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# IMPACT OF ENTERIC INFECTIONS ON COGNITIVE DEVELOPMENT: FIELD AND ANIMAL STUDIES OF PROTECTION BY ApoE4

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## SUMMARY

Although diarrhoeal diseases in early childhood remain important causes of mortality, the long-term impact of enteric infections, with or without overt diarrhoea, may impair child growth and development. Repeated dehydrating or malnourishing infections can compound other causes of mortality and may also have lifelong consequences for those who survive. This includes a cognitive decrement that may average up to 10 IQ points by 7-9 years of age that can be attributed to diarrhoeal illnesses in the first 2 years of life alone. The cognitive function most affected is semantic, rather than phonemic, fluency, a deficit also seen in early Alzheimer's (vs. Parkinson's) dementia. These effects may be lifelong and may have selected for surprising "survival" or "thrift" genetic alleles like ApoE4 that we have shown to *protect* against the cognitive deficits seen in children with heavy diarrhoea burdens as well as providing protection against the growth and histopathologic impact of enteric infections in a murine model of malnutrition and infection.

In this overview, we review these long-term growth and cognitive effects of early childhood diarrhoea, the Alzheimer-like deficits seen and the surprising protection provided by the ApoE4 allele that increases Alzheimer risk in later life, a new example of "antagonistic pleotropy". We also show this protection in our murine model of cryptosporidiosis using targeted transgenic mice with the human E4 allele and how the molecular mechanism of this benefit can lead to novel interventions to break the vicious cycle of diarrhoea and malnutrition and their devastating consequences for child development.

## BETTER COGNITION AND IQ CORRELATE WITH FEWER INFECTIONS

Over 200 million of the world's children less than 5 years of age fail to achieve their developmental potential, are stunted and live in poverty. These children do poorly in school and go on to have low incomes, high fertility and provide poor care for their children, contributing to what Dr. Grantham-McGregor has called "intergenerational transmission of poverty" (*Grantham-*

*McGregor* et al., 2007). Eppig and colleagues recently suggested that the "Flynn Effect" of improving IQ with development correlates with decreasing "infectious diseases burden", even when controlling for GDP per capita, education, temperature and malnutrition; i.e. the recognized correlation of nutritional deficiencies with IQ is lost when the effects of infectious diseases are removed which they interpret as suggesting that the malnutrition-IQ link appears to occur through infectious diseases (*Eppig* et al., 2010). Whether there is an effect of infections on cognitive development that is independent of their effects on malnutrition is less clear. Although this is a controversial area, our data on diarrhoeal illness correlations also appear to be independent of stunting (<1HAZ) (which is itself also correlated with diarrhoea in the first 2 years of life) (*Pinkerton* et al., 2012).

## INFECTION OR STUNTING IMPAIRS COGNITION

Convincing support for the effects of infection on child development came from albendazole trials in Kenya and Jamaica, suggesting that intestinal helminths impair growth and cognitive development. These were the basis of our studies of heavy early childhood diarrhoea burdens in Fortaleza. Stephenson and colleagues reported that, among Kenyan schoolchildren, even a single dose of albendazole showed a benefit in fitness, appetite and weight and height gains within 2 to 4 months when compared with double blinded placebo-treated controls (*Stephenson* et al., 1989, 1990, 1993). Similarly, in studies of schoolchildren in Jamaica, *Nokes* et al. (1992) showed improved fluency (long term memory and retrieval) and digit span backwards/forwards (from WISC, involves attention and distractibility) 2 months after a 3-day course of albendazole (vs. placebo). Since HAZ-2 may be a surrogate for early childhood diarrhoea burdens (*Dillingham* and *Guerrant*, 2004), it may also be relevant that Chang and co-workers described better arithmetic scores at 10 years old with higher HAZ-2 (height for age Z scores at 2 years old) (*Chang* et al., 2011). Although these brief anti-helminthic treatments did not necessarily eradicate intestinal geohelminths (and certainly did not prevent common re-infections), the 2-3 log reduction in major geohelminths provide impressive evidence for the importance of heavy intestinal nematode infections in the physical and cognitive development of schoolchildren. Our own data from Northeast Brazil also suggests that geohelminth infections in early childhood (i.e. from birth to 2 years old) have important, lasting effects on subsequent child growth and development (*Moore* et al., 2001). We and others are now exploring whether stunted children may also be at greater risk for later obesity, diabetes and metabolic syndrome in what we have called a 'collision of bad water with bad diets' (*DeBoer* et al., 2012).

The importance of stunting on cognitive development is clear from several studies (*Grantham-McGregor*, 1995). In studies of nonverbal intelligence at 8-11 years old in the Philippines, Mendez and Adair's group has shown that moderate to severe stunting (i.e. HAZ-2 <-2) at 2 years is associated with a 0.25 to 0.61 standard deviation lower mean cognitive test Z score by age 8 ($p<0.001$) (*Mendez* and *Adair*, 1999). They also noted that stunting (i.e. progressing from -1.5 to -3.3 HAZ) at 2 years correlated with progressive delays in age at starting school from 5 to 8+ years, and that, even

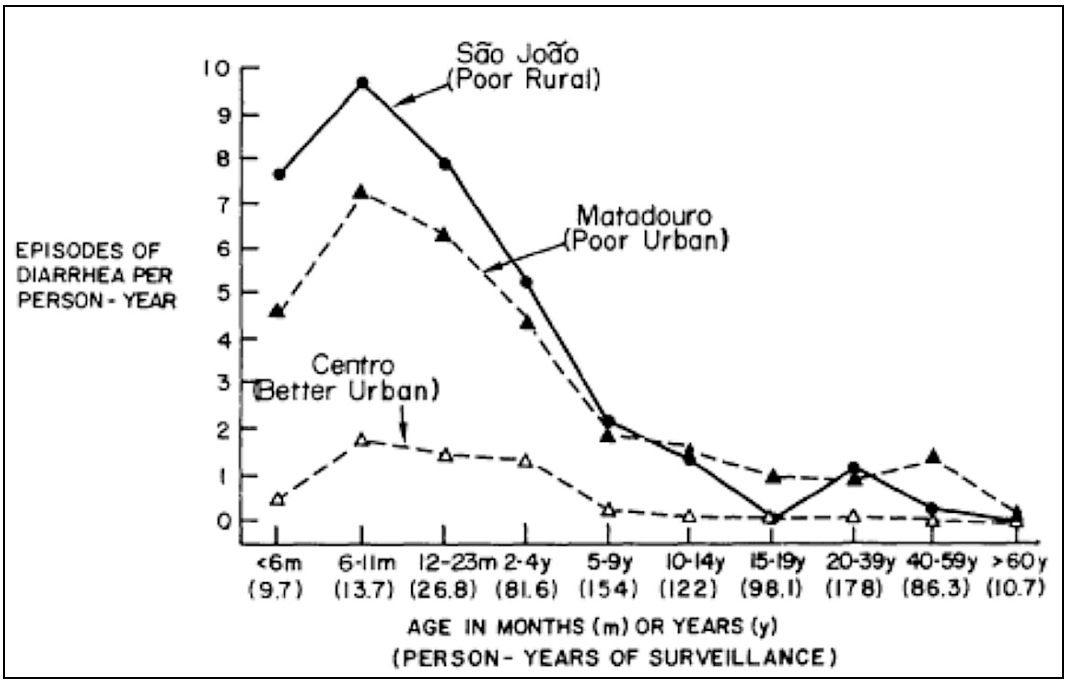



**Figure 1**: Age specific diarrhoea attack rates in rural and urban communities (Pacatuba and Goncalves Dias) in Northeast Brazil (Guerrant et al., 1983; Lima et al., 2000).

though schooling (achieving 2-6 years of education) improves mean cognitive test scores, there remains a 30% decrement in the stunted children at 11 years; i.e. stunting limits what education can accomplish!

## THE CRITICAL 4-24 MONTH AGE "WINDOW" FOR CHILD DEVELOPMENT

Leonardo Mata, in studying The Children of Santa Maria Cauque (*Mata*, 1978) described how even impoverished children often start off on their growth curves reasonably well, only to fall progressively off with the onset of diarrhoeal and other common early childhood infections over the critical first 2 years of life. Precisely the same pattern of fall-off in linear growth (i.e. HAZ) occurs worldwide, with remarkably similar findings in Asia, Africa and Latin America described by Victora and Shrimpton (*Victora* et al., 2010) in 2010, much as they had shown nearly a decade earlier in 2001 (*Shrimpton* et al., 2001).

Feeding studies also confirm the importance of early childhood (3 years) in cognitive development. For example, in the two Guatemalan villages given an "atole" vegetable-protein supplement (163kcal with 11.5g protein) vs. "fresco" sugar sweetened beverage (59kcal and no protein) in two control villages in INCAP studies from 1969-1977. A follow-up 25-35 years later showed that those in the supplemented villages had a 10% better IQ, even after, adjusting for schooling (*Stein* et al., 2008), and that the 25-42 year old males had 46% higher wages, with the females having 8-20% higher reading, Raven scores and schooling if they had

been in the supplemented villages compared with the non-supplemented villages some 40 years before. However these benefits were seen only in those who had been in the supplement program in their first 2-3 years of life, showing the crucial timing of early life in cognitive development (*Hoddinott* et al., 2008).

We also have found increasingly impressive correlations in our Fortaleza studies of early childhood diarrhoea (ECD) that heavy diarrhoea burdens in the first two years of life are associated with impairments in cognitive function (TONI, WISC-III coding and digit span and WRAML mazes) several years later (*Guerrant* et al., 1999; *Niehaus* et al., 2002). Furthermore, these heavy diarrhoea burdens in the first 2 years of life also correlate with impaired schooling (both age at starting school and age-four-grade) several years later (*Lorntz* et al. 2006). Early heavy diarrhoea burdens are also associated with stunting at 2 years of age in our as well as in other multi-country studies (*Moore* et al. 2001; *Checkley* et al., 2008) and, as noted above, stunting at the second birthday (HAZ-2) is clearly associated with impaired cognition as assessed later in life. Although some suggest that the effects of diarrhoea on cognition may be only through its effects on stunting, as noted above, like Eppig, we find that the association of diarrhoea with cognitive impairment remains even when controlling for anthropometry. Thus heavy early childhood diarrhoea burdens clearly associate with lasting effects on stunted growth, and, in turn on impaired cognition. Whether the cognitive impact of early childhood diarrhoea is independent of (i.e. even greater than) the also significant effects of diarrhoea on stunting remains controversial. Nevertheless, the huge effects of early childhood diarrhoea on child growth and development are critical to recognize and ameliorate.

The reasons for the first 2 years of life being so critical are likely at least two-fold: first is the obvious vulnerability to the heaviest burdens of diarrhoea and enteric infection (with rates clearly highest in that age range) (*Guerrant* et al., 1983; *Lima* et al., 2000; *Fischer Walker* et al., 2012) (Figure 1) Second is the rapid development of brain growth and synaptogenesis and myelination in humans in this window from birth to 2 years. Unlike some other species in which brain development occurs predominantly *in utero*, human infants are born with small brains and sparse synapses relative to their near adult levels of brain weight-for-height and even synaptic density by 2 years of life (*Dobbing* and *Sands*, 1973; *Corel*, 1975; *Rice* and *Barone*, 2000; *Thompson* and *Nelson*, 2001) (Figure 2). Not only does the human brain double in size in the first year of life, by 3 years of age it reaches 80% of its adult volume (The Urban Child Institute: Baby's brain begins now, Conception to age 3. URL: www.theurbanchildinstitute.org/why-0-3/baby-and-brain, 2012; Nowakowski, 2006). Furthermore, synapse formation occurs predominantly between birth and 2-3 years of age (by which time the human brain has nearly 200% of its adult number of synapses); after which "blooming and pruning" gradually eliminate nearly half of these synapses throughout childhood and adolescence (*Corel*, 1975; *Huttenlocher*, 2002). These anatomic observations are even more apparent when one considers the impressive incapacity of the human new-born infant compared with the remarkable appearance of motor and cognitive skills including walking, running, talking and personality which develop by the child's second birthday.

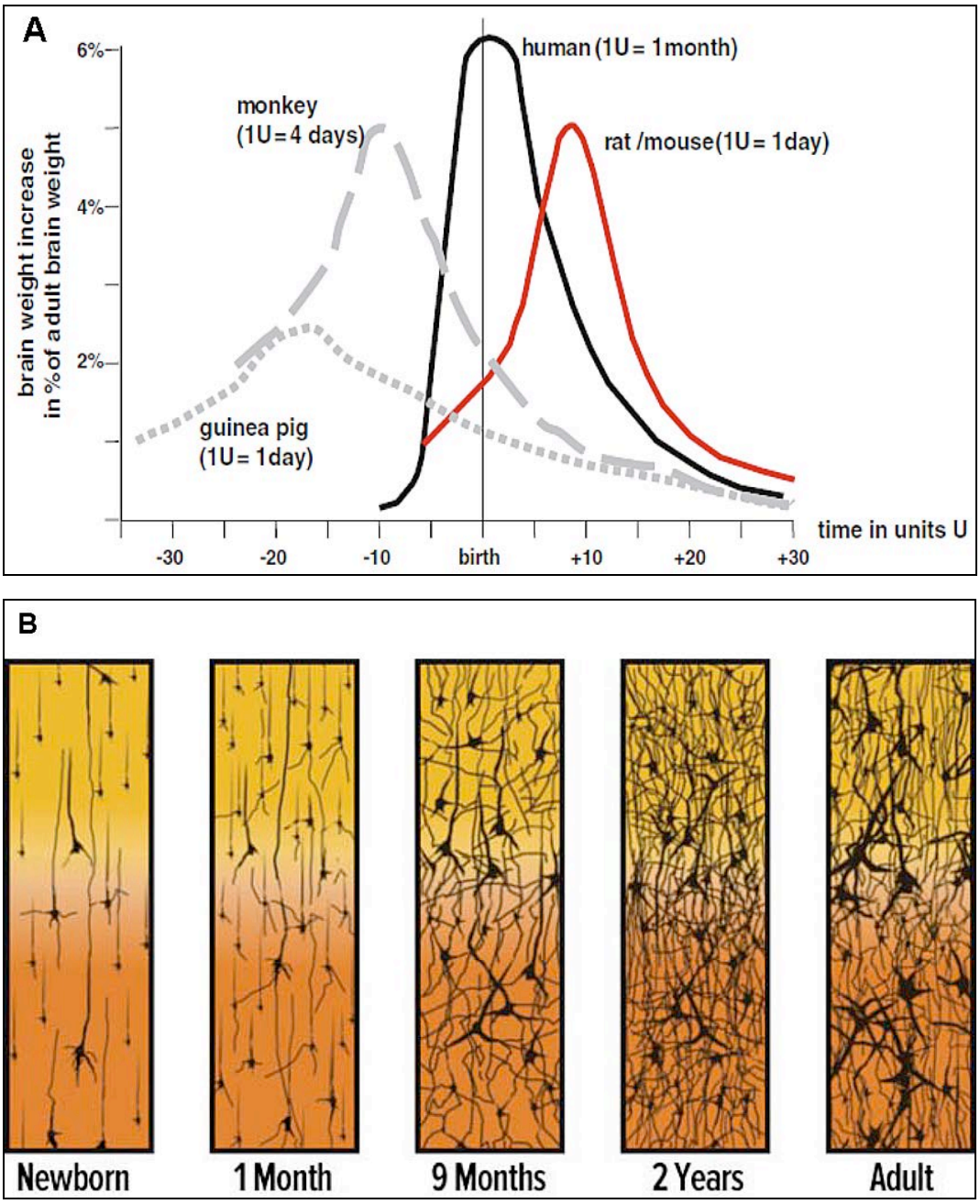
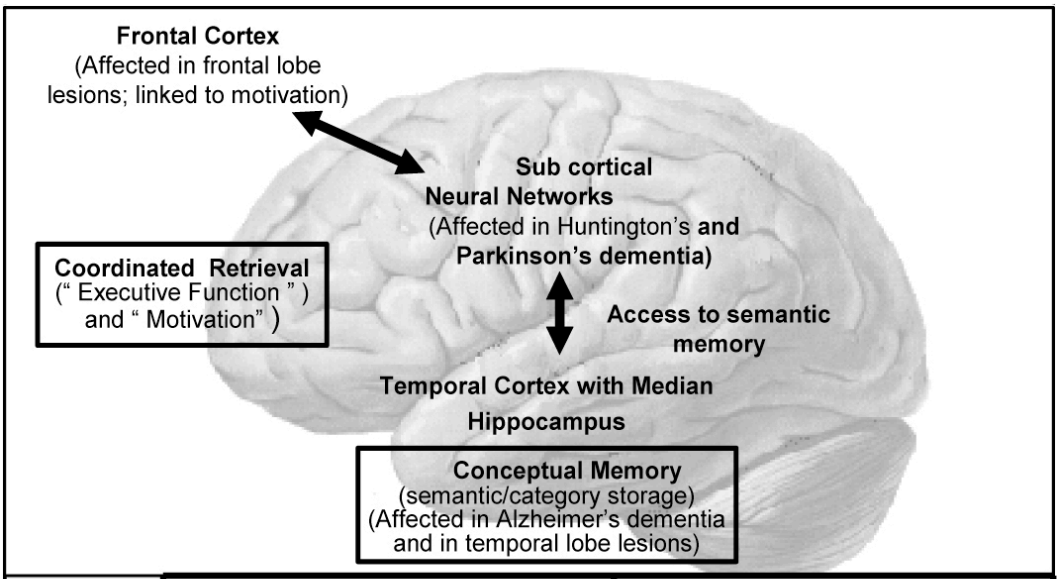



**Figure 2**: Age of brain development in humans and other animals, showing that most human brain development (by A: weight or by B: synapse formation) occur in the first 2 years of life. Adapted respectively from *Dobbing* and *Sands* (1973) and from *Corel* (1975).




| Function | Coordinated Retrieval ("Executive Function" and Motivation") | Conceptual Memory (Long-term semantic/category storage) |
|---|---|---|
| Site | Frontal Cortex<br>Sub cortical Networks | Temporal Cortex<br>(with median Hippocampus) |
| Main Diseases | Huntington's/Parkinson's diseases<br>Frontal lobe or sub cortical lesions | Alzheimer's disease<br>Temporal lobe lesions<br>***? Early Childhood Diarrhea*** |
| Test(s) | Phonemic and semantic performance | Semantic performance only |

**Figure 3**: Brain regional development and function.

## EARLY CHILDHOOD DIARRHOEA AND GIARDIASIS IMPAIR SEMANTIC FLUENCY (LIKE ALZHEIMER'S DISEASE) AND ApoE4 PROTECTS

When we examined the specific areas most affected by early childhood diarrhoea, it was striking that these were predominantly functions similar to those lost in early Alzheimer's disease. These include higher executive function and semantic (vs. phonetic) fluency (*Patrick* et al., 2005; *Oria* et al. 2009), with brain regions that likely differ from the predominant ones involved in phonemic fluency or in Parkinson's dementia (Figure 3).

Because of the semantic fluency predominance of the deficits, we examined the ApoE4 allele frequencies and found, to our initial surprise, that ApoE4 was protective of the cognitive function and against diarrhoea, the former only in the children with heavy diarrhoea burdens (*Oria* et al., 2005, 2007, 2010). Some early investigations into this link are beginning to support a host benefit of ApoE4 against enteric protozoa. The positive correlation

between *Giardia lamblia* and diarrhoea ($p<0.01$) in ApoE4 negative children in our study was lost in the presence of an ApoE4 allele ($p=0.53$) (*Oria* et al., 2005). Moreover, the ApoE4 allele appears to be protective against cognitive impairments due to early *Giardia* infections in Egypt (*Yahya* et al., 2009a, 2009b), although these findings should be further confirmed with larger numbers and better control for confounding factors. The association of potential protection against symptoms with this parasite by the host ApoE4 allele may be related to the *Giardia*'s requirement to obtain host cholesterol for its own growth (since *Giardia* is unable to synthesize cholesterol) thus needing to divert host cholesterol to the parasite from the intestinal milieu, which may deleteriously affect the developing brain (*Oria* et al., 2007). These effects may require an evolutionarily conserved LDL-receptor pathway, which is found to be down-regulated in ApoE4 carriers (*Mahley* and *Rall*, 2000). Recently, a putative *Giardia lamblia* low-density lipoprotein receptor-related protein (GlLRP), a type I membrane protein, which shares the substrate N-terminal binding domain and an FXNPXY-type endocytic motif with human LRPs, was identified (*Mahley* and *Rall*, 2000; *Rivero* et al., 2011).

In addition, Colton and colleagues (*Colton* et al., 2001; *Czapiga* and *Colton*, 2003) have shown that ApoE4 increases NO production in microglial macrophages by stimulating arginine uptake via an arginine-selective cationic amino acid transporter (CAT1). Hence we examined the ability of arginine to enhance parasite killing with *Cryptosporidium* infections in our murine model (*Castro* et al., 2012) and also the ability of targeted transgenic C57Bl6 mice with the human ApoE4 allele to resist cryptosporidial infections or their growth impairment in our murine model. We found that arginine is the most effective "anticryptosporidial drug" we have encountered in our murine model, with a 10-fold reduction in the number of parasites per milligram of intestinal tissue. These effects were only partially blocked by L-NAME (NG-nitro-arginine methyl ester) (*Castro* et al. 2012) or by BEC (S-(2-boronoethyl)-L-cysteine) (*Castro* et al., unpublished data), suggesting that the protection from arginine was through both the iNOS and the arginase pathways to kill the parasite and repair epithelial cell injury respectively. Furthermore, in studies that are being submitted for publication elsewhere, C57BK6J ApoEko mice expressing the human ApoE4/4 gene under murine ApoE promoter lost less weight, had better villus-to-crypt ratios and shed fewer parasites than ApoE 3/3 target replacement and wild-type mice, suggesting that understanding how ApoE4 may be protective can open novel approaches to better controlling protozoal infections. Interestingly, supporting the concept that ApoE4 may improve innate immunity against enteric pathogens, data from the Tsimane population in lowland Bolivia, (an indigenous forager-farmer population living under conditions resembling pre-industrial European populations with high infectious morbidity, high infection and inflammation, and shortened life expectancy) when bearing ApoE4 show lower serum C-reactive protein levels (*Vasunilashorn* et al., 2011), suggesting that ApoE4 bearers had lower rates of environmental-related infections.

Our data support that ApoE4 behaves as antagonistic pleiotropy, where the presence of ApoE4 in our gene pool was associated with increased needs for fat energy storage and improved innate immunity adaptations against enteric

infections in times where those were critical for human survival in the pre-industrialized era. However this once beneficial gene becomes potentially quite detrimental in the presence of longer life expectancy, reduced physical exertion and westernized diets. There are other postulates and literature evidence for an antagonistic pleiotropy described for ApoE4 during human early development and in adulthood (*Prentice* et al., 2005; *Alexander* et al., 2007; *Finch* and *Morgan*, 2007; *Beeri* et al., 2009; *Chang* et al., 2011).

## EFFECTS OF MALNUTRITION AND OF MICRONUTRIENTS ON BRAIN ANATOMY AND FUNCTION

Especially vulnerable in the first two years of life in humans (analogous to the first few weeks of life in rodent models) is the postnatal brain plasticity, particularly in dynamic hippocampal, neocortical and cerebellar regions (*Frankova* and *Barnes*, 1968; *Rice* and *Barone*, 2000). It is these areas that are potentially altered by nutrients or micronutrients in critical developmental windows (*Georgieff*, 2007; *Pinero* et al., 2001; *de Souza* et al., 2011). Hence we examined the effects of malnutrition and of zinc and glutamine therapy in our murine model of litter clustering induced malnutrition. There we found that malnourished mice (with breast-milk restriction by increased litter size) had growth impairment and deficits in early post-natal behaviour ontogeny, associated with reduced serum and brain zinc levels. In addition, we found reduced hipocampal GABA levels and reductions in hippocampal synaptophysin (as a marker for synaptic density) expression, associated with malnutrition. Furthermore, malnutrition-induced CA-1 neuronal hypertrophy was found with litter size clustering, likely related to CA-1 cell death changes. All effects improved with glutamine and/or zinc treatment (Ladd et al., 2010).

We next examined the effects of zinc, vitamin A and glutamine supplementation on the growth and cognitive responses in 213 undernourished children from the favela in Fortaleza and found that lower vitamin A and glutamine levels were associated with disrupted intestinal barrier function (by lactulose/mannitol absorption ratios). There was also a significant correlation between vitamin A supplementation of apolipoprotein E4(+) children and improved lactulose/mannitol absorption ratios. In addition, only the ApoE4 positive children (37/213, 13.9%) who received glutamine supplementation (10 day glutamine treatment either with or without zinc or vitamin A supplementation) showed significant positive Pearson correlations between the changes in height-for-age z-scores over four months with delayed verbal learning scores, along with correlated changes over the same period in weight-for-age z-scores and weight-for-height z-scores that were associated with non-verbal intelligence quotients (*Mitter* et al., 2012).

## CONCLUSIONS

In conclusion, we find profound and lasting effects of early childhood enteric infections and diarrhoea on children's growth and cognitive development. While these "vicious cycles of poverty" are only now being dissected

at the levels of molecular causality, genetic predispositions and potential novel interventions can already be designed that hold promise for interrupting these vicious cycles of diarrhoea, stunting, cognitive impairment and poverty.

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