The Relationship between Nutritional Status and Allocentric Orientation in 31-36-Months-Old Toddlers

Ilaria Lisi
MRes Cognitive Neuroscience
Donders Institute, Radboud University

Abstract

Poor dietary quality and obesity have been linked to impaired cognitive abilities, including spatial orientation. Spatial orientation can be achieved either via egocentric orientation (using one's own body as the primary reference frame) or allocentric orientation (using spatial relationship between objects as the primary reference frame). Allocentric orientation is a more advanced skill than egocentric orientation. Previous studies show that 30-month-old toddlers are unable to use allocentric orientation, while 35-month-old toddlers are. Here, we investigate differences in allocentric orientation abilities in a sample (N=23) of 31-36-month-old toddlers using a previously established virtual reality paradigm. We then related allocentric orientation abilities to nutritional status, measured as a compound score of Body Mass Index (BMI) and dietary quality. A repeated measures ANOVA with age and nutritional status as covariates and trial type (egocentric vs allocentric) as the within-subjects factor, revealed no significant main effects of age or nutritional status on task performance. Albeit the lack of significance of these effects, their associated effect size was of medium magnitude, indicating that significance may not have been reached due to the small sample (N=23). Follow-up exploratory analyses indicated that allocentric orientation abilities increase with age. Nutritional status was also strongly, albeit non-significantly, positively related to allocentric task performance. This relation was mostly driven by dietary quality, rather than by BMI. Given the small sample size and the lack of significance of the results found, the trends found should be treated as exploratory and should be replicated by future, better powered studies.

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Introduction

Unhealthy dietary habits are strongly related to the development of noncommunicable diseases such as diabetes and cancer (World Health Organization, 2019). Globally, there has been a surge in the consumption of highly caloric, nutrient poor, foods especially in children (World Health Organization, 2019). This dietary pattern, characterized by high consumption of red and processed meat, sugar-sweetened beverages, fried and refined food, is often referred to as 'Western Diet' (WD) and is one of the main contributors to the current global obesity epidemic (World Health Organization, 2019). The consumption of a WD and its often-associated high Body Mass Index (BMI) have been shown to pose a threat to brain health. Indeed, these factors have been linked to mental illness (e.g. Fiedorowicz et al., 2016; Scott et al., 2008), brain structure changes (Jacka et al., 2015; Val-laillet et al., 2009) and, most relevantly, to impaired *cognitive functions* (e.g. Dahl et al., 2009; Francis and Richard, 2011; Kanoski et al., 2007).

Given the detrimental consequences of a suboptimal diet, the increase in obesity and WD consumption in children under 5 is particularly alarming (World Health Organization, 2019). The importance of proper nutrition for cognitive function, combined with research showing that the developing brain is particularly susceptible to dietary insults (Boitard et al., 2014), highlights the importance of investigating the link between nutritional status and the development of cognitive abilities in children under the age of 5. Here, we investigate the relationship between spatial cognition, dietary quality, and BMI in 31-36-months-old toddlers.

Nutrition and Spatial Cognition

Nutrition impacts brain function and structure through the microbiota-gut-brain axis, a multifaceted bidirectional communication system between the microbial gut and the brain involving endocrine, immunological and neural signaling (Rhee et al., 2009). Several mechanisms are involved in the microbiota-gut-brain axis (for a review see: Cryan et al., 2019), such as neuroinflammation.

Obesity (Odegaard and Chawla, 2013) and poor nutrition (Minihane et al., 2015) have been shown to lead to a low-level chronic inflammation state. Sustained peripheral inflammation can then lead to neural inflammation by inducing cytokine release in the brain (Dantzer et al., 2008) and disrupting the structural integrity of the blood brain barrier (BBB) (Kanoski and Davidson, 2011).

The hippocampus is particularly sensitive to neuroinflammation derived from poor nutrition (Kanoski and Davidson, 2011). Consumption of a WD has been shown, in rodents, to lead to neurophysiological changes in the hippocampus, including reduced brain derived neurotropic factor, increased neuroinflammation, and structural BBB changes (Kanoski and Davidson, 2011). Moreover, rodents fed a high-fat diet show impairments in hippocampal long-term potentiation, key cellular basis of learning and memory (Karimi et al., 2013; Stranahan et al., 2008). In humans, a high BMI in midlife is a risk factor for hippocampal atrophy in late life (Jagust et al., 2005; Raji et al., 2010).Given the susceptibility of the hippocampus to dietary changes, it is not surprising that the cognitive abilities impaired in cases of obesity and poor nutrition are often hippocampal dependent.

Hippocampal dysfunction in animals is often assessed via spatial learning tasks, with the most widely used being the Morris Water Maze (MWM; Morris, 1984). In this task, the animal has to learn over several trials to use distal cues to orient itself inside a circular pool of water in order to find a hidden platform. Animals with hippocampal damage are severely impaired in learning and remembering the platform's position.

Increasing evidence shows that rodents fed high-fat-high-sugar (Stranahan et al., 2008), high-fat-high-carbohydrate (Yu et al., 2010) or high-sugar (Jurdak et al., 2008) diets have poorer MWM performance compared to animals fed a low-fat-high-complex-carbohydrate diet. Moreover, feeding rodents a WD has been shown to impair hippocampal-dependent place recognition after merely 5 days; one month of WD triggered large inflammatory changes in the hippocampus, strongly correlated to poor behavioral performance (Beilharz et al., 2014).

Interestingly, spatial cognition impairments in WD fed animals are observable before significant increases in body weight (Kanoski and Davidson, 2011). This suggests that differences in body weight are not necessary for the short-term cognitive impairments caused by WD consumption. This is of particular note for human studies investigating the relationship between diet and cognition. While BMI can be a more objective measure than self-reported dietary intake, it may not always be predictive, *in and of itself*, of diet-related changes in brain function. This may be one of the reasons why, depending on whether BMI or dietary quality is used as a measure of nutritional status, research on the effects of nutritional status on children's cognitive function yields inconsistent findings. Indeed, poor dietary quality has been consistently linked to impaired cognitive abilities, ranging from abstract reasoning to cognitive control (Cohen et al., 2016). On the other hand, while some studies have related a high BMI to an increased risk for attention deficit hyperactivity disorder (e.g. Lam and Yang, 2007) and to a decrease in academic effort (Ramaswamy et al., 2010), others have failed to find any association between BMI and children's cognitive abilities (Gunstad et al., 2008). Studies examining the relationship between nutritional status and brain function should take this into account when deciding which predictive variables to introduce in their research.

The alarming rate at which obesity and WD diet consumption are increasing in children may be particularly problematic given that childhood is a critical period of neurodevelopment and neuroplasticity, especially of the hippocampus (Spear, 2000). Indeed, a seminal animal study has reported that rodents consuming a high-fat diet for 8-10 weeks showed impairments in spatial navigation abilities, tested using the MWM, compared to their chow-fed littermates. The high-fat diet also lead to a significant increase in body weight and promoted increased hippocampal inflammation (Boitard et al., 2014). Importantly, the marked cognitive impairments and the increased inflammation were only visible in *juvenile* and not adult, rats. This shows that hippocampal-dependent abilities as spatial cognition are particularly sensitive to the influence of poor dietary quality during early development.

To the best of our knowledge, the relationship between diet, BMI and spatial cognition has not yet been investigated in toddlers.

Spatial Cognition in young children

Exceptions notwithstanding, successful spatial orientation and navigation can be supported by adopting two fundamental reference frames to represent location (Learmonth et al., 2001).

In an *egocentric* reference frame, locations are represented in relation to the observer (Bremner, 1978). Egocentric coding is employed either or both when observers are stationary and when they can use path integration, the ability to update and track movements by integrating optic flow, vestibular, and proprioceptive cues (van den Brink and Janzen, 2013). Twelve-month-old toddlers can rudimentarily use path integration and egocentric orientation for coding objects' locations (Schmuckler and Tsang-Tong, 2000). Egocentric orientation is mostly subserved by the precuneus, inferior and superior parietal cortices, and the frontal cortex (e.g.Committeri et al., 2004; Galati et al., 2000; Zaehle et al., 2007).

In an *allocentric* reference frame, locations are coded independently from the observer's position and are instead coded based on inter-objects relations (Bremner, 1978). Allocentric coding is often considered a more advanced orientation strategy, and it is mostly prevalent in later stages of development (e.g. see Fernandez-Baizan et al., 2019). Allocentric orientation starts being preferentially chosen as the main orientation strategy only during early school age (Bullens et al., 2010). Allocentric orientation is mostly subserved by the *hippocampus* (e.g. Zaehle et al., 2007), region in which neurons with allocentric properties have been found both in rats (Taube et al., 1990) and monkeys (Rolls and O' Mara, 1995). In humans, hippocampal maturity has been associated with increased allocentric memory abilities (Lambert et al., 2015).

During development, younger toddlers have been shown to primarily use egocentric-related spatial cues for orientation (i.e. path integration), while older toddlers seem to be able to also use allocentric information (e.g. see Fernandez-Baizan et al., 2019). In particular, Bremner and colleagues (1994) report an improvement in the efficiency of spatial cues use for orientation between the ages of 24 and 36 months.

Given the presented evidence of a sensitive period between 24 and 36 months for the development of spatial cues used for orientation (e.g. Bremner et al., 1994) a study from our group has investigated orientation abilities in 30-35 month old toddlers (van den Brink and Janzen, 2013). The study was a virtual reality (VR) paradigm, in which participants had to locate a target after a rotational transformation. VR paradigms have been shown provide sufficient visual information to prompt spatial updating processes following perspective changes (Wraga et al., 2004) even in toddlers as young as 6 months-old (Schmuckler and Jewell, 2007). Van den Brink and Janzen (2013) showed that, while 35-month-olds were able to use allocentric orientation to perform the task when needed, 30-month-olds children relied solely on egocentric coding.

Of note, individual differences in daily living skills (assessed through the Vineland-Screener questionnaire) predicted orientation success. Indeed, toddlers who were more independent relative to their peers had better performance on the task. Van den Brink's and Janzen's study (2013) shows that the developmental period between 30 and 35 months is highly sensitive for spatial cue use, and that younger children are not able to use allocentric cues, while older children are. Importantly, the results also highlight that on top of chronological age, individual differences contribute in determining successful use of visual cues for orientation.

The Current Study

To summarize, the hippocampus has a prominent role in spatial cognition and in the development of allocentric orientation abilities. Dietary quality and obesity have been shown to impact hippocampal-dependent abilities, especially during development (Boitard et al., 2012).

Here, we aimed at investigating the relationship between nutritional status and spatial abilities in toddlers (31 to 36 month-olds). Nutritional status was assessed as a compound score of dietary quality indicating adherence to the Dutch Healthy Diet Index (measured through a parent-directed Food Frequency Questionnaire; Lee et al., 2013) and of BMI. We used a modified version of Van den Brink and Janzen's (2013) task and investigated whether individual differences in nutritional status play a role in predicting toddlers' ability to use allocentric orientation. Differently from Van der Brink and Janzen's (2013) study we used age as a continuous rather than dichotomous variable for a more nuanced understanding of the role of age in spatial cognitive abilities.

The task included two trial types. In side congruent trials (SCon), above chance performance could be achieved employing either egocentric or allocentric orientation. As this trial type does not allow to infer the type of strategy used, it mainly constituted a sanity check to ensure participants understood task instructions and were not performing at random. In side incongruent trials (SInc), employing egocentric coding would result in below chance performance. Employing allocentric coding would result in above chance performance.

We hypothesized that, in accordance with Van den Brink and Janzen's (2013) results, ability to use allocentric coding would develop with age. Performance on SCon trials should thus not be related to age, while performance on SInc trials should increase with age from below to above chance. We also hypothesized nutritional status to be positively related to allocentric orientation abilities. Again, performance on SCon trials should not be related to nutritional status, while performance on SInc trials should increase from below chance to above chance as nutritional status increases.

As this was the first study to investigate the role of nutritional status in the development of spatial abilities in toddlers, we could not make a directional hypothesis regarding the interaction of age and nutritional status with certainty. If the interaction was significant, we would have two possibilities. It could be that as nutritional status increases so does performance on SInc trials, but that the slope of this relationship decreases as age increases. Thus, at later age points toddlers' task performance would not benefit by a good nutritional status as they have already developed allocentric orientation abilities; good nutritional status would be beneficial for the development of allocentric orientation at earlier age points. Alternatively, we could see the slope of the relationship to increase with age. This would indicate that poor nutrition delays the achievement of developmental milestones such as allocentric orientation abilities. Finally, it could be that the interaction is not significant, indicating that the age and nutritional status have additive effects on allocentric orientation abilities.

Methods

Participants

Twenty-three children took part in the experiment; participants were aged 31-36 months old (*M*: 33.61, *SD*:1.49) and 13 were female. We recruited participants via the Baby Research Centre (BRC) in Nijmegen, the Netherlands. Both over-the-phone and in-person screening were carried out to ensure participants fit with inclusion criteria (Appendix I). Parental signed informed consent was collected. All procedures were approved by the local Radboud University Nijmegen Ethics Committee for Behavioral Research (ECSW-2019-107R1). At completion of the experiment, parents were compensated 20 Euros for their child's participation. It should be noted that the desired number of participants (N=50) could not be reached as testing was interrupted due to COVID-19 restrictions.

Stimuli

The stimuli used in the present study were the same employed by van den Brink and Janzen (2013). However, here we did not include trials containing landmarks as they were shown in the original experiment to be distracting to participants

Stimuli consisted of 4 movies featuring a purple animated bird (target) appearing to the front of the screen, turning around and flying to hide in one of two identical trees. The trees were situated, at different distances, into one of four different types of 3D environments (Figure 1). All stimuli were designed via Blender, an open source animation suite (www.blender.org). Stimuli were presented using Presentation software (Neurobehavioral Systems, Inc.).

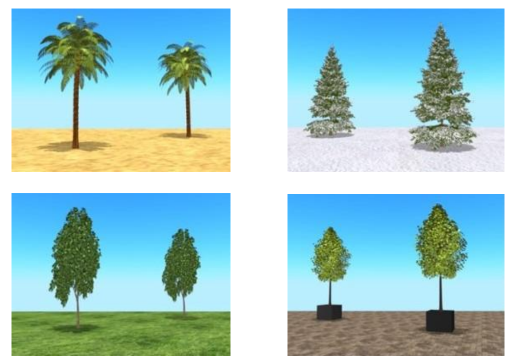

Fig. 1. **3D Environments** | Depicted are the four types of virtual 3D environments (beach, snow, park and square) used in the current study. (Images adapted from: van den Brink and Janzen, 2013)

Once the bird had hidden itself in the tree, a camera shift mimicked a self-motion path (duration: 4s). This led to a perspective change of 90°, to the left or the right of the center of the visual scene (see Figure 2). During the camera rotation, all objects in the environment temporarily disappeared from sight, preventing toddlers from keeping their eyes fixed on the target's hiding position. Position of the hiding tree (left/right and front/back) and turn (90° left/right) were fully counterbalanced. For further details regarding the camera movements please refer to the stimuli section in Van den Brink's and Janzen's (2013) paper. For an example of the experimental trials, the reader can refer to the online video provided in the supplementary material.

In total, the experiment contained 32 trials. In half of the trials, the tree in which the bird hid in was on the same side of the participant's body before and after the camera rotation; these were labeled Side Congruent trials (SCon, Figure 3a). Correct performance on SCon trials could be achieved either by egocentric or allocentric spatial coding. In the other half of the trials, (Side Incongruent; SInc, 3b), the final hiding position was on the opposite side of the participants' body compared to the hiding position before the camera turn. Correct performance on the SInc trials could only be achieved by allocentric spatial coding. Reliance on egocentric coding to respond on SInc trials would result in

Fig. 2. **Camera Path** | Depicted is an example of the camera path during a trial. The first panel shows the top view of the camera path, indicating camera angle and position at five points. The second and third panels show the initial and final position of the camera, resulting in a 90° turn to the left of the center of the visual scene. (Image and caption adapted from: van den Brink and Janzen, 2013)

below-chance performance. As SCon trials do not allow to distinguish the spatial orientation strategy used, they were primarily employed to check that participants understood task instructions and were not performing at random.

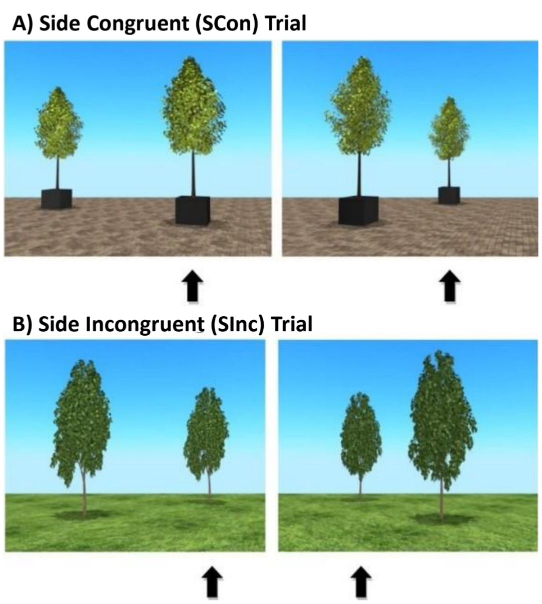

Fig. 3. **Trial Types** | The stills from the experimental movie depict the two trial types included in the present study. In Side Congruent trials (SCon, A) the initial and final target position are on the same side of the participant's body. In Side Incongruent trials (SInc, B), the initial and final target position are on the opposite side of the participant's body. Reliance on egocentric coding to respond on SInc trials results in below chance performance (Image and caption adapted from: van den Brink and Janzen, 2013)

Body Mass Index (BMI) Percentiles

As a measure of body fat, we calculated participants' BMIs. In children, BMI is calculated based on their height, weight, age in months and gender. Height and weight measurements were completed by the experimenters after the testing session. BMI was calculated using the Centers for Disease and Control Prevention's (CDC) BMI calculator for children (https://www.cdc.gov/healthyweight/bmi/calculator.html). The CDC calculator used expresses BMI as a percentile; indeed, given the variability of height and weight during growth, children's BMIs need to be compared to a reference population of the same age and gender. According to the Centers for Control Disease and Prevention, a toddler aged 31-36 months is considered underweight if he or she falls below the 5^{th} centile, overweight if he or she is between the 85^{th} and 95^{th} centile and obese if he or she is above the 95^{th} centile.

Dietary Quality- Dutch Healthy Diet adherence

To assess dietary quality, parents were asked to fill in the Food Frequency Questionnaire (FFQ; Lee et al., 2013). The FFQ assesses dietary type by querying the frequency of consumption of listed food items over a specified period of time (here, over the past month). Completion of the FFQ lasted approximately 25 minutes. Data from the FFQ was analyzed by Wageningen University, and yielded information about adherence to the Dutch Healthy Diet Index (Lee et al., 2013).

Dutch Healthy Diet (DHD) adherence scores can range between 0 and 100, with a higher score meaning better adherence to the Dutch guidelines for a healthy diet (Dutch Health Council, 2015). In particular, the FFQ assessed adherence to the Dutch dietary guidelines regarding the consumption of: cooked and raw vegetables ($\geqslant$ 75g/d), fresh fruit ($\geqslant$ 150g/d), whole grains ($\geqslant$ 90g/d), legumes ($\geqslant$ 4g/d), nuts and seeds ($\geqslant$ 15g/d), unsweetened dairy products ($\geqslant$ 300g/d), fatty and non-fatty fish ($\geqslant$ 7g/d), fats and oils ($\geqslant$ 30g/d), red and processed meat ($\leqslant$ 21g/d), sugary beverages ($\leqslant$ 150g/d), sugary spreads (max. once a day).

Below is an example of a question from the FFQ:

"In the past month, how often has your child eaten rusk, crispbread or crackers?"

A Never
B Once a month
C 2-3 times a month
D Once a week
E Twice a week
F 3 days a week
G 4 days a week
H 5 days a week
I 6 days a week
J 7 days a week

Socio-Economic Status (SES)

In order to control for possible confounding variables, we collected information on parental education which could range from 1 (Elementary School) to 5 (University). The highest of the two parents' education level was used as a proxy of SES.

Procedure

All procedures were carried out in accordance with the regulations of the Baby Research Center of the Radboud University (Nijmegen, The Netherlands). Parental informed consent was collected and exclusion criteria checked prior to starting the experiment. toddlers were seated on a high chair, or on their parents' lap depending on the child's preference, in front of a Hewlett-Packard 23 inch LCD touch screen monitor. A LG web cam was fixed on top of the monitor to record the child's face and eye movements for the purpose of excluding non-attended trials (on average 13.5% of all trials). Throughout the experiment, one of the parents was sitting on one side of the child and the experimenter on the other. Participants were informed that the game they were about to play involved a bird named Pico, which they watched fly and hide behind one of the two trees present on the screen. Upon hiding, a camera shift led to a change in perspective of 90° either to the left or the right of the center of the visual scene (see *Stimuli*). Thereafter the child had to indicate, by touching the monitor, which tree the bird was hidden in. Cartesian coordinates of the touch were recorded to store information about correct and incorrect responses.

Following the touch response, Pico would fly out of the correct tree providing the child with feedback on the correct hiding position. If the participant had chosen the correct tree, the bird chirped while flying toward the camera; otherwise, the bird made no sound. The first trial was an example trial completed by the experimenter to clarify the procedure to participants. In between each trial, as an incentive, children were allowed to stamp an offline paper sheet (Appendix II); after every 4 trials, children got to choose a sticker and place it on the sheet. In total, participants could complete up to 32 trials but if the child was inattentive or uninterested, a number of 16 trials was deemed adequate for analyses.

At completion of the experiment, height and weight of children were measured to later calculate BMI. Afterwards, parents were explained the procedure to fill in the FFQ at home and received compensation for their child's participation in the study. The overall experimental procedure lasted for around 1 hour.

Data Analyses

Nutritional Status was calculated as a compound score of the standardized scores of BMI centile and DHD adherence. In adults, BMI is inversely related to DHD scores (Looman et al., 2017). Thus, nutritional status (NS) was calculated as:

NS= -zBMI + zDHD.

To investigate the link between age, NS and task performance we performed a Repeated Measures ANOVA with trial type (SCon and SInc) as the within-subject factor, standardized age and nutritional status as covariates and accuracy (% of correct answers) as the dependent variable. A series of post-hoc analyses were completed to further understand the trends in the data. Given the small sample size, all post-hoc analyses were exploratory in nature. We performed post-hoc one sample t-tests by creating bins determined by age to investigate age-related differences in relation to chance level for accuracy on SCon and SInc trials. We also performed two exploratory regression analyses, one on accuracy on SCon trials and one on accuracy on SInc trials, using age, gender, SES and nutritional status as predictors. Finally, two correlation analyses were performed between BMI and accuracy on SCon and SInc trials and between DHD adherence and accuracy on SCon and SInc trials. All independent quantitative variables used in the analyses were standardized.

RESULTS

Descriptive Statistics

The average BMI centile of the sample was 65 (*SD*: 27.5). According to the Centers for Control Disease and Prevention categorization (see *Body Mass Index (BMI) Percentile*), none of the tested toddlers were underweight, 5 were overweight, and 3 were obese. In our sample the average DHD adherence score was 66.2 (*SD*: 11.3); the mean nutritional status score was 0.05 (*SD*:1.5).

Repeated Measures ANOVA

A one-sample t-test with overall task accuracy as the independent variable revealed that participants performed significantly above chance (50%) (t(22)=4.06, p=0.001, $M_{accuracy}$ =65.16%), indicating that overall participants understood task instructions.

To assess the relationship between nutritional status and the development of spatial orientation, we performed a Repeated Measures ANOVA with trial type (SCon and SInc) as a within-subject factor, standardized age and nutritional status as covariates and accuracy (% correct) as the dependent variable. We found a significant main effect of trial type with a large effect size (F(1,19)=7.761, p=.012, η_p^2=.290). There were no significant main effects of age (F(1,19)=.595, p=.450, η_p^2= .030) or of nutritional status (F(1,19)=2.298, p=.146). The latter, however, had a medium effect size (η_p^2=.108). The age*nutritional status interaction was non-significant (F(1,19)=1.252, p=.277, η_p^2= .062).

The trial type*age interaction showed a trend towards significance and a medium effect size (F(1,19)=3.65, p=0.071, η_p^2=.161) while the trial type*nutritional status interaction (F(1,19)=.497, p=.489, η_p^2=.025) and the three-way trial type*age*nutritional status interaction (F(1,19)=.712, p=.409, η_p^2=.036) did not.

Follow up analyses

Trial type

One sample t-tests for each trial type revealed that participants performed significantly above chance and with a large effect size on Scon trials (t(22)=5.98, p<0.001, Cohens'd= 1.25, $M_{accuracy}$ =75.44%) and at chance on SInc trials (t(22)=.77, p=.45, Cohens'd= 0.16, $M_{accuracy}$ = 54.89).

Age

While the trial type*age interaction only showed a trend towards significance, its medium effect size indicated that it could be the case that significance was not reached due to the small sample size (Schäfer and Schwarz, 2019); we thus decided to carry out exploratory post-hoc tests to investigate age differences on task performance. We formed three age categories (31-32 months, 33-34 months and 35-36 months) based on the frequencies of each age group. One sample t-tests with accuracy as the independent variable showed that participants aged 31-32 months performed significantly above chance with a large effect size on SCon trials ($t(4)$=5.522, p=0.005, Cohens'd= 2.47, $M_{accuracy}$=81.7) and at chance with a medium negative effect size on SInc trials ($t(4)$=-1.42, p=.229, Cohens'd= -.64, $M_{accuracy}$=34.94,). Participants aged 33-34 months performed significantly above chance with a large effect size on SCon trials ($t(10)$= 4.04, p=0.002, Cohens'd= 1.22, $M_{accuracy}$=75.98,) and at chance with a small effect size on SInc trials ($t(10)$=.468, p=.650, Cohens'd= .14, $M_{accuracy}$=54.67). Participants aged 35-36 months performed above chance with a trend towards significance and large effect sizes on both SCon ($t(6)$=2.19, p=.071, Cohens'd= .86, $M_{accuracy}$=70.07) and SInc ($t(6)$=2.149, p=.075, Cohens'd= .81, $M_{accuracy}$=69.50) trials. Task performance per age group and trial type is depicted in Figure 4.

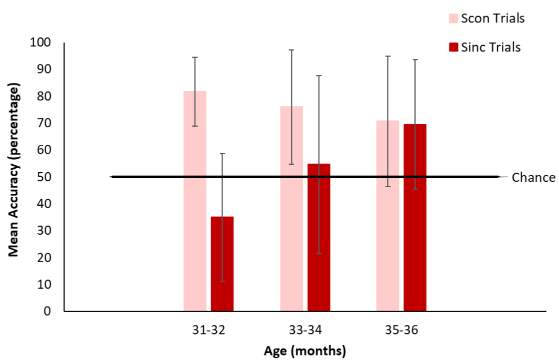

Fig. 4. **Task Performance by Age** | Mean accuracy scores on SCon (pink) and SInc (dark red) trials per each age group. Error bars represent standard deviations from the mean. The horizontal black line indicates chance level.

Nutritional Status

Correlation analyses showed no significant correlation between nutritional status and accuracy on SCon trials ($r(21)$=1.36, p= .542, R^2 = .018). Albeit non-significant, the correlation between nutritional status and accuracy on SInc trials had a medium effect size ($r(21)$=.323, p= .121, R^2 = .104). The trends found are depicted in Figure 5.

Two exploratory regression analyses, one on accuracy on SCon trials and one on accuracy on SInc trials, were conducted with nutritional status, age, gender and socio-economic status as predictors. Results of the regression using accuracy on SCon trials as the criterion revealed no significant effect of the predictors ($F(4,16)$=.396, p=.808, R^2=.09). Results of the regression using accuracy on SInc trials as the criterion again revealed no significant effect of the predictors ($F(4,16)$=1.72, p=.195). In this case, however, the much larger effect size (R^2=0.3) indicates the possibility

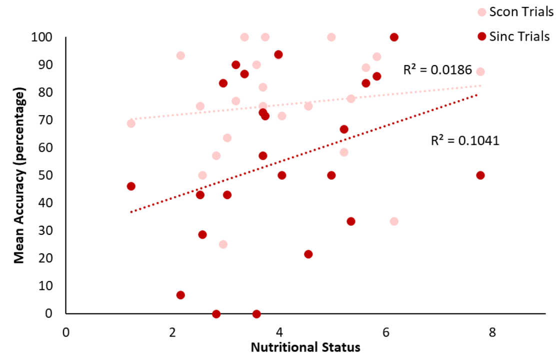

Fig. 5. **Correlation between Nutritional Status and Task performance** | Relationship between Nutritional Status (NS) and mean accuracy on SCon (pink) and SInc (dark red) trials. Please note that raw data nutritional status scores ranged from -2.77 to 3.77. Here, for visualization purposes, we have shifted all data points of 4 units to avoid displaying negative values. In the graph, a low nutritional status score indicates poor nutritional status.

that the lack of significance has not been reached due to the small sample size (N=23). Simply for exploratory purposes, the standardized β weights for each predictor in both regression models are presented in Table 1. Due to the lack of significance of the overall models p values are not reported.

Table 1. Standardized β weights for each predictor of the two linear regression analyses on accuracy on SCon and SInc Trials

Predictor	SCon Trials	SInc Trials
Nutritional Status	.061	.467
Age	-.214	.302
Gender	.052	-.348
Socio Economic Status	.131	-.058

To further control for potential confounds, we checked the correlation between the standardized scores for Socio-Economic Status and nutritional status. Based on Grubb's test, one outlier was removed. The correlation was non-significant ($r(21)$=.281, p=.217).

BMI and Dietary Quality

We then investigated the possible separate contributions of BMI and dietary quality to task performance, for each trial type.

The correlations between BMI centile and accuracy on SCon ($r(21)$=-0.14, p=.949, R^2=.0002) and SInc (r=-.087, p=.692, R^2=.007) trials were not significant (Figure 6).

Dietary quality, assessed as adherence to the Dutch Healthy Diet (DHD), was also not significantly correlated with accuracy on SCon trials ($r(21)$=.191, p=.383, R^2=.03). However, the correlation between DHD score and accuracy on SInc trials showed a trend towards significance and a medium effect size ($r(21)$=.398, p=.060, R^2=.15) (Figure 7).

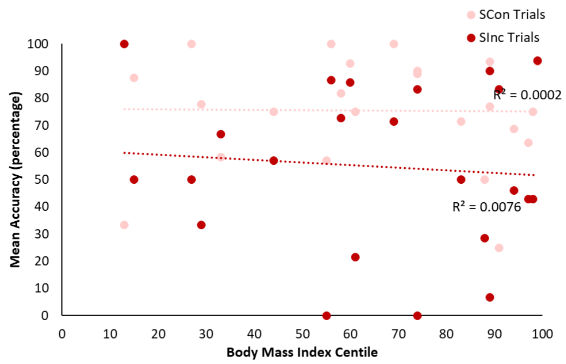

Fig. 6. **Correlation between BMI and Task performance** | Relationship between Body Mass Index (BMI) and mean accuracy on SCon (pink) and SInc (dark red) trials.

Fig. 7. **Correlation between Dietary Quality and Task performance** | Relationship between dietary quality, assessed as adherence to the Dutch Healthy Diet (DHD) and mean accuracy on SCon (pink) and SInc (dark red) trials. A higher score on the Dutch Healthy Diet Index indicates better dietary quality

Discussion

This study investigated the role of nutritional status and age in the development of allocentric spatial orientation abilities in 31-36 months old toddlers. Nutritional status was assessed as a compound score of dietary quality (adherence to the Dutch Healthy Diet; Dutch Health Council, 2015) and body-mass index (BMI).

We found that increasing age was associated with an increase in performance on trials requiring the use of allocentric orientation. Specifically, the age-related increase in performance was from below chance (indicating reliance on egocentric orientation) to above chance (indicating ability to employ allocentric orientation). Results also suggest that with increasing nutritional status, performance on trials requiring allocentric orientation increases from below to above chance. Trends from follow-up exploratory analyses suggest that nutritional status uniquely predicts performance on trials requiring allocentric orientation after controlling for potential confounding factors as age, gender and social-economic status. Finally, correlational exploratory analyses indicated that while BMI was not significantly related to task performance, Dutch-Healthy Diet adherence scores showed a positive correlation trending towards significance with performance on trials requiring allocentric orientation.

Age

The age-related data replicates the findings of van den Brink's and Janzen's (2013) study. While the main effect of age was non-significant, its interaction with trial type showed a trend towards significance and a medium effect size. One of the main advantages of effect sizes is their independence from sample size: they can express the size of an effect regardless of the size of the study (Schäfer and Schwarz, 2019). Given the medium effect size found, we suspect that the interaction would have reached significance had we had a bigger sample.

We compared toddlers' performance to chance using one sample t-tests. On average, participants performed above chance on SCon trials, which indicates that they understood task instructions and were not performing at random. Three participants showed below or at chance performance on SCon trials; however, given the already limited sample we decided not to remove them from the analyses. Older (35-36 months) children showed above chance performance SInc trials, indicating their ability to use allocentric coding for spatial orientation. On the other hand, below-chance performance on SInc trials indicated that 30-31-month-olds are unable to use allocentric orientation and instead rely on egocentric orientation. Again, these effects only showed a trend towards significance but a large effect size, so the reasoning above applies for the interpretation of these analyses too.

The response pattern found (above chance performance on SCon trials and below chance on SInc trials) suggests that younger toddlers tend to choose the spatial position of the tree prior to the viewpoint manipulation. Such response strategy indicates the use of egocentric coding (e.g. choosing the tree at their right before and after the camera rotation).

However, the use of other strategies cannot be excluded. For example, it could be that younger toddlers were unaware of the viewpoint manipulation; to give the impression of a 3D environment, there was a subjective size difference between the two trees (see Figure 3). If the younger children were unaware of the viewpoint manipulation, they could have based their responses on the subjective size difference between the two trees (i.e., if the bird hid itself in the tree in the back, which was 'small', they would choose the 'small' tree after the camera rotation). In the task used, such decision-making strategy would have resulted in below chance performance on SCon trials and above chance performance in SInc trials. As this pattern was not present in our data, we can exclude that participants based their decision making on the subjective size of the trees and confirm instead that younger children were aware of the viewpoint manipulation but unable to employ allocentric coding.

It should be noted that while the present study shows that 34-35 months old participants are better able to use allocentric orientation than 30-31 month-olds, sophistication of allocentric orientation use develops throughout childhood at least until early school age (Bullens et al., 2010). Nevertheless, in accordance with previous literature (Bremner et al., 1994; van den Brink and Janzen, 2013), this study indicates that spatial cues use development can be observed within a short 5-6 months period and that allocentric orientation abilities increase with age. Given the hippocampal-dependence of allocentric orientation abilities (e.g. Zaehle et al., 2007), it is possible that the gradual acquisition of allocentric orientation skills reflects the maturation of the hippocampus with age (Bullens et al., 2010).

Nutritional Status

There was no significant main effect of nutritional status. The medium size magnitude of its effect size indicates again the possibility that the lack of significance rises from our study being underpowered. As the following interpretation of the data is based on this assumption, it remains speculative and should be treated as such. Exploratory correlation analyses revealed a medium effect size positive correlation between nutritional status and performance on SInc trials but not on SCon Trials (see Figure 5). Correct performance on SCon trials could be achieved using both egocentric or allocentric coding (or even alternative strategies); the lack of a correlation between SCon performance and nutritional status, indicates that nutritional status is not related to spatial cognition *per se*. The positive correlation between performance on SInc trials and nutritional status, instead, indicates that as nutritional status increases so does the ability to use allocentric orientation. Indeed, while below chance performance on SInc trials indicates reliance on egocentric orientation, above chance performance indicates allocentric orientation abilities.

The trends found in our data show that performance on allocentric orientation requiring trials increases from below to above chance as nutritional status increases. The development of allocentric orientation is tightly linked to hippocampal maturation (Lambert et al., 2015; Zaehle et al., 2007). Previous animal literature has shown that the hippocampus is particularly sensitive to poor dietary quality and obesity (Kanoski and Davidson, 2011), especially early in life (Boitard et al., 2012). Thus, should it be the case that significance was not reached due to the small sample, our results would indicate that poor nutritional status in toddlers can lead to poorer use of hippocampal-dependent spatial orientation strategies. These results would be in line with animal literature suggesting that spatial orientation is impaired in cases of obesity and poor dietary quality (e.g. Boitard et al., 2012).

Some potential confounders that could explain our results can be ruled out. For example, social economic status could be a confounding variable. One widely used measure of socioeconomic status is parental education. Indeed, a spurious correlation between nutritional status and orientation abilities could arise if parents with a higher education level both feed their children a more nutritious diet and support earlier development of more advanced spatial abilities, e.g. via educational games or activities. However, the correlation between nutritional status and parental education level was found to be non-significant.

Another potential confounding variable could be gender. Indeed, previous studies both in adults (Chamizo et al., 2011) and children (Joshi et al., 1999; but see Nardini et al., 2006) have shown that males are better able to use allocentric orientation than females are. However, the trends from our exploratory post-hoc regression analysis seem to suggest that gender did not significantly predict task performance on SInc trials and that nutritional status was the strongest predictor of performance. Nevertheless, as these results were non-significant, they should be interpreted with caution and be replicated by future confirmatory research.

The fact that this is a behavioral study prevents us from stating with certainty that the effect of nutritional status on spatial orientation is due to the impact of nutritional status on the hippocampus. However, the regression analysis on SCon trials yielded quite different predictors' weights from that on SInc trials; this may be due to a dissociation in the predictive value of nutritional status between hippocampal dependent (allocentric) and non-hippocampal dependent (egocentric) orientation abilities. However, as we did not image brain activity during task performance, this interpretation remains highly speculative and should be further investigated. To further ascertain this relationship, neuroimaging studies should investigate the relationship between allocentric orientation abilities and nutrition.

The invasiveness and constraints of traditional neuroimaging research (e.g. functional magnetic resonance imaging, fMRI) make it not suitable for use with participants in the age range of the present study. Thus, the use of less invasive neuroimaging methods, such as functional near infrared spectroscopy (fNIRS) is suggested. However, fNIRS only allows to image cortical brain areas so it would not be particularly informative regarding the role of the hippocampus in the relationship between nutritional status and diet. Nevertheless, while a shared fronto-parietal network is involved in both egocentric and allocentric orientation, the two are to some extent subserved by different cortical areas. For example, allocentric orientation uniquely involves the bilateral ventral visual stream (e.g. Galati et al., 2000). As cortical areas have also been shown to be sensitive to diet-derived neuroinflammation (Guillemot-legris and Muccioli, 2017), an investigation of the cortical correlates of allocentric orientation abilities in relation to nutritional status would introduce an interesting dimension to the discussion on the role of diet in toddlers' spatial cognition. Given the evidence that allocentric orientation develops at least until early school age (Bullens et al., 2010) it would also be interesting to investigate the relationship between nutritional status and allocentric orientation in an older age group, more suitable for fMRI studies.

The three-way interaction between NS, age and trial type was not significant. As posited in the *Introduction*, a non-significant interaction indicates that nutritional status and age have an additive, rather than interactive, effect on task performance.

It could also be the case that the present study is too underpowered to detect the effects of a three-way interaction. Alternatively, it may be that the 5 months age difference investigated in the current study is too short to observe any significant interaction between age, trial type and nutritional status. As this is the first study investigating the link between nutritional status, age and spatial cognition in humans, and such small age differences are difficult to investigate with animal models, further research is needed to assess whether age and nutritional status interact in predicting allocentric orientation abilities.

Should future research reveal that nutritional status and age interact in predicting allocentric orientation abilities, the direction of such interaction should also be assessed. Indeed, it could be that as age increases, the relationship nutritional status and allocentric abilities decreases; this would indicate that at an earlier age, good nutritional status supports an earlier achievement of developmental milestones, but it does not impact as much older children, who have already developed allocentric abilities. Alternatively, the relationship between nutritional status may increase as age increases; this would indicate that while at an earlier age nutritional status does not impact the development of allocentric abilities, at later time points a poor nutritional status can delay the achievement of developmental milestones.

BMI and Dietary Quality

Given the small sample size, we did not include BMI and dietary quality measures separately in the ANOVA, but we decided to investigate possible trends in the data by using exploratory correlation analyses.

There was no relation between BMI and spatial orientation abilities, neither on SCon nor on SInc trials. This may seem in contrast with the findings on nutritional status, as one would expect a higher BMI to correspond to lower nutritional status. Moreover, some studies have shown associations between BMI and specific constructs of cognitive functions (Ramaswamy et al., 2010). However, our results are in line with previous animal research suggesting that diet-induced hippocampal impairments can occur prior to, or independently from, changes in body weight (Kanoski and Davidson, 2011). Indeed, other studies in children have failed to find a relationship between BMI and cognitive functions (Gunstad et al., 2008). BMI is often used in research as a proxy of body fat and an indicator of health. While this may be true at the population level (Green, 2015) our results support previous literature in suggesting that BMI is not a strong predictor when it comes to diet-related changes in cognitive functions. It could also be the case that we did not have enough obese (N=3) or overweight (N=5) participants to show an association between cognition and BMI. Moreover, as the inverse relationship between BMI and cognitive functioning is most often consistently found in adults (Gunstad et al., 2008), it could be that weight affects toddlers differently from adults. Further research is necessary to understand the role that BMI plays in toddlers' cognitive functioning.

Dietary quality (measured as adherence to the Dutch Healthy Diet, DHD) was not significantly related to performance on SCon trials. However, the correlation between DHD scores and accuracy on SInc trials tended towards significance and had a medium effect size. This trend indicates that allocentric orientation abilities increase with dietary quality. Again, the correlation only shows a trend towards significance, so caution should be implemented when interpreting these results. Should it be the case that significance was not reached due to the small sample size, given that allocentric orientation is hippocampal dependent, these results would be in line with previous animal research showing that poor dietary quality is related to impaired hippocampal-dependent spatial orientation (Boitard et al., 2012; Kanoski and Davidson, 2011).

Alternative explanations are possible. For example, dietary quality has been related to metal flexibility and task shifting (Cserjési et al., 2007). It could be that participants with poorer diet found it harder to switch between the two types of trials resulting in lower task performance. However, this would have resulted in lower task performance on both trial types; we only found an association between dietary quality and performance on trials requiring allocentric orientation. Another possible confounding mechanism is attention; indeed, poor dietary quality is inversely related to attention (Liang et al., 2014). Nevertheless, we excluded non-attended trials, so performance on the trials included should not be impacted by general attention levels.

Relevance

Early childhood is a period during which the brain and cognitive functions are developing, and as such are particularly sensitive to environmental factors. Our study, together with previous literature, suggests that dietary quality is one such environmental factor to impact cognitive development in early childhood. Nutrition is one of the most important sources of epigenetic modulation, and it can affect gene expression at levels of transcription, translation and post-translational modifications (Dauncey, 2012). This is of high relevance for public health as nutrition is potentially modifiable and as such it could be used throughout life, and especially during childhood, to support optimal brain and cognitive development.

Here, we show an effect of nutritional status on spatial abilities, in particular on the ability to use allocentric orientation in toddlers. Spatial orientation is a particularly important skill for toddlers' day-to-day life, for example allowing them to locate their parents when needed (van den Brink and Janzen, 2013).

Moreover, spatial abilities are linked to quantitative reasoning skills; indeed, longitudinal studies have shown that increased spatial abilities are associated with proficiency in mathematics and science (Wai et al., 2010). These skills are highly relevant in today's society, where numeracy, the ability of analyzing and interpreting data, and critically assessing complex problems are increasingly requested abilities.

Given the impact of nutrition on spatial abilities, and the fact that these underpin the development of skills highly relevant for today's society, it is of paramount importance to further investigate how nutrition impacts spatial abilities in the developing brain.

Limitations and Future Research

The main limitation of the current study is the small sample size. Given the medium size effects found both for age, nutritional status and DHD scores, such limitation seems to be the main cause of the lack of significance in the presented results. Recruitment of toddlers for participation was found particularly challenging; future studies should consider this when conducting research on young children. It should also be noted that due to the COVID-19 crisis all testing was interrupted, preventing us from reaching the desired number of participants for the analyses. Given the small sample, the results and interpretations presented in this paper should be treated as encouraging trends to explore with further, better powered, research.

Another limitation of this study is that we did not include variables in our analyses that have previously been shown to predict allocentric orientation abilities, such as language or motor skills (see Van den Brink & Janzen, 2013). Studies with larger sample sizes could introduce multiple predictors to better understand the role that nutrition plays in predicting allocentric orientation abilities in toddlers.

On top of the above-mentioned suggestions, future research should focus on investigating whether animal research on the mechanisms through which diet influences hippocampal-dependent abilities (e.g. inflammation) can be replicated in humans. As neuroinflammation cannot be non-invasively measured in humans, collection of peripheral inflammation biomarkers (e.g., through saliva samples) should be considered.

Conclusions

To conclude, the present study investigated the relationship between nutritional status and the development of allocentric orientation abilities in 30-36 months old toddlers using a VR task. The small sample of the study only allowed us to find trends in the data, rather than significant results, so these should be interpreted with caution and should be replicated by better-powered research. The trends found, however are promising, and well embedded in the context of previous research. We found that between 31 and 36 months of age toddlers progressively show the ability to use allocentric cues for orientation. We also found trends suggesting that nutritional status (measured as a compound score of BMI and dietary quality), predicts performance on allocentric-orientation requiring trials, but not on trials that did not require allocentric orientation. These findings are in line with previous animal research showing a negative impact of obesity and poor dietary quality on cognitive abilities such as spatial cognition, especially early in life. Nutritional status seems to predict allocentric-orientation abilities even when controlling for potential confounds such as gender, social economic status, and age. The main driver of the relationship between nutritional status and allocentric spatial abilities was nutrition, rather than BMI. Indeed, while BMI was not related to task performance, dietary quality positively correlated to allocentric orientation abilities.

Supplementary material

The supplementary video of the task used in this study can be found online at: https://www.youtube.com/watch?v=cWg85G-dk-s&feature=youtu.be

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