

n-3 Fatty acids and cognitive and visual acuity development: methodologic and conceptual considerations¹⁻⁴

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ABSTRACT

Several randomized clinical studies in infants born preterm and at term have explored the effects on visual acuity development of postnatal supplementation with various sources of docosahexaenoic acid (DHA). Higher visual acuity after DHA supplementation is a consistent finding in infants born preterm. For infants born at term, the results are less consistent and are better explained by differences in sensitivity of the visual acuity test (electrophysiologic tests being more sensitive than subjective tests) or by differences in the amount of DHA included in the experimental formula. Differences in the sensitivity of the test may also be relevant in discussions of whether the effects of DHA on visual acuity are transient or persistent. A smaller number of studies have attempted to study the effects of DHA on cognitive development. The major focus of this article is to review the types of methods that have been used to evaluate the effects of DHA on cognition and to provide the rationale for measures that are a better conceptual fit. Research is needed 1) to probe the effects of variable DHA exposure on infant and child development, 2) to measure outcomes that better relate to preschool and school-age cognitive function, and 3) to reinforce, and in some cases demonstrate, links between specific infant and preschool measures of cognitive development. We strongly encourage collaborations with developmental cognitive neuroscientists to facilitate these research goals. *Am J Clin Nutr* 2006;83(suppl):1458S-66S.

KEY WORDS Cognition, n-3 fatty acids, vision, development, infants, children

INTRODUCTION

Human infants can synthesize long-chain polyunsaturated fatty acids [LC-PUFAs: arachidonic acid (20:4n-6) and docosahexaenoic acid (DHA; 22:6n-3)] from their precursors [linoleic acid (18:2n-6) and linolenic acid (18:3n-3), respectively] (1-3). However, autopsy results show less DHA in the frontal cortex of infants born at term and fed infant formulas that contained α -linolenic acid than in infants fed human milk, which contains both α -linolenic acid and DHA (4, 5).

Randomized clinical trials (RCTs) of DHA supplementation and exploratory research over the past 2 decades have shown that supplementation with LC-PUFAs can positively affect both visual and cognitive outcomes, although the results from such trials are not entirely consistent, particularly in studies of the latter. In this article, we will briefly review the contemporary literature with an eye to the explication of null results and then propose directions for future research. Our proposal for future research

will be based on a developmental cognitive neuroscience perspective, with the goal of pinpointing specific brain systems and cognitive processes on which LC-PUFAs might have effects.

LC-PUFAs AND VISUAL DEVELOPMENT

The first randomized studies of infants focused on visual development, because it was already known that diets that reduced retinal and brain DHA could affect retinal electrophysiology and visual acuity of nonhuman primates (6, 7). Comprehensive reviews of retinal electrophysiology and visual acuity development have been published that focus on the work with animal models (8) and with infants (9-14). Because DHA begins to accumulate in the brain in the last trimester of gestation (15), it follows that infants who are deprived of the intrauterine environment during these critical weeks would be more likely than infants born at term to show benefits of DHA supplementation on visual acuity. Thus, the study of supplementation of infants born preterm serves to elucidate the value of LC-PUFAs in development.

Preterm and term studies

The results of studies in which infants born preterm were supplemented with formulas containing DHA show that supplementation results in higher retinal function (16) or visual acuity than in controls fed formula without DHA (17-22). The study by Bougle et al (23) is an exception, but the experimental groups in that study were very small. Conversely, the results of studies of DHA supplementation in infants born at term are mixed, with some researchers reporting increases in visual acuity (24-32) and others reporting no effects (33-38).

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Both subjective (eg, Teller Acuity Card procedure) and electrophysiologic (eg, sweep visual evoked potential) assessments of visual acuity have been used in published RCTs, which also have included a wide range of DHA concentrations in the experimental formulas. The sensitivity of the method used and the dose of DHA provided may be factors in the ultimate findings of the trials. However, when comparing infants born preterm with those born at term, the differences in results may be better explained by differences in brain development (and related brain DHA accumulation).

Interpretation of effects

Some researchers have reported transient improvements in visual acuity in infants born preterm as well as in those born at term. For example, using Teller Acuity Card procedures, Carlson et al (17–19, 27) found evidence of the benefit of DHA supplementation at 2 mo of age when testing infants born preterm and infants born at term, but not in subsequent evaluations at 4 (in 3 of the 4 studies), 6, 9, and 12 mo of age (ages corrected for prematurity). SanGiovanni et al (10) conducted a meta-analysis of 12 studies of the effects of DHA supplementation on visual acuity in infants born at term. They concluded that the evidence from RCTs utilizing behaviorally based methods showed statistically significant, positive effects of supplementation only at 2 mo of age; evidence from nonrandomized trials utilizing behaviorally based methods showed significant differences that extended through 4 mo of age. In the electrophysiologic-based studies reviewed, acuity differences were also significant at 4 mo of age. The effects of supplementation were not significant when the infants in the reviewed studies were older (eg, 6 through 12 mo).

Since the review by SanGiovanni et al (10), data have been reported that suggest that electrophysiologic methods may be more sensitive to the effects of DHA supplementation than are the behaviorally based methods. Researchers who have found higher visual acuity with DHA supplementation when using visual evoked potentials (most notably the trials of Birch and collaborators) report measurable effects that persist to at least 12 mo of age (32, 39). The contrast between studies done with subjective (eg, the Teller Acuity Card procedure) and those done with electrophysiologic (eg, visual evoked potentials) measures of visual acuity suggest that the positive effects of supplementing the diet with DHA on visual acuity may persist, although they become more subtle with age and more difficult to detect with the subjective, behaviorally based tests.

From the perspective of developmental cognitive neuroscience, a pattern of seemingly transient or diminishing effects of DHA on any aspect of the visual system (visual acuity being only one aspect of vision) over time would not be surprising. The visual system is an experience-expectant system (40), in that prior input from the environment has a substantial and significant contribution to the development and refinement of the system. Visual acuity shows extremely rapid improvement over the first 6 mo of life, but improvement reaches a temporary plateau between 6 and 12 mo of age, after which development proceeds at a more gradual pace (41) until fully mature levels of acuity are attained between 4 and 6 y of age (42). One might expect, then, that the effect of any intervention on visual acuity would be more readily detected during the period of most rapid development (birth to 6 mo). Furthermore, with the exception of situations of extreme deprivation, it is reasonable to expect that visual acuity

would continue to progress along its developmental course, even in unsupplemented individuals.

Whether the effects of LC-PUFA supplementation on visual acuity are transient or more persistent, the effects may be conceptualized as producing an accelerated developmental course. Several modern developmental perspectives (most notably, developmental systems theory) (43, 44) would predict that accelerated visual development in the early months of life might well confer an advantage in cognitive development. The accelerated maturity of the visual acuity pathway might influence the development of the structure and organization of the higher cortical pathways, and these differences would be reflected in the efficiency with which visual information is utilized by higher-order cognitive functions.

LC-PUFAs AND COGNITIVE DEVELOPMENT

Lipids make up 60% of dry brain matter (45), and of the fatty acids, DHA is one of the most plentiful and is highly concentrated in the membranes (46) and vesicles of the synapse (47). During periods of DHA (or DHA-precursor) deprivation in adult mammals, concentrations of DHA in the central nervous system are retained even while DHA is depleted in other organ stores (48), which attests to the importance of DHA in the central nervous system. However, during development, the central nervous system is vulnerable to DHA deficiency: rats fed diets deficient in α -linolenic acid during pregnancy and lactation, when transfer of DHA from the dam to the fetus or pup is high, do show loss of brain DHA (49). Thus, the general capacity of the neural system to maintain optimal concentrations of DHA does not extend to an ability to achieve optimal concentrations: if sufficient DHA or its precursors are not available to the organism during development of the neurons and the retina, DHA concentrations are lowered relative to development under sufficient conditions (48, 50).

Thus, in an organism that is not otherwise consuming optimal amounts of LC-PUFAs, DHA might optimize development if provided during the period of normal increase in brain DHA. For humans, this period extends from ≈ 24 wk of gestation to ≥ 2 y of age (15, 51). Any change in diet that affects brain DHA could potentially affect all functions involving the brain and not just visual acuity development, as has been pointed out previously (52, 53). In the following section, we briefly review the literature on the effects of DHA on cognitive development and offer potential explanations for the varied results.

Preterm and term studies

Several comprehensive reviews of this literature have appeared in recent years (9, 11, 12, 54–56), and the reader who wishes more detail about individual studies should refer to the initial studies or these reviews. For the purposes of our discussion, we will focus on a few general concepts related to this body of literature and, in general, restrict our references to RCTs, although this is in no way meant to discount the value of exploratory or quasi-experimental work. For the most part, the researchers who carried out RCTs used measures of global cognitive development, such as the Bayley Scales of Infant Development (57) or Brunet-Lezine's Scale (58). These trials have yielded mixed results in both preterm and term samples. Relative to infants fed control formulas, infants supplemented with DHA formulas showed either improved global cognitive function (59–63) or no significant differences (21, 33, 34, 61,



64–68). Language production and comprehension in relation to DHA supplementation have been assessed by using the MacArthur Communicative Development Inventory (69) in 14-month-old infants with mixed results (21, 34, 68, 70). In one study, supplemented infants who were born at term were not significantly different from those fed control formula (34). However, in another study of infants born at term, the group of infants supplemented with only DHA (compared with supplementation with DHA and arachidonic acid) actually showed lower language production, as reported on the MacArthur Communicative Development Inventory, than did those fed the control formula (68). The effect was not evident when these infants were 39 mo of age (71). In contrast, in another multicenter trial, infants born preterm had higher vocabulary production at a corrected age of 14 mo when fed formula with DHA (21).

Several possible explanations for the inconsistent results exist. In an early review, Morley (72) articulated 4 primary issues, one being that effects do not exist. Among the other possibilities, she mentioned inadequate sample sizes and variable composition of study and control formulas, which have been echoed in various other reviews by other scientists. Last, she suggested that the inconsistent results could be related to the use of inappropriate measures of cognition. Here we will focus on this last possibility. Our rationale for this is as follows: 1) Evidence suggests that various cognitive functions develop independently, even during infancy (73). 2) Certain types of interventions differentially affect specific cognitive functions. For example, prenatal exposure to alcohol affects aspects of infant visual attention, but not infant memory; prenatal exposure to polychlorinated biphenyls affects infant memory, but not infant attention (74). 3) Researchers have shown that specific cognitive outcomes (eg, problem solving, attention, and processing speed in infancy; distractibility in toddlers; and attention in preschool and school-aged children) are related to DHA intake or the amount of DHA in circulating cells or plasma lipids (75–79; P Willatts, JS Forsyth, C Agostoni, J Bissenden, P Casaer, G Boehm, unpublished observations, 2003). 4) It was recently reported that, at school-age, children who were exposed to higher levels of DHA as infant participants in RCTs show differential developments in cognition (79, 80; P Willatts, JS Forsyth, C Agostoni, J Bissenden, P Casaer, G Boehm, unpublished observations, 2003), even though measures conducted in infancy did not detect any benefit (79, 80). Thus, studies that use measures of specific aspects of cognition as outcomes seem warranted and in need of extension.

Specific processes of cognitive development

Early global measures of development, such as those commonly used in research of DHA supplementation (eg, the Bayley Scales of Infant Development or Brunet-Lezine's Scale), are a general rubric against which one can judge overall development. These tests were originally designed to identify those who were developing nontypically as quantified by tests of age-normed milestones. As such, the global measures do not allow for the assessment of specific independent cognitive processes (such as attention, memory, inhibition, or higher-order functions), and they may not be sensitive to manipulations that produce specific effects. In the field of cognitive psychology, the discontinuity between the global measures of infant development and childhood outcomes has long been known (81–83), and contemporary

researchers have shown that studies of specific processes evidence continuity and are better able to predict childhood abilities (84) than are the global assessments.

Two specific processes thought to underlie early cognitive development are memory and processing speed (85–87). There is further evidence to suggest that these cognitive processes in infancy are related to cognitive outcomes in childhood (84, 85, 88, 89). Thus, as was proposed previously (73, 90), it is possible that differences between infants supplemented with DHA-enriched formula and those fed control formula may not be evident when global measures are used but might be detectable if outcomes focus on specific abilities that underlie cognitive development.

Indeed, DHA is implicated in processes that underlie cognitive development. Improvements in synaptic efficiency (90) and transmission speed (91) theoretically aid in the efficiency with which information is processed. DHA is concentrated in neuronal membranes with particularly high concentrations in synaptosomal preparations (46). DHA may also influence the timing of myelination (if the response to DHA of children with peroxisomal disease reflects a normative relation between DHA accumulation and brain myelination) (92). In addition, DHA enhances the function of *N*-methyl-D-aspartate channels (93), which may in turn alter long-term potentiation in the hippocampus (94), a plasticity process thought to be integral in the establishment of an explicit memory trace. Thus, it is plausible that DHA has a role in increasing the speed with which information is acquired, and it may enhance the efficacy with which such information is retained.

Evidence of this potential enhancement of neuronal efficiency is found in the exploration of specific processes. Infants supplemented with DHA, relative to those fed standard formula (21, 76, 77, 95, 96), and those who apparently have been exposed to higher levels of DHA (on the basis of some biochemical marker) (75) have been reported to show increased novelty detection at 6 mo of age (21); better problem solving abilities at 9 (77) and 10 (76) mo of age; faster information processing at 4 (97), 6.5 (96), 9 (96), and 12 (95, 96) mo of age; more mature information processing abilities at 4, 6, and 8 mo of age (75); and more mature orienting and sustained attention at 12 and 18 mo of age (75). It would seem imperative, then, that the study of the benefits of dietary LC-PUFAs during development be continued through the evaluation of the effects of supplementation on specific cognitive processes or parameters. Proper methods and motivation for the exploration of infant attention have been detailed previously (73) and so will not be reiterated here. However, we will extend the proposal of work on specific processes to include measures of memory and higher cognitive functions.

Whereas the infant paradigms used in the specific process research to date (habituation, paired comparison, and means-end problem solving) are sensitive enough for use with infants in the first 7 or 8 mo of life, it is not clear whether such measures are truly appropriate beyond the first year (98). Important neurobehavioral changes occur toward the end of the first year of life, and it is important that the paradigms used to assess cognitive function be appropriate to tap these emergent abilities. A knowledge of the neural structures in which DHA is highly concentrated and the emergent cognitive functions subserved by those structures can be used to suggest measures appropriate for assessing the effects of DHA in later infancy and toddlerhood. [Note: Although regional differences in brain DHA have been observed



(92), DHA is present in all brain regions and accumulates in brain at a higher rate than reported in most other organs and tissues (99, 100).] Keeping in mind the due cautions when comparing humans and other species, histologic data from a DHA-depleted rat model indicate a significant reduction in DHA in the frontal cortex (101), and volumetric studies indicate reduced hippocampal cell size (102), relative to a DHA-adequate group. In conjunction with these anatomical findings, DHA-depleted rat models have been shown to be deficient in hippocampal- and frontal-based memory and learning tasks (103), and DHA-supplemented rats experience improvement in memory and learning (104–111).

Hippocampal and frontal areas have been shown in humans to be integral to memory, attentional control, and higher-order cognition. We therefore posit that measurement of such constructs would be critical in the evaluation of the effects of supplementation with DHA or other LC-PUFAs. The studies in which DHA supplementation was shown to have effects on specific processes hint at this relation in that tasks such as means-end problem solving (76, 77) and distractibility (75) rely on the frontal lobe function *voluntary attentional control* (or precursors thereof) (112). Thus, between 9 and 12 mo of age, we see the beginnings of the relation between DHA and the cognitive functions thought to be subserved by the frontal areas of the brain.

In humans, toward the end of the first year of life, the hippocampus becomes more adult-like in function (113), and the frontal lobes reach adult levels of metabolism (114). It also seems reasonable to propose that older infants be assessed for those precursor processes that underlie higher-order cognition, such as working memory, attentional control, and inhibitory control, and that these functions be followed by using a longitudinal or developmental approach, given that frontal-based executive functions change and mature through at least the preschool period (115). In what follows, we describe several tasks and paradigms that we believe would prove useful in the assessment of the effects of DHA supplementation on cognition. Because a detailed description and review of these paradigms and their empirical possibilities is beyond the scope of this article, nutrition researchers might best seek collaborations with developmental cognitive neuroscientists in endeavors of this sort.

Memory

Researchers have made great strides in the development of paradigms that allow for the assessment of memory in young or nonverbal participants (116). Several of these paradigms capitalize on the infants' natural tendency to imitate: the infant is shown a sequence of actions through the use of props and is subsequently encouraged to imitate the actions either immediately (elicited imitation) or after a delay of minutes to months (deferred imitation). Imitation tasks have been shown to measure hippocampal-based explicit memory (117–121), which is the type of memory on which one relies for recall of everyday events and facts. Performance of children at 6 y of age on an analogue picture-sequencing task and on a standardized memory assessment has been shown to be related to their imitation performance at 20 mo of age (TD DeBoer, CL Cheatham, E Stark, P Bauer, unpublished observations, 2005), thereby providing evidence of continuity between the infant and child measures.

In typically developing infants, the ability to recall these sequences (eg, to "Build a Gong," the infant hangs a metal plate on an A-frame-supported bar and rings it with a small mallet) begins to develop between 9 and 10 mo of age and continues into the

second year of life (122). The time frame coincides with the age at which developments are apparent in the hippocampus and its connections to the frontal lobes, structures that underlie explicit memory functioning (113, 123–127). These structures not only accumulate significant amounts of DHA, but brain DHA accumulation continues through the second year of life (15). Therefore, performance on imitation paradigms assessed at the end of the first year as well as during the second year of life would be of great import to the DHA story.

Executive function

In addition, optimal DHA concentrations are important to cognitive processes supported by the frontal areas of the brain (103–111, 128–131). These processes, known as "executive functions," can be characterized as the integration and control of attentional and response components with various aspects of working and long-term memory. Such integration gives rise to constructs such as inhibitory control, voluntary deployment of attention (eg, attention span and distractibility), use of rules and strategy in thinking, and planning (112). Information about a toddler's ability to self-regulate attention (flexibility) can be garnered from the distractibility paradigms used by Colombo et al (75). Those authors showed that toddlers whose mothers had higher DHA contents at the time of their births were better able to maintain their focus on a toy than were toddlers whose mothers had lower DHA contents at the time of their births. Moreover, LC-PUFA supplementation of children with attention-deficit/hyperactivity disorder reportedly ameliorates symptoms, which include inattention, impulsivity or lack of inhibitory control, and hyperactivity (132).

The animal literature provides further support for the notion that fatty acids serve a role in higher-order cognitive functions. Rodents' frontal areas subserve the same types of cognitive functions as do humans' (133), and several tasks provide assessment of processes that are relatively similar to the executive functions that are routinely measured in humans. These include delayed alternation, radial arm maze, and delayed nonmatching to sample tasks (134). Rats deficient in DHA do not perform as well as DHA-adequate rats on such tasks (135, 136). Furthermore, such tasks are associated with frontal area dopamine function (136). In adult rats, after more than one generation of α -linolenic acid-deficient diets, dopamine concentrations in frontal cortex decreased relative to those in controls (137), and, in the first 7 d of life, dopamine concentrations in the cerebral cortex, hippocampus, and striatum were lower than in DHA-adequate rats (135). More modest reductions in brain DHA, on the order of magnitude observed with formulas without DHA compared with human milk (5), resulted in abnormal effects on dopamine-related behaviors in adult rats, some of which were not reversed by normalizing brain DHA after weaning (50). Dopamine in nonhuman primates (138) and humans (139) is an important modulator of executive functions. Indeed, dopamine in the human frontal lobes reportedly increases working memory capacity (140). The animal and the human data converge to suggest the utility of executive function assessment in DHA research.

These higher-order cognitive functions reach full maturity at ages well beyond the ages to which most RCTs have extended. Successful completion of means-end tasks (76, 77) and the developing ability to voluntarily focus attention and inhibit a turn to a distractor (75) are precursors to emergent executive skills (112) that can be measured between 10 and 18 mo of age and that have



been shown to be sensitive to DHA concentrations. Although testing for these skills can begin as early as 9 or 10 mo of age, continued testing in the preschool years with more sophisticated paradigms is needed to get a complete account of the development of these executive abilities.

For example, in infancy, the precursors of the executive functions working memory and inhibitory control have been assessed by using the A-not-B task (141): an infant who has twice recovered a hidden object will experience difficulty retrieving the toy from a second location after even a short delay (seconds), even though the concealment occurs in clear view of the infant. Successful completion of this task relies on both inhibitory control (infant must inhibit the prepotent reach to the first hiding place) and working memory (infant must keep in mind where the object was hidden), which are both executive functions. Developmental researchers have found that the basic ability emerges by 12 mo of age (115), but with increases in task complexity, even 3-y-olds make the error (142). Thus, continued testing into the preschool years is required for a complete developmental picture.

Several paradigms have been used to test the development of inhibitory control, rule and strategy use, and working memory at later ages. For example, in a variant of the Stroop task, the child is instructed to say "day" when shown a picture of a moon and to say "night" when shown a picture of the sun (143). Thus, the child must inhibit the prepotent responses and engage working memory to hold the rules in mind. Success on Stroop-type tasks develops between 3 and 6 y of age. Another task is the dimensional card sort in which the child sorts cards alternating between 2 dimensions: either by the color of the object on the card ("Can you show me where the red ones go?") or by the type of object on the card ("Can you show me where the stars go?") irrespective of color (144). Typically developing 4-y-olds can successfully respond after the rule changes.


Psychophysiological approaches often employ the go/no-go task, which is easily computerized. In the computer version of this task, the child presses a button in response to visual cues according to the rules of the game, which requires inhibition to some cues. Another computerized task, the Children's Continuous Performance Task (C-CPT) (145) may be of value to DHA researchers given the potential relation between DHA and attention (75, 78, 132), because it has been shown to be sensitive to attentional control issues (146–148). The C-CPT was developed for use with adults and elementary-age children (145), but has been adapted for preschool-age children (149, 150). In the C-CPT, the child must discriminate between target and nontarget stimuli and respond to targets according to some rule, much like the go/no-go task. Both tasks are appropriate for a wide range of ages and for use in electrophysiological protocols.

Thus, from these executive function task descriptions and the review of the neuroscience behind executive function development, one can see that the effects of DHA supplementation may not be clear until the participants reach childhood. In general, the number of rules, the requirements for attentional shifting, and the need for inhibitory control can be manipulated to increase the complexity and, therefore, the degree of challenge inherent in the executive function tasks, which also allows the use of variants of the same task across a wide range of ages. Normative data for several of the executive function tests have been published for the 7–12-y-old age range (151). Certainly, as DHA-supplemented infants who participated in RCTs grow older, we have a unique opportunity to assess their developing abilities on these executive function tasks.

CONCLUSIONS

From our review of the published results of the RCTs of LC-PUFA supplementation on visual and cognitive development, 2 themes have emerged with respect to understanding, optimizing, detecting, and documenting the effect of LC-PUFAs on visual and cognitive domains. The first theme has to do with the timing of supplementation. The literature on sensitive periods in development has generally determined that periods of rapid development are those in which environmental interventions would have the greatest effect (152, 153). In keeping with this point, any RCTs involving supplementation should be synchronized with the normal developmental course of central nervous system uptake of LC-PUFAs during the early part of the life span. This translates to ensuring that LC-PUFAs are readily available to the fetus during the last trimester of pregnancy and to the child through the second year of life.

The second theme involves strategies for assessment and the choice of dependent variables to document the effects of LC-PUFAs on visual and cognitive development. We strongly recommend a comprehensive developmental cognitive-neuroscience approach to this issue. Cues from the comparative literature (with appropriate caution) indicate those neural substrates that are most affected by LC-PUFA supplementation. Careful reading of this literature should guide the human researcher toward those behaviors most appropriate to assess in RCTs of supplementation. Given that the effects of LC-PUFA supplementation may well be specific (or perhaps simply more evident in targeted assessments), global assessments of early cognitive function (eg, the Bayley Scales of Infant Development) may not be sensitive enough to detect the effects of LC-PUFA supplementation consistently. Collaboration with developmental cognitive neuroscientists will allow the expansion of LC-PUFA research into realms of cognition not yet explored but which show theoretical promise for the explication of the true effects of supplementation.

As noted before, investigators continue to look for answers to many questions related to dietary DHA and infant and child development. These include the importance of timing to the accumulation of DHA (in utero, during infancy, and in toddlerhood and acknowledging the plausibility that maternal DHA status plays a role), the amount of DHA that might be optimal (acknowledging the possible influence of antecedent or subsequent exposure), and the degree to which observed effects of inadequate DHA exposure may later be remediated. The choice of more specific measures of cognitive development needs to be a priority in these investigations. 

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