed to be a homogenous scattering medium, therefore any attenuation of light in each chromophore specific wavelength can be assumed to be due to absorption by oxy-Hb and deoxy-Hb (Fallgatter and Strik, 1997). A modified Beer-Lambert law incorporating the optical differential path-length factor is used to calculate changes in concentrations of oxy-, deoxy- and total Hb in micro molar units (Pellicer and Del Carmen Bravo, 2011).

A number of different types of NIRS systems exist, each with their own set of advantages and disadvantages, although all systems share common attributes that make it a particularly attractive alternative to fMRI, widely perceived to be the current "gold standard" in neuroimaging. All NIRS systems benefit from high temporal resolution due to the fact that they can sample at 50 Hz or more. In addition, due to the fact that NIRS monitors both oxy- and deoxy-Hb in tandem (as opposed to just the comparative concentrations of oxy-Hb and deoxy-Hb with respect to each other, as in the fMRI blood oxygen level dependent [BOLD] signal), the pattern and time course of hemodynamic response can therefore be measured with a high degree of accuracy. Attachment of the diodes to the head using a simple and relatively comfortable headband also allows for extended recording over several hours. The portability of NIRS systems even accommodates accurate measurement while participants move around, supporting advances in the study of cortical control of gait, for example, but allowing for more ecologically valid paradigms overall (Wang et al., 2008; Kurz et al., 2012). On the other hand, with NIRS spatial resolution is limited to approximately 2 cm as the source and photodetectors must be spaced at a sufficient distance to allow sensitive measurement of tissue absorption (Fukui et al., 2003).

NIRS systems vary in complexity ranging from simple one or two channels to arrays of several dozen that cover the whole head. Several different systems are also available: "time domain" systems emit short bursts of photons, with the temporal distribution providing information about tissue absorption and scattering; "frequency domain" systems emit amplitude-modulated light and record amplitude decay and phase shift; and the most common measurement technique is a "continuous wave" (CW) system that emits light continuously at constant amplitudes, and only the amplitude decay is measured (Strangman et al., 2002). Frequency and time domain systems offer deeper cortical illumination than CW systems—which only illuminate the top 2–3 mm of the cortex (Chance et al., 1988)—as well as the ability to produce absolute values for changes in concentrations of the chromophores. However, compared to CW systems, they are bulkier and much more expensive, rendering these systems less accessible and applicable to a less diverse range of paradigms (Ferrari and Quaresima, 2012). Recent "quantitative" systems have similar advantages in terms of cost and practical considerations as earlier CW systems but more importantly resolve the measurement issue by collecting light at several increasing distances from the light source, allowing the exact calculation of the path length and thereby the absolute quantity of hemoglobin in underlying tissue.

VALIDITY OF NIRS EXAMINATIONS

Despite the limitations of the technique, NIRS has been successfully used to image activation in the frontal cortex (e.g., Fallgatter and Strik, 1997, 1998) and other areas of the brain including the temporal, visual and parietal cortices (e.g., Jasdzewski et al., 2003; Schecklmann et al., 2008a). NIRS has also been particularly valuable as neurovascular assessment tool and for the identification of brain injury in all age groups from neonates (Grant et al., 2009; Lin et al., 2013) to adults (Cohn, 2007; La Monaca et al., 2010; Len and Neary, 2011), and for the evaluation of cognitive function across the lifespan as well as in abnormal functioning. Advances in the field of neonatal and infant cortical functioning have been made possible with NIRS, as the technique is truly non-invasive and can accommodate a certain degree of movement. Evaluations of infant hemodynamic response following simple visual and auditory stimuli have been followed up more recently by studies that have used whole head multi-channel arrays to characterize activation during much more complex tasks including object, face and motion processing and social communication (reviewed in Lloyd-Fox et al., 2010). NIRS has also been used to investigate age-related changes in cerebral oxygenation elicited by sensory stimuli or during cognitive tasks. Reduced hemodynamic response has been generally observed in older, compared to younger participants, as well as subtle differences in cortical control across the cohorts (Schroeter et al., 2003; Kameyama et al., 2004; Safonova et al., 2004; Harada et al., 2007; Kim et al., 2011).

NIRS has also proven to be a sensitive research technique in patients with neurodevelopmental or neurological conditions. For example, reduced hemodynamic response has been demonstrated in the prefrontal cortex in schizophrenia (Shimodera et al., 2012; Taniguchi et al., 2012) as well as in children and adults with Attention Deficit Hyperactivity Disorder (ADHD) (Schecklmann et al., 2008b; Xiao et al., 2012). Prefrontal reductions in tissue oxygenation have also been observed in mild cognitive impairment and dementia (Arai et al., 2006; Herrmann et al., 2008) as well as similar reductions in parietal regions (Zeller et al., 2010). Given these demonstrations, consideration has been given to the possibility of using NIRS as a tool in early disease detection or for supporting accurate clinical diagnosis. For example, one research group in Japan have been able to characterize hemodynamic response to a verbal fluency task in different disease states (Fukuda and Mikuni, 2012). In a similar vein Takahashi et al. (2010) were able to identify differences in prefrontal hemodynamic response to a response inhibition task in responders and non-responders to treatment with methylphenidate (used to treat the symptoms of ADHD),

raising the possibility of being able to use NIRS to tackle the issue of over prescription of this drug in children.

Although the number of publications to date is, as yet, still limited, NIRS has also been successfully applied in pharmacological studies where the CBF effects of a wide range of pharmaceutical interventions have been assessed across the lifespan. For example, the use of NIRS in this field has enabled easy and accurate monitoring of neonatal cerebral oxygenation following administration of routine drugs including anesthetic (Vanderhaegen et al., 2010) and non-steroidal anti-inflammatories (Garner et al., 2012), both of which have been shown to reduce cerebral oxygenation. Reductions in the prefrontal CBF response to tasks have been observed following methylphenidate in children with ADHD (Weber et al., 2007), and also in normally developing children following anti-histamines (Tsujii et al., 2009, 2010). Interestingly, Tsujii et al. (2010) were able show the difference in cerebral oxygenation following sedating and non-sedating anti-histamine variants, with reductions in CBF only being observed for the sedating variety. In adults, NIRS has been used to show decreased frontal lobe oxygenation following sumatriptan administration in migraine sufferers (Watanabe et al., 2011) and also following the vasopressor agent phenylephrine, wherein the technique has been instrumental in providing evidence in support of sympathetic regulation of cerebral circulation (Brassard et al., 2010; Ogoh et al., 2011). Conversely, increased frontal CBF has also been demonstrated following the vasodilator vinpocetine in stroke patients (Bonoczk et al., 2002).

THE APPLICATION OF NIRS IN NUTRITION INTERVENTION STUDIES

NIRS has been shown to be a feasible method with which to measure hemodynamic response and CBF in the superficial cortex and as a sensitive measure of change in cerebral oxygenation following pharmacological agents. With the growing interest in the contribution of nutrition to cognitive health across the lifespan, the application of NIRS in the field of nutritional neuroscience is a valid and natural extension of the technique. To date, however, only a small number of studies have assessed the cerebral hemodynamic effects of dietary components, and the majority of these have only collected data from the prefrontal cortex. In terms of acute administration studies, two studies have demonstrated the vasoconstricting properties of caffeine. An early study presented by Higashi et al. (2004) found reductions in CBV during the post-treatment rest period compared to the control (no treatment) rest period but found no difference between hemodynamic response to cognitive tasks between the treatment and control test periods, despite performance on the tasks being improved following caffeine. However more recently, Kennedy and Haskell (2011) reported a prefrontal decrease in total Hb during task performance following an ecologically valid dose of caffeine (75 mg). The latter study benefitted from a double-blind, placebo-controlled design, with continuous monitoring during the absorption and testing periods as well as increased statistical power resulting from monitoring NIRS outcomes during multiple completions of the same tasks. This approach has been applied by the same research group to acute investigations of the effects of polyphenols. Examples include demonstrations of a dose-related increase in total Hb and deoxy-Hb during cognitive tasks following 250 mg and 500 mg of resveratrol (Kennedy et al., 2010) and a decrease in total Hb following a single dose of epigallocatechin gallate, the principal polyphenol found in green tea (Wightman et al., 2012). The only study to apply a multi-channel, multi-site approach assessed the immune system marker and CBF effects of acute administration of soybean peptide using a frequency wave NIRS system (Yimit et al., 2012). The authors of this placebo-controlled crossover study reported immediate (30 s) post-treatment (resting) increases in frequency amplitude across several areas of the brain, including the premotor cortex, primary motor cortex and dorsolateral prefrontal cortex.

NIRS has also been successfully applied to chronic intervention studies. Jackson and colleagues assessed the effects of 12 weeks' administration with fish oil in healthy young adults (18–35 years) on prefrontal oxygenation in two separate double-blind placebo-controlled parallel group studies. The first of these revealed that administration with docosahexaenoic acid (DHA)-rich fish oil, but not eicosapentaenoic acid-rich fish oil, was associated with task-related increases in oxy-Hb and total Hb (Jackson et al., 2012b), whilst the second, larger study was able to demonstrate a dose-related effect of DHA-rich fish oil on the same parameters (Jackson et al., 2012a). Watanabe et al. (2002) also examined cerebral oxygenation in healthy young adults during a mental arithmetic task before and after five days' supplementation with creatine. Although these authors reported that creatine led to decreased oxy-Hb and increased deoxy-Hb in comparison with the pre-treatment hemodynamic response to the task, they did not conduct any comparisons between the placebo and treatment groups.

METHODOLOGICAL LIMITATIONS AND FUTURE RECOMMENDATIONS

The above collection of studies provide strong support in favor of NIRS as a sensitive technique for monitoring changes in hemodynamic response/CBF following the administration of nutrients or dietary components. As yet, however, the application of NIRS in a nutritional neuroscience setting has been limited by the shortcomings of the methodology and also by intrinsic difficulties in applying a relatively novel technique in a field that has only really flourished in the past few decades itself. For example, Tsujii et al. (2011), in a placebo-controlled counterbalanced crossover trial, set out to assess the neural correlates of the effect of alcohol on response inhibition, a process that has previously been associated with activity in the inferior frontal cortex (IFC). These authors used NIRS to assess hemodynamic response in the IFC during a Go/No-Go task prior to and after consuming alcohol and were able to demonstrate for the first time the contribution of the right IFC in inhibitory control of pre-potent responses following alcohol. In addition, the authors reported a negative correlation between increased concentrations of oxy-Hb in the right IFC and false alarms on the task. This type of focused and hypothesis-driven research is simply much more difficult to conduct when the *in vivo* physiological and cognitive effects of the nutritional interventions in question are not as well established, certainly in comparison to pharmacological agents. In addition, given that complications arise from the placement

of the optodes on parts of the head that are covered in hair, the research to date has tended to concentrate on examining CBF and hemodynamic responses in the prefrontal region, measured through the hair free forehead. This shortcoming may contribute to the fact that, hitherto, the nutritional intervention studies listed above have failed to find a direct relationship between the observed changes in hemodynamic response/CBF and concomitant significant changes in cognitive function. Furthermore, the depth penetration issues of the NIRS signal—at least with the most prevalent CW NIRS systems—places a further limitation on the areas of the brain that can be assessed. On a positive note, the advent of newly developed quantitative NIRS systems will address the current issue of not being able to report absolute concentration changes in the chromophores, which again has placed limitations on nutritional intervention study designs thus far. With the development of more complex protocols incorporating multi-channel, multi-site monitoring along with the continued investment in the development of the NIRS systems, it is hoped that these challenges will be overcome in future studies.

One last point to note is the current lack of standardized statistical analysis approaches for NIRS assessment, similar to those applied in more well-established neuroimaging techniques. At present there are no universally adopted methods for the data analysis used in the production of topographical maps, or even agreement about determining the statistical significance of hemodynamic changes (Elwell and Cooper, 2011). Further, papers do not always present both oxy- and deoxy-Hb, despite evidence that that they should be equally valued as markers of cerebral oxygenation, and should therefore be reported together as standard.

Overall, NIRS as a neuroimaging technique offers several advantages in nutritional intervention studies over more commonly used methods. Given the increasing demand for functional activation data as a secondary endpoint the fact that NIRS is low cost and practically ready to use "off the shelf" offers a real opportunity for enhancing nutritional intervention studies. However more hypothesis-driven research in the area is a necessity, and the fact that NIRS can easily be combined with other imaging techniques may be one way in which to address this issue. Moreover, the development of quantitative NIRS systems brings with it the real possibility of conducting the type of long-term research that can be informative about the modulating effects of nutrients on the brain across the lifespan.

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Neuroimaging, a new tool for investigating the effects of early diet on cognitive and brain development

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Nutrition is crucial to the initial development of the central nervous system (CNS), and then to its maintenance, because both depend on dietary intake to supply the elements required to develop and fuel the system. Diet in early life is often seen in the context of "programming" where a stimulus occurring during a vulnerable period can have long-lasting or even lifetime effects on some aspect of the organism's structure or function. Nutrition was first shown to be a programming stimulus for growth, and then for cognitive behavior, in animal studies that were able to employ methods that allowed the demonstration of neural effects of early nutrition. Such research raised the question of whether nutrition could also programme cognition/brain structure in humans. Initial studies of cognitive effects were observational, usually conducted in developing countries where the presence of confounding factors made it difficult to interpret the role of nutrition in the cognitive deficits that were seen. Attributing causality to nutrition required randomized controlled trials (RCTs) and these, often in developed countries, started to appear around 30 years ago. Most demonstrated convincingly that early nutrition could affect subsequent cognition. Until the advent of neuroimaging techniques that allowed *in vivo* examination of the brain, however, we could determine very little about the neural effects of early diet in humans. The combination of well-designed trials with neuroimaging tools means that we are now able to pose and answer questions that would have seemed impossible only recently. This review discusses various neuroimaging methods that are suitable for use in nutrition studies, while pointing out some of the limitations that they may have. The existing literature is small, but examples of studies that have used these methods are presented. Finally, some considerations that have arisen from previous studies, as well as suggestions for future research, are discussed.

Keywords: early diet, brain development, cognition, neuroimaging, programming

INTRODUCTION

The fact that the study of nutrition is basic to brain science often goes unrecognized by neuropsychologists and cognitive neuroscientists who tend to regard it as a variable of little or no interest. Most fundamentally, and throughout life, energy to fuel the brain comes from dietary intake, mainly in the form of glucose. Other elements in the everyday diet also influence brain function by providing resources for the maintenance of central nervous system (CNS) activity; Greenwood and Craig (1987), for example, describe some ways in which food intake can affect neurochemistry: by providing precursors for the synthesis of neurotransmitters; by providing vitamins and minerals that serve as essential co-factors in enzyme activity during synthesis; by providing dietary fats that can affect properties of the nerve cell membranes. Variations in neural processes such as these, resulting from dietary intake, may have demonstrable cognitive consequences.

In addition to these ubiquitous effects across the human lifespan, nutrition has an important role to play in the initial development of the brain. Indeed, according to Walker (2005), nutrition is possibly the single environmental variable that can have the widest range of effects on brain development. Nevertheless, it is important to remember that it is one of many other environmental factors such as socioeconomic status, maternal attachment, and level of parental education that play an important role in neural development. Recently, epigenetic factors (i.e., modifications to the genome that do not involve changes in nucleotide sequence) have been shown to be modifiable by environmental factors, making this a candidate mechanism for the mediation of the effects of early experience on the development of the organism (Zhang and Meaney, 2010; Blaze et al., 2013). There are two features of nutrition that emphasize its important role: Rosales et al. (2009) have pointed out that nutrition is unique in that it can directly modify genetic structure and also mediate how genetic factors are expressed. It is also more amenable to modification than many other factors.

One of the hallmarks of developmental neuropathology is the idea that a stimulus will have its greatest effect on the structures and processes that are developing at the time it is applied (Dobbing, 1981). In other words, the CNS is most vulnerable to environmental influence, such as nutrition, when it is in a state of change and plasticity is at its greatest; in the case of

the human infant, this period of rapid growth and development, known as the "brain spurt," extends from the beginning of the third trimester of pregnancy until ∼2 years of age (Gilmore et al., 2012). Georgieff (2007) states that the brain is particularly vulnerable to the influences of nutrition between 24 and 42 weeks of gestation. During the growth spurt, the brain follows an invariant sequence of developmental events, beginning with neuronal proliferation and migration, followed by neuronal differentiation, the formation of connective patterns, subtractive processes in the form of neuronal death and synaptic elimination and, finally, myelination (de Graaf-Peters and Hadders-Algra, 2006). By the age of ∼2 years, the volume of the human brain has reached 80–90% of adult size (Knickmeyer et al., 2008). This rapid period of growth has become a focus of research into how variation in nutritional intake may affect subsequent brain development. This does not mean, of course, that nutrition cannot exert effects at other times but envisages a continuum across the lifespan with variations in vulnerability at different ages. Many studies have investigated the important role played by maternal nutrition via the placenta on brain development (Morgane et al., 1993). Antonow-Schlorke et al. (2011), for example, reported that even moderate restriction of maternal nutrition during pregnancy, to a degree not uncommon in the developed world, resulted in major cerebral development disturbances in baboons.

There has been a recent explosion of research activity exploring the early origins of adult disease hypothesis (Barker et al., 1993; Lucas et al., 1999) that, in its original form, stated that low growth rates *in utero* and during infancy were associated with high death rates from cardiovascular disease. This has generated increased interest in infant nutrition. Early studies by McCance (1962) are relevant in this context. The size of the litter in which new-born rats spent the first 3 weeks of life was manipulated. Not surprisingly, those reared in small litters, with proportionally more of the mother's milk supply per individual, were heavier and longer at the end of this time than those from larger litters. At this point, all animals were allowed unlimited food but the smaller animals showed no catch-up growth and this persisted throughout life, so that they never attained the size of the larger; in fact, the differential tended to increase with age. A period of under-feeding of the same duration but introduced at 9 weeks in animals that had been developing normally also resulted in smaller size but, in these circumstances, the re-introduction of a normal feeding regime resulted in rapid weight gain until they attained the "normal" size expected. Studies of this type illustrated that an event early in life, during a limited developmental window, could "programme" long-lasting differences, i.e., that nutrition could act as a programming stimulus for growth. While much of the evidence for a link between early growth and adult outcomes has been observational, a series of studies based on randomized controlled trials (RCTs), reported that early nutrition was shown to programme health-related outcomes such as blood pressure (Singhal et al., 2008), obesity (Singhal et al., 2010), and atherosclerosis (Singhal, 2006). These results raise the question of whether early nutrition could also programme cognition and its underlying neural bases.

While many nutrients have a role to play in brain development, certain ones seem to be of particular importance during fetal and neonatal life: protein, iron, zinc, selenium, iodine, folate, vitamin A, choline, and long-chain polyunsaturated fatty acids (LCPUFAs) (Georgieff, 2007). Each of these has generated a research literature examining relationships with cognition/brain, but these will not be reviewed here where the main concern is the role of neuroimaging in nutrition studies. It is interesting to note, however, that Dobbing (1981) has suggested that all nutrient deficiencies exert their influence through a common pathway of growth restriction of the brain in some form.

THE HISTORY OF NUTRITION STUDIES

The study of how nutrition early in life might affect cognition is a long-standing area of research but the form that such investigations take has changed dramatically over time. Initially, the emphasis was on determining minimal nutrient requirements and preventing deficiencies, usually in developing countries and often in areas of severe malnutrition (Lucas et al., 2001). These observational studies reported associations between malnutrition and cognitive behavior but their interpretation was confounded by factors such as poverty, poor parental education, and low levels of environmental stimulation that in themselves could affect cognitive development, making interpretation of the role of nutrition difficult.

There was a clear need for RCTs to establish the causative role of nutrition. Although direct extrapolation to humans was not appropriate, the animal literature was suggestive. Smart (1986), for example, reviewed 165 studies, mainly in rodents, examining the effects of early under-nutrition on subsequent cognitive behavior such as maze learning. RCTs of nutrition in early human life began to appear around 30 years ago. The most frequently used supplement in these trials has probably been LCPUFAs and systematic reviews of studies in both term (Simmer et al., 2011) and preterm infants (Schulzke et al., 2011) have recently appeared. Breastfeeding has also generated a lot of interest and while it is not possible to randomly allocate infants to breastfeeding, Kramer carried out a RCT (PROBIT) that assigned mothers to groups that received either extra encouragement to extend the duration of breast feeding or the standard level of advice (Kramer et al., 2008). Lucas carried out pioneering studies with random assignment to either high or low nutrient diets in terms of protein/calorie content in preterm infants (Lucas et al., 1984); Morgane et al. (2002) describe protein as the most critical component of dietary intake for the development of neurological function. This large cohort has been followed up in a series of studies examining the effects of early diet not only on cognition (Lucas et al., 1990, 1998; Isaacs et al., 2009), but also growth (Lucas et al., 1984), cardiovascular function (Singhal, 2006) and bone health (Fewtrell et al., 2009). These RCTs marked a shift in the context of early nutrition research to include studies set in developed, industrialized societies consuming a typical Western diet where lack of calories is not often a problem.

The demonstration of these cognitive effects raised the possibility that nutrition might be affecting the underlying neural substrates either in terms of structure or function. Again, the animal literature was suggestive. Early nutrition was reported to have effects both at the whole brain and cellular level: cortical thickness (Katz et al., 1982), number of neurons (Fish and Winick, 1969),

myelination (Dobbing, 1964), and dendritic development (Salas et al., 1974; Cordero et al., 1986) for example. These discoveries were made possible, however, by methods not available for use in living human subjects. Some limited information was provided by human *post-mortem* studies; Benítez-Bribiesca et al. (1999), for example, reported dendritic spine pathology in the brains of infants severely malnourished in early post-natal life. Electroencephalography (EEG) provided some *in vivo* information; breast-fed infants weaned between 4 and 6 months, to either a control or LCPUFA supplemented formula, demonstrated more mature visual evoked potential (VEP) acuity when tested at 1 year if they were in the supplemented group (Hoffman et al., 2003). It was only with the advent of neuroimaging, however, allowing examination of the brain *in vivo*, that these questions could be widely addressed in human subjects. We are entering a new era of research methodology where we can examine the effects of early nutrition at both the behavioral and neural levels.

While neuroimaging held the promise of demonstrating changes in the brain attributable to nutrition, the advent of MRI scans did not allow this initially because of the nature of the effects expected to occur. The introduction of post-natal feeding, even in very preterm infants, does not occur before a gestational age (GA) of 23/24 weeks. By this time, neuronal proliferation and migration are reaching an end and nutritional intervention, therefore, is unlikely to affect these processes. Instead, the subsequent developmental events of synaptogenesis, glial cell proliferation, and myelination are vulnerable. This means that anomalies in brain structure associated with early nutrition are unlikely to be frank lesions (because no tissue has been destroyed) but rather, "quantitative deficits and distortions" (Dobbing, 1985). These more subtle morphometric anomalies occurring at the level of neuronal organization are not detectable by visual inspection of the scans, the usual clinical method of interpretation. Dobbing (1981) pointed out that these diffuse deficits, of serious consequence to society as a whole, would remain difficult (and maybe impossible) to demonstrate in humans. This skepticism was only assuaged when powerful post scan-acquisition processing techniques became available, providing a robust method for investigating subtle differences in the living brain.

THE USE OF NEUROIMAGING TO STUDY THE EFFECTS OF EARLY NUTRITION

When used in well-designed studies, neuroimaging may be a useful tool in helping to elucidate the effects of dietary intake. For example, breastfeeding in infancy has been shown to be associated with better cognitive outcome later in life, (e.g., Anderson et al., 1999; Drane and Logemann, 2000), although the evidence is not unanimous (Der et al., 2006). It would be informative if we could determine what changes in underlying brain structure were associated with these cognitive differences, with possible practical consequences in the production of infant formula.

While offering the prospect for studying hypotheses generated by both the animal and human literatures, there are certain constraints when using neuroimaging with infants and children that do not apply in adult studies. Although MRI studies are acceptable for use in a pediatric population, because they are non-invasive and do not involve the use of ionizing radiation, scanning demands a degree of immobility in order to avoid movement artifacts and this may not be possible in young children. While sedation is an option in clinical situations, this is not so when scans are being obtained for research. In practice, this restricts the ages at which children can be studied. In the new born and for the first few months after birth, MRI scanning can be carried out during natural sleep following feeding ("feed and wrap") but then becomes more difficult until the age of four or five. Various techniques such as pretraining in a mock-up scanner and/or playing audio books/music or showing cartoons can be used to try to lower the age of viability but, in reality, MRI does not become a truly reliable option again until early school age. It is unfortunate that the use of MRI is at its most difficult at the very time when the brain is developing most rapidly and when we expect nutrition to be exerting maximum effects. Other imaging techniques, discussed below, can provide useful information during this "silent" period and cognitive/behavioral measures may be obtained. Because of the above restriction, published studies tend to look at long-term effects associated with infant diet; one might argue that effects that persist are of greatest importance anyway.

The most usual form of post-natal nutritional study is the experimental intervention trial when groups differing in the amount or type of nutrient given as a supplement at one time point are then compared on outcome measures at one or more subsequent times. The most easily interpreted of such studies are RCTs and some such studies with both cognitive and MRI outcomes have started to appear in the literature (see below). Observational trials where, for example, we obtain scans and look at relationships with current diet, are also possible but are less informative with regard to causation, especially since nutrition is associated with a wide variety of other variables such as socio-economic status. Two large collaborative studies funded by the European Union (EARNEST and NUTRIMENTHE: www.project-earlynutrtion.eu and www.nutrimenthe.eu) have followed up a large number of experimental studies (but with no original intention to conduct long-term follow-up) and several of these have included neuroimaging (White et al., 2013) with results due to appear in the near future.

NEUROIMAGING METHODS

Although most neuroimaging studies of early nutrition reported so far have used structural (or anatomical) MRI, the only method that is ruled out in children for research purposes is positron emission spectroscopy, because of the radiation involved (although it has been used in clinical situations). A description of the basic methods is given below in case these are not familiar to nutritionists. [For more detailed information see Paus (2010) or ILSI, if published]. A good review of more recent developments in both scanning protocols and analytic techniques is given by Vasung et al. (2013).

STRUCTURAL MRI

The choice of MRI method depends on the hypothesis being investigated, as well as the age of the subjects. The main distinction is between "structural/anatomical" scans that image the brain anatomy resulting from genetic and environmental factors

up to the point of scanning, and "functional" scans (fMRI) that reflect present physiology, by measuring changing oxygenation levels in the cerebral blood supply. If our interest is in long-term effects of infant diet, then we are more likely to collect structural scans, although early diet could also influence the way the brain processes information as reflected in fMRI. Short-term studies, such as those looking at the immediate effects of breakfasts varying in macronutrient content, are more likely to measure concurrent functional effects.

There are also choices to be made within these categories since there are different MRI imaging protocols available; the choice will depend on what method of data analysis is to be used post-acquisition, and that, in turn, is partly determined by the research hypothesis. In the study of morphometry and volumetrics, the most widely used structural protocol results in a 3D data set of T1 weighted images. MR imaging involves aligning the protons in the nuclei by placing them in a magnetic field and then perturbing that field and, hence, the protons; T1 refers to one aspect of the time (relaxation time) it takes for the nuclei to return to their initial aligned state. These scans are particularly suitable for anatomical analyses because they provide sharp contrast between gray and white matter. Once the scans are acquired, a wide variety of analysis techniques is available. Automated methods such as Freesurfer (Fischl et al., 2002) provide measurements of the volumes of anatomical structures and of other features such as the thickness of the cortex (Fischl and Dale, 2000) or the degree of cortical gyrification in newborns (Dubois et al., 2008). These quantitative data can then be analysed statistically to determine differences between groups or can be correlated with other measures such as cognitive outcomes. Another widely-used technique is voxel-based morphometry (VBM; Ashburner and Friston, 2002), designed to determine local differences in the distribution of gray and white matter in the brain between groups, such as those with different early dietary interventions, which can be compared to determine if there are areas of the brain where they differ in tissue type distribution; correlations between gray or white matter and other measures (e.g., nutrient measures) can be examined.

A more recently developed structural protocol is diffusion tensor imaging (DTI), used to examine aspects of the microstructure of white matter (primarily), often not apparent on standard MRI, by measuring the diffusivity of water molecules in the brain tissue. The brain is anisotropic and water flow is constrained by anatomical features such as the degree of myelination of the axons. Metrics sensitive to various aspects of diffusion are produced; fractional anisotropy (FA), for example, reflects the directionality of water flow, and FA maps can be analysed in similar ways to T1 scans, using VBM to look for differences between groups of scans. DTI can be used to measure both the degree of myelination and also density in unmyelinated nerve fibers. Because nutrition is likely to affect both dendrites and axons, known collectively as neurites, a recently-developed diffusion MRI technique called Neurite Orientation Dispersion and Density Imaging (NODDI; Zhang et al., 2012) may be well-suited for use in early nutrition studies. Another popular voxel-based method of analysis is tract-based spatial statistics (TBSS; Smith et al., 2006), part of the FMRIB Software Library (FSL: www.fmrib.ox.ac.uk). This method creates white matter "skeletons" consisting of voxels common to all scans within a group and then compares these between groups. DTI data can also be used in tractography studies (Feigl et al., 2013) that visually represent the neural tracts and networks underlying functional connectivity by using 3D modeling techniques.

Magnetic Resonance Spectroscopy (MRS) is also based on structural scans but the emphasis here is on measuring concentrations of metabolites in the brain (a wide variety but commonly including aspartate, choline, creatine, glucose, and taurine). It is a non-invasive method for studying the metabolism of tissue *in vivo* (Gadian, 1995). In this MR application, the radiofrequency pulse in the magnetic field picks up signals from different metabolites depending on the nuclei under study. Signals are obtained from a defined region of interest and chemical spectra produced. Changes in the metabolic concentrations measured by these spectra occur over the lifespan and data can be compared with known population values at different stages of life. It has been widely used clinically (e.g., in the study of epilepsy) but, although it would seem to be of particular relevance to nutrition (for example, in studies of phospholipids in neuronal membranes), studies are sparse. Sizonenko et al. (2006) used MRS to measure creatine using MRS in babies with *in utero* growth restriction (IUGR), and showed marked increases at term compared to control infants.

FUNCTIONAL MRI

Functional studies make use of the fact that brain activity is linked to changes in cerebral blood flow and the level of oxygenation in the blood. Areas of the brain that are active require oxygen and, as a consequence, blood flow increases to these areas; the blood oxygenation level falls as neural activity continues. fMRI uses the different magnetic properties of oxygenated and deoxygenated blood to image areas of brain activity. A study by Akitsuki et al. (2011), although not conducted in children, shows how this method could be used to study the relationship between elements of nutrition and cognitive function in younger populations. Six young adult males took part in a repeated measure, counterbalanced crossover study that used fMRI to study how the nutritional quality of breakfast affected cognitive function and the brain. A nutritionally balanced breakfast produced higher brain activation than either water or a sugar/water mixture in the medial aspect of the prefrontal cortex during the performance of a working memory task. This illustrates how short-term responses to nutrition might be measured but it is also possible that early changes in brain structure due to nutrition may lead to differences in how cognitive tasks are carried out in the long-term.

Originally, fMRI studies involved measuring the brain's response to some external stimulus designed to activate different regions, as in the Akitsuki et al study (2011). More recently, fMRI imaging has been used to acquire images in the absence of external stimulation (resting state) in order to detect brain regions that are highly correlated with each other temporally, described as the brain's default network. This resting fMRI is potentially a useful technique in young children (Fransson et al., 2011; Gao et al., 2011) because they are not required to pay attention nor cooperate in carrying out cognitive tasks. Resting state functional connectivity studies (Knickmeyer et al., 2008; Gao et al.,

2009), along with DTI tractography, used in conjunction with new methods of data analysis such as graph theory (Bullmore and Sporns, 2009), are all consistent with current conceptions of brain function as a network where connections between neural areas are of importance (e.g., Jung and Haier, 2007), rather than the older emphasis on modularity of structure. No studies have yet been done in children but early diet might well be seen to impact the nature of the resting state default network and its development.

OTHER METHODS

One of the original techniques for studying brain function is EEG, used to measure the electrical activity of the brain from electrodes placed on the surface of the head. While temporal resolution is good (<1 ms), providing a direct measurement of brain activity, spatial resolution is less so. Magnetoencephalography (MEG) is a more recent development that measures brain electromagnetic activity. Both are non-invasive and easily repeatable as well as being less sensitive to movement than MRI, making them suitable for use with even quite young children. They can be used to record brain activity while carrying out tasks, or measure responses to environmental stimulation (event-related potentials—ERPs), sometimes using paradigms that do not require the child to actively attend (e.g., oddball tasks). They are suitable across the life span for measuring how early intervention might have affected brain processing and also for studying the immediate effects of nutrient intakes (such as caffeine in adults).

Near Infrared Spectroscopy (NIRS), sometimes called optical imaging, is another method suitable for use in children. Like fMRI, it makes use of hemodynamics, but the energy source in this technique is near-infrared laser or LED light shone through the skull. The light is diffused through the upper layer of the cortex and is then absorbed by optodes positioned on the skull. Oxygenated and deoxygenated hemoglobin have different light absorption properties and NIRS makes use of this information to produce measures that correspond strongly to the fMRI BOLD signal (Blood Oxygenation Level-Dependent; see below). Like EEG, temporal resolution is superior to spatial. It has many of the advantages of EEG for use with children although, to date, it has been used mainly in a clinical context. Its advantages, along with recent improvements in the metrics it produces, indicate that it could be a useful tool in the study of early nutrition's effects on the brain and cognition but studies have yet to be reported.

EARLY NUTRITION STUDIES THAT HAVE USED NEUROIMAGING TECHNIQUES

If we eliminate studies in clinical populations, such as anorexics, relatively few using neuroimaging to examine the effects of early diet have so far been reported in the literature, but that can be expected to change in the future. Although the ultimate aim would be to define dietary intake for optimal brain/cognitive development, the research is still at the "proof of principle" stage.

The most widely used technique has been structural MRI. MRI showed reduced intracranial and cortical gray volumes in infants with IUGR (Borradori Tolsa et al., 2004) compared to preterm infants matched for GA but with appropriate intrauterine growth; there was also a correlation between cortical gray matter volume and a behavioral developmental measure of attention. Scans were obtained soon after preterm birth and again at term equivalent age and it is interesting to note that the IUGR infants showed no catch-up growth in total intracranial and cortical gray matter volumes in this time period (reminiscent of McCance's rats) despite the rapid brain development that is occurring. Lodygensky et al. (2008) used MRI to obtain volume measurements of the hippocampus and demonstrated a reduction in IUGR infants at term age compared to matched non-IUGR infants. In this case, the hippocampal measurements were related to all six domains of a preterm behavioral assessment. Sizonenko et al. (2006), in a study using MRS, reported that babies with IUGR, reflecting deficient prenatal nutrition, showed marked increases in creatine at term compared to control infants. These results clearly demonstrate alterations in brain tissue volumes, both total and regional, and in metabolite concentration in infants who have been growth restricted *in utero*. While nutritional deficiencies no doubt play a role in the genesis of the effects on brain tissue, it is likely that other factors in the stressful intrauterine environment that these infants inhabit are also implicated; it is difficult to disentangle these. At present, preterm infants have a limit of viability (50% survival rate) of around 23–24 weeks GA so postnatal nutrition will always be introduced during the second major phase of brain growth, resulting in differences mainly in white matter. IUGR, however, can occur during the first phase and this may be why we tend to see gray matter differences, rather than white, in such infants.

Several neuroimaging studies have been carried out in adolescents who were members of the original preterm cohort RCT of infant feeding reported by Lucas et al. (1984). All infants were randomly assigned to either a high or low nutrient diet for the duration of their hospital stay. The low nutrient diet was either banked breast milk from donors or the formula that was in standard use at the time, while the high nutrient diet was an enriched formula prepared for the study that contained extra protein and calories (for full details of formulas see Lucas et al., 1998). Mothers were asked if they planned to breastfeed or not. If not, their infants received their randomized diet as their sole feed (100%). If mothers chose to breastfeed, then the assigned formula was used only if supplementary feeding was required; depending on the success of breastfeeding, the percentage of expressed maternal breast milk in the infant's diet could range from 0 to 100% of the total intake (%EBM). Follow-up studies to examine cognitive development took place at 18 months, using the original Bayley Scales of Infant Development (BSID; Bayley, 1969; Lucas et al., 1990) and 7.5–8 years when IQ was measured using the Wechsler Intelligence Scale for Children-Revised UK edition (WISC-R, UK; Wechsler, 1974) (Lucas et al., 1998). In both studies, there were differences in favor of the group that had received the high-nutrient diet. The effects were more marked in boys [as in animals (Smart, 1986)] and, at 7.5–8 years, Verbal IQ (VIQ) showed a greater difference between groups than did Performance IQ (PIQ), a differential often seen in nutrition studies. These cognitive effects have been shown to extend into adolescence (Isaacs et al., 2009) and formed the basis for the generic hypothesis that the impact of early nutrition on IQ would be related to selective growth restriction of specific brain structures. A more focused hypothesis was suggested by the findings of Abernethy

et al. (2004) who showed that growth restriction in the caudate nucleus was associated with lowered IQ in preterm subjects; we speculated that nutritional effects might be implicated. The participants chosen to take part in the imaging studies had all been born at or below 30 weeks GA, were neurologically normal (history/examination at 7.5–8 years) and were all attending mainstream schools. The period between their preterm birth and term birth at 40 weeks, a time when the major brain spurt is occurring), was spent *ex utero* in these infants; this exposure to environmental influences, at an early stage of brain development, might be expected to increase their vulnerability to dietary effects.

Full IQ testing was carried out on 76 adolescents (mean age = 15y9m) of whom 38 each had received a high or low-nutrient formula as preterm infants. Conventional multi-slice imaging, as well as MPRAGE volume acquisition, was obtained on a 1.5T Siemens Vision system. Freesurfer was used to obtain volumes for total brain volume (TBV) as well as a series of subcortical gray matter structures including the caudate nucleus and hippocampus. Consistent with prior results, the high nutrient group had significantly higher VIQ than the low-nutrient group (Isaacs et al., 2008). In addition, the mean volumes for all structures were higher in the high-nutrient group but only significantly so for the left and right caudate nuclei. (TBV and age at scan were controlled in these analyses). Further, both caudate nuclei showed strong relationships to VIQ (and not PIQ). Because of gender differences in the cognitive data, preplanned gender analyses were carried out that found that the effect of diet on caudate volume was gender-specific, shown only by males, and that caudate volume was associated with VIQ in males but not in females. While some nutrition studies have reported gender differences, many have not examined this factor, so that the pervasiveness of effects is largely unknown. If we accept that prenatal under-nutrition may be seen as a form of stress, then the animal research in rats demonstrating that the male brain is more vulnerable to the effects of stress becomes relevant (Sizonenko et al., 2006; Zuena et al., 2008; Weinstock, 2011). The finding also reinforces the recommendation by the US National Academy of Sciences that gender should be considered in the design and analysis of studies in all areas and at all levels of biomedical research (Wizemann and Pardue, 2001). While the study by Isaacs et al. (2008) demonstrated relationships between early diet and both brain structure and cognition, it is important not to over-interpret this to mean that the caudate is the only neural structure affected by early nutrition; other aspects of brain structure not examined here may also be vulnerable.

A second study in the same cohort investigated the relationships among breast feeding, neural structure and cognition in a group of 50 adolescents (Isaacs et al., 2010), children of mothers who had chosen to breastfeed and whose EBM% intake, therefore, varied between 0 and 100% (see above). We correlated %EBM with brain volumes and IQ scores; note that in this study there is no comparison of groups but relationships amongst neural structures, a cognitive variable and a dietary variable are explored with correlation. We know that gray and white matter volumes (GMV; WMV) follow different trajectories of development (Toga et al., 2006; Groeschel et al., 2010) and so absolute volumes of these tissues vary by age. Correlation coefficients, however, are independent of absolute values and as long as the ranks of the individuals' volume and EBM% measurements remain constant, the correlation is unchanged. %EBM was correlated significantly with TBV and WMV, left and right, but was not associated with GMV. When TBV was held constant by covarying it (i.e., determining if differences would be found in specific volumes if TBV was the same for everybody, i.e., relative rather than absolute volumes), we found that %EBM was correlated significantly and positively with WMV and significantly but negatively with GMV. We found the same pattern in boys and girls considered separately as in the group as a whole, although correlations between %EBM and WMV were larger in boys than in girls. In this group of preterm infants it appears that increasing the percentage of breast milk in the early diet increases the amount of white matter relative to gray. How is this related to cognitive function? Overall, there was a significant correlation between %EBM and VIQ only. Here, however, boys and girls showed divergent patterns: all three IQ measures [VIQ, PIQ, and FS (Full-Scale) IQ] were significantly related to %EBM in boys but not in girls. There were no differences in IQ scores between boys and girls but it seems that the percentage of breast milk in the infant diet may affect IQ in boys differently than in girls. Finally, we considered the relationship between the neural volumes and IQ scores. The group as a whole showed no relationship between the IQ measures and TBV but both VIQ and FSIQ were related significantly to WMV. In boys, VIQ was related to TBV and, along with FSIQ, to WBV. There were no significant relationships in girls and no correlations with GMV in any group. This finding is in contrast to results reported by Kafouri et al. (2013) who found that duration of exclusive breastfeeding correlated with gray matter rather than white. Their measure of gray matter, however, was cortical thickness in contrast to cortical volume as in the study here and this, along with a different metric for measuring breast milk consumption, may help to explain the difference. The most likely explanation seems to be related to the timing of the nutrition event, since the infants were born at term rather than preterm when different developmental processes are occurring in the brain.

An interesting study by Tan et al. (2008) used a different approach. They point out that children born preterm are not only at risk of later developmental difficulties in attention, literacy, numeracy, and motor coordination, but are also smaller and lighter with lower head circumference compared to their peers. Head circumference is relevant because relationships between it and both TBV and IQ have been reported (Deary, 2012). The aim of the study was to examine the relationships among developmental outcomes, protein/energy intakes during the first 4 weeks of life, and post-natal growth up to term age, using quantitative MRI at 40 weeks GA to examine brain structure. The study took the form of a RCT of hyper-alimentation (diet containing macronutrients above the recommended values) in which 142 infants born before 29 weeks' gestation were randomly assigned to receive either standard or enhanced parenteral/enteral nutrition between weeks 1 and 4. At term age of 40 weeks they were scanned using the "feed and wrap" method ($n = 65$) on a 0.5T scanner and quantitative data were then extracted from the scans. At 3 and 9 months post-term, the infants' neurodevelopment was measured using the BSID-II; 81 were assessed at 3 months and 71 at 9 months. There were no differences between diet

groups in the incidence of gross abnormalities seen on MRI nor in TBV and cortical brain volume or in T2 relaxation times, a MRI measure that is known to decrease with maturation. There were, however, significant correlations between TBV and both energy intake (positive) and energy deficit, defined as the difference between actual and recommended daily intakes (negative), across the pooled groups. CBV and T2 times correlated with a measure of head circumference and both volumes correlated with weight and height at 9 months but with neither birth weight nor head circumference. Analyses of the BSID-II data showed that the mental index (MDI) was correlated with length, TBV, and T2 relaxation time after adjustment for confounding factors. The motor index (PDI) was significantly related to weight. Therefore, while neither the growth nor the MRI measures differed after the dietary intervention, important relationships among growth, neurodevelopment, and brain measures were shown across the whole group. The authors conclude by saying that reducing the energy deficit by improving early nutrition in preterms may improve the growth and maturation of the brain. They also suggest that quantitative MRI measures have the potential to predict both mental and motor outcomes.

The above studies looked at outcome at some future point after early intervention. A study by Taki et al. (2010), however, considered brain structure in terms of concurrent diet in a group of healthy children; although not "infant" diet, this study is included in view of the paucity of studies as an illustration of how neuroimaging can be incorporated into studies of nutrition. Given findings of the positive effects of eating breakfast on cognitive function in children (Benton and Jarvis, 2007), the authors looked at the relationships among breakfast staple type (in terms of glycaemic index—GI), GMV and WMV in the brain and IQ in a group of 290 healthy 5–18 year olds. Participants were divided into three groups depending on their habitual breakfast diet: (1) the "rice" group ate boiled white rice, (2) the "bread" group ate white bread, (3) the "both" group ate one or the other on different days. T1 weighted images were acquired on a 3T scanner, and then analysed using VBM. They confirmed their hypothesis that the "rice" group (low GI) would have greater gray matter than the higher GI "bread" group by demonstrating a significantly higher gray matter ratio (GMV divided by intracranial volume) in the former. This group also had significantly larger gray matter volumes in several regions, such as the left superior temporal gyrus (corrected for intracranial volume and other possible confounding factors), The "bread" group had larger white and gray matter volumes in several regions, including the right fronto-parietal region. Cognitively, the "rice" group had a significantly higher Perceptual Organization score (an Index score from the IQ test). The authors conclude that breakfast staple type affects GMV and WMV and cognitive function (possibly because of the GI differences), and that an optimally nutritious diet is important for brain maturation during childhood and adolescence. Correlational studies, of course, report associations between variables and it must be borne in mind that these do not imply causation.

The oldest existing method for studying brain activity directly, EEG, has also been used in several studies of early nutrition. A study by Li et al. (2010) used ERPs and behavioral measures to study how early diet might influence CNS activity. Data were obtained from 130 healthy full-term infants who were divided into three dietary groups: breastfed ($n = 40$); milk-based formula ($n = 51$); soy-based formula ($n = 39$). Measures were obtained at both 3 and 6 months of age; infants had been on the same diet since they were at least 2 months old and until at least 6 months of age. Behavioral development was assessed at both time points using the BSID and feeding information was collected from 3-day feeding records obtained monthly. ERP responses to language sounds, described as a brain measure of information processing, were collected and measures of ERP amplitude and latency were derived. In terms of behavioral development, all three groups of healthy term-born infants were, perhaps not surprisingly, within the normal range. Differences between diet groups were described by the authors as marginal and transient, but suggested some behavioral advantage for the breastfed group early in development. Females generally improved their scores across the study period more than did males. Although significant group differences were found in the ERP measures, the effect sizes were small. Latency effects in the breastfed group were associated with better developmental scores at 6 months, suggesting that neural maturation might have been more advanced at this point. No differences between the two formula types were found on development or brain activity in this study.

Finally, another EEG study investigated the effects of eating breakfast on the efficiency of the neural networks engaged during mental arithmetic tasks in children aged 8–11 years (Pivik et al., 2012). The authors point out that there is a general consensus that a network of both frontal and parietal brain regions is activated when calculation tasks are being carried out. While many factors influence the ability of an individual to carry out these tasks, they express surprise that nutritional status at the time of assessment is not generally considered as one of them. Mental calculation, for example, has been shown to be sensitive to variations in nutrient intake, such as glucose, as well as the interaction of glucose with macronutrients such as proteins and fats (Dye et al., 2000). The study group consisted of 116 healthy children who had all fasted overnight while staying at the research facility prior to completing the experimental protocol the following day. On awakening, all children completed a battery of cognitive tasks, including mental calculation, and were then randomly assigned to either a breakfast or fasting group; the former consumed a standardized breakfast while the latter continued to fast. The children then repeated the cognitive battery; EEG recordings from frontal and parietal electrode placements were made during both testing sessions. Summarizing, the results showed that the efficiency of the neural networks engaged during mental calculation tasks was enhanced in the group who had eaten breakfast compared to the fasting group; those who continued fasting had to expend greater mental effort in undertaking the tasks. Furthermore, this neural activity was associated with a higher number of correct responses on the mental calculation task.

We are clearly in the very early stages of studying the effects of nutrition on brain variables using neuroimaging but the above examples give some indication of what might be done. Perhaps they are best thought of as proof-of-principle papers at the beginning of a research route that aims eventually to document

the characteristics of a diet optimal to the brain/cognitive development of individuals.

CONSIDERATIONS

In view of the findings from the animal literature, as well as what we know about the effects of environmental influences during human brain development, the results so far obtained from neuroimaging studies in nutrition are biologically plausible. Conceptually, it seems fruitful to consider nutrition as an early programming stimulus for the brain and, hence, cognition, much as McCance (1962) did for growth.

Demonstrating causal effects of early nutrition, rather than just associations, depends on obtaining data from randomized, controlled trials. These are easier to conduct in older children and adults where permission can be obtained to randomly assign the participants to different conditions, e.g., nutritional supplements or meals that vary in macronutrient composition. Even here, however, RCT data may not be straightforward to obtain. There are some interesting data concerning cognitive behavior and high-fat diets in rodents (Greenwood and Winocur, 1990; Winocur and Greenwood, 1999), for example, but it would not be possible to randomly assign human subjects to a high-fat diet that could have adverse consequences for health. The difficulties multiply when trying to carry out studies of early diet in infants and young children, where randomly assigning infants to different dietary groups is unethical in most circumstances. Even when a large RCT such as that of Lucas et al. (1984) is conducted, long-term follow-up is subject to high rates of attrition, making interpretation difficult (for discussion of this problem see Fewtrell et al., 2008). The additional constraints imposed when collecting neuroimaging data in young children necessarily restrict the number of studies that can be carried out and will remain a difficulty with this sort of research, although future imaging methods and protocols may make this more viable. Careful study design may also get around these difficulties. The PROBIT study by Kramer et al. (2008) that looked for effects of breastfeeding on cognition by comparing groups randomized to breast feeding encouragement vs. standard care (resulting in greater duration in the former) suggests ways that information (including neuroimaging) can be obtained.

The timing of the stimulus in programming studies is crucially important. The infants in the Lucas cohort were fed their assigned diet only for the duration of their stay in hospital, (a mean of 4 weeks). After discharge, the infants were fed according to the parents' choice. This may seem a short period to have brought about marked effects in brain structure but there are many examples in the animal world where much shorter periods of stimulation, provided that they occur during vulnerable stages of development, cause permanent change. Perhaps the best known example is imprinting, first described by Spalding and elaborated by Lorenz. Birds show life-long following behavior to the first moving visual stimulus they are exposed to during a brief critical period shortly after hatching, adaptive because it is usually the mother. A single dose of phenobarbital administered to a neonatal rat has lifetime effects on the activity of p450 cytochrome mono-oxygenase activity (Bagley and Hayes, 1983) if administered during a short critical period, but the same dose only induces a period of drowsiness at any other time. Exposure to valproic acid in rats has been shown to cause maximum later autistic-like alterations in social behavior if administered on embryonic day 12 (Kim et al., 2011). The term "critical periods" was used to describe these times with strict temporal boundaries during which specific effects on various aspects of animal behavior were observed. In fact, further research indicated that these boundaries were not as invariant as first thought (Michel and Tyler, 2005) In the case of humans, with a protracted period of brain development and complex behaviors, we are unlikely to see such rigidly defined periods and so the use of the term "sensitive period" seems more appropriate (Johnson, 2005). For example, early squint can affect life-long vision by resulting in conditions like amblyopia (Adams and Sloper, 2003) but the period during which the visual areas of the brain are sensitive extends from birth to around 2 years of age but with no strict upper limit. We do not yet know the parameters of the sensitive, period for dietary effects on brain/cognition in humans; this remains to be empirically determined. Defining this period is of extreme importance, however, especially for infant feeding policy, and needs to be prioritized in nutrition research but the difficulties in obtaining ethical approval in those who are normal and healthy have already been pointed out. Developments in neuroimaging technology are occurring all the time and new methods may become available in the future that will solve some of these problems.

The issue of timing is closely related to the course of brain development. As mentioned earlier, the sequence of steps in brain development is invariant but the timing may differ between regions so that one process may be at its peak in one area of the brain while another is at its peak in a different region (Huttenlocher and Dabholkar, 1997). Two outcomes follow from these principles: (1) the *same* stimulus applied at different times (e.g., nutrition at varying gestational ages) might lead to different brain effects because the neural areas at peak development are not the same, (2) *different* stimuli applied at the same time might have the same effect on the brain. Another important timing principle is that many developmental processes in the brain have the opportunity of occurring only at certain chronologically defined times (Dobbing, 1985), and if one process fails to develop optimally, later processes may be affected. If the opportunity for optimal development is missed, then it does not come again and any effects can be permanent, altering the trajectories of both brain and cognitive development. A study by Banich et al. (1990) looked at the cognitive development of children who had suffered damage to one hemisphere of the brain before birth resulting in congenital hemiplegia. Their IQ scores kept pace with healthy controls up to the age of 6 years but from then on, although their cognitive scores continued to increase, they did so at a slower rate than in controls, so that their developmental trajectories diverged. Interestingly, the same did not apply in children in whom the onset of hemiplegia occurred in post-natal life. It might be that the effects of non-optimal nutrition, during a limited period, have similar effects on subsequent development; neuroimaging studies, particularly longitudinal, could be used to delineate the brain regions involved.

The emergence of two types of specific effects of early diet on subsequent brain structure/cognition should be noted as areas

for future research. The first is the differences found between girls and boys, where it appears that the brain's vulnerability to nutritional influences, at least in some cases, differs by gander. There are many references in the non-imaging nutrition literature to gender effects, indicating that they should be examined more thoroughly. It is recommended that all future studies be powered to conduct gender group analyses, so that important effects are not cancelled out by looking only at total groups. The second specific effect is that on the verbal aspects of cognitive function (reflected by the VIQ) rather than the non-verbal (PIQ), again widely reported in the nutrition literature (Lucas et al., 1998; Horwood et al., 2001). This is often assumed to be due to cultural factors since these are known to be correlated with intelligence test scores, particularly VIQ. A study by Edmonds et al. (2010), however, indicates that this might be too easy an explanation. They found that the differences in birth weight (reflecting prenatal nutrition) between members of monozygotic twin pairs (twins derived from a single ovum and known as "identical") were related to difference in their VIQ scores but not PIQ, with a research design that controls, of course, for potentially confounding factors such as socio-economic status, parental IQ, level of parental education, and genetic makeup.

Timing differences may contribute to explaining these specific effects of nutrition. If the timetable of early brain development were different in boys and girls, then the application of a stimulus (nutrition) might have an effect in the group in which a structure was still developing but not in the group where this period had passed. Various aspects of neural development have been shown to differ between males and females both in infancy (Vasileiadis et al., 2009; van Kooij et al., 2011) and later (deBellis et al., 2001; Sowell et al., 2002). Determining this depends on having detailed information about prenatal and early post-natal brain development in boys and girls separately but such data are surprisingly difficult to find. What is needed are detailed descriptions of brain development such as those of Chi et al. (1977), at the level of individual sulci and gyri, but with separate data for boys and girls. Similarly, the specific effects on VIQ might occur if the neural substrates of PIQ were developed very early or very rapidly compared to those subserving VIQ—the latter would remain vulnerable for a longer time and at a different period. Interaction between these two processes could produce differences between genders in specific aspects of cognition.

We pointed out that gender-specific effects could be masked if only the total group is considered, leading to a potentially erroneous conclusion that there was no demonstrable effect of nutrition. A similar situation might arise if only a sub-group of the population is deficient in the nutrient under study and responds to supplementation; a large effect of nutrition in this group could be masked by no effect in the non-responders. These issues are discussed by Benton and Buts (1990) with regard to studies of the effects of vitamin/mineral supplementation on intelligence. Having measures of the status of the individual with regard to the nutrient under study both before and after supplementation (e.g., plasma LCPUFA levels from a blood sample) enables us to determine whether the supplementation worked, and also look at sub-groups in terms of original status to see if the neural effects differ.

Most nutrition studies have focused on two main time periods in the lifespan, infancy/early childhood and old age. The emphasis has been different in both, optimal conditions for development in early life and protection against decline in the elderly, but both have in common that they are times of change in the brain when it is seen as more vulnerable to intervention. Despite the fact that the changes that become apparent in old age may be the culmination of processes that have been occurring throughout the lifespan, this has often been treated as a discrete epoch. Recent findings indicate that another important period of change in brain development, although not as dramatic as the original brain spurt, occurs in adolescence (Ment et al., 2009; Ramsden et al., 2011). This is also an important time nutritionally as adolescents start to make food choices for themselves, independent of the home environment. Since neuroimaging at this time is subject to fewer restraints than in childhood, it is suggested that studies in this age group might be a particularly fruitful focus for research. Nutrition is probably best seen as having an influence on brain and behavior throughout life (Gomez-Panilla, 2008) but with some periods of enhanced vulnerability when the brain is undergoing rapid change.

With the advent of the non-invasive imaging techniques described here, our knowledge of how the young brain develops in both normal and abnormal conditions has increased. Vasung et al. (2013) describe what they consider to be the three major aspects of this structural development. One is the series of dynamic changes that take place in the thickness of the cortex over development. Shaw et al. (2008), for example, describe both thickening and thinning of the cortex over time and demonstrate different thickness patterns in groups with varying IQ; those with superior IQ at young adulthood had thinner cortices than those in other groups (a useful warning that interpreting brain findings must not be based on naïve assumptions such as bigger always means better). Second is the progressive folding of the cortex that takes place, resulting in complex patterns of gyri and sulci. The third aspect is the establishment of patterns of connectivity between brain regions that underlie functional networks, both intrinsic (resting state fMRI) and those that develop with experience. Individual differences in cognition may be related to individual differences in these dimensions of brain structure. Studies focused on how early nutrition affects these different aspects of development would be of great interest.

Neuroimaging studies can add substantially to our body of knowledge about the early effects of nutrition but they must be used cautiously and interpreted with care. The novelty of neuroimaging has led to a plethora of studies outside the field of nutrition, including some whose conclusions seem ill-founded. It cannot be stressed enough that the most complex neuroimaging techniques available are no substitute for good research design—it is all too easy to be dazzled by the technology. Even the oldest methods are still relatively new and there is continuing discussion about what how some of the measures, in wide use, should be interpreted, e.g., the BOLD signal in fMRI (Logothetis and Wandell, 2004). Some have questioned the validity of post-acquisition analysis such as VBM (Bookstein, 2001). There are statistical considerations with voxel-based methods entailing as they do very large numbers of computations, making adequate

correction essential. Although automated procedures have generally replaced manual ones for volumetric measurements, these should not be used uncritically since they are sometimes prone to error. Not all of the neuroimaging measures used have been adequately validated as nutritional markers. And, as pointed out above, interpretation of the brain findings must not be based on naïve assumptions.

Relating cognitive outcomes to the brain findings adds another dimension of possible error. Although there has been a tendency in the past for nutrition studies to measure only IQ, this is short-sighted and may miss important effects. IQ is a complex, composite measure and the same overall values can be arrived at by very different combinations of underlying sub-test scores. In addition, groups with the same IQ score may show marked differences in specific cognitive abilities. Too often, it is concluded that nutrition has no effects because the IQ scores between groups do not differ but specific cognitive effects cannot emerge if they are not measured. A study that looks at the effect of one nutrient can only come to selective conclusions regarding the effects of that particular nutrient on the brain/cognition. In the breastfeeding study described above, for example, it would be incorrect to conclude that the female brain is not vulnerable to the effects of nutrition; we can conclude that the effects of breast milk on the brain are more pronounced in boys and that their breast milk intake is related to IQ in a way not seen in females, but studying another nutrient might lead to gender-specific effects in females.. Negative brain findings are sometimes attributed to the fact that the imaging methods available at present are not sensitive enough to demonstrate effects; this may be true but sometimes the failure to demonstrate effects means that there are . . . no effects.

The number of studies so far has been small largely because many centers have not had both nutrition and scanning data, but this is changing and a significant number of reports are expected in the near future. The advent of neuroimaging has allowed us to answer questions that would have been difficult to pose even relatively recently. As long as we employ neuroimaging sensibly and responsibly we should expect many more insights into the effects of early diet on the brain and cognition to emerge.

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