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Effects of cow milk versus extensive protein hydrolysate formulas on infant cognitive development

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Abstract

Little research has focused on infant developmental effects, other than growth, of formulas that differ substantially in the form of protein. To examine development of infants fed formulas differing in free amino acid content, we randomized 0.5-month-old infants (n=79) to either a control group who fed only cow milk formula (CMF) during the first 8 months (CMF8), or to one of two experimental groups: one experimental group fed extensively protein hydrolyzed formula (EHF) for 1–3 months during first 4.5 months (EHF1-3) of life, and the other fed EHF for 8 months (EHF8). The Mullen Scales of Early Learning were administered monthly from 1.5 to 8.5 months to assess fine (FM) and gross (GM) motor control, receptive (RL) and expressive (EL) language, visual reception (VR), and an early learning composite (ELC). Across the 5.5–8.5 month time period, when compared to CMF8 infants, GM scores in EHF1-3 infants averaged 1.5 points higher (95% CI: 0.1, 3.0) and in EHF8 infants 2.2 points higher (95% CI: 0.3, 4.0). Similarly, VR scores averaged 1.9 points higher (95% CI: 0.1, 3.8) in EHF1-3 infants and 2.2 points higher (95% CI: –0.2, 4.5) in EHF8 infants. EHF8 infants RL scores averaged 1.8 points lower (95% CI: 0.1, 3.6) than CMF8 infants. These data suggest that the form of protein in infant formula may impact cognitive development and that the higher free amino acid content in breast milk may be a contributing factor to the differential cognitive development between breastfed and CMF-fed infants.

Clinical Trial Registration: clinicaltrials.gov NCT00994747.

Keywords

cognitive development; extensive protein hydrolysate formula; cow milk formula; free amino acids

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Disclosure

JAM, JCT, and MAP report no conflicts of interest.

Introduction

Although infant formulas are considered suitable feeding alternatives, breast milk remains the gold standard since it contains nutrients needed for optimal growth, neurodevelopment, and health, including macronutrients, minerals, vitamins, flavors, and other components, some of which remain unknown or uninvestigated. During the 1970s, research focused on the compositional differences between human milk and infant formulas in the amounts and types of macronutrients (Hawley et al. 1978; Jensen et al. 1978). For example, breast milk is a rich source of long-chain polyunsaturated fatty acids (LCPUFAs), a major component of retinal and brain tissue; most infant formulas at the time had lower or no concentrations of these fatty acids (Hoffman et al. 2009). A series of randomized control trials (RCTs) investigated the dose effect of differing concentrations of LCPUFAs in cow milk infant formulas (CMF) on development, in particular, visual acuity. A meta-analysis of this body of research concluded significant benefits of LCPUFA supplementation of infant formulas, at concentrations approximating that found in human milk, on infant visual acuity at several stages of development within the first year of life (Qawasmi et al. 2013). In other words, at least for visual development, the concentration of LCPUFA in infant formula was sufficient to explain some of the observed differential outcomes on neurodevelopment based on early feeding mode. Other compositional differences between human milk and infant formulas, including milk proteins (e.g., α -lactalbumin, lactoferrin, osteopontin, milk fat globule membrane proteins) and biologically active peptides derived from protein digestion, have also been the focus of research (Lonnerdal 2014; Raikos and Dassios 2014; Wada and Lonnerdal 2014). These proteins are naturally occurring in human milk but are either not present or present in very small amounts in most CMFs. In both short- and long-term studies, the addition of these proteins to infant formula affected a variety of outcomes, including immunity, nutritional status, neurodevelopment, and mineral absorption (King et al. 2007; Timby et al. 2014; Trabulsi et al. 2011; Veereman-Wauters et al. 2012).

For an eligible infant formula to be approved for use in the United States, the manufacturer must establish via a growth-monitoring study (minimum of 4 months duration) that the formula supports normal physical growth (using National Center for Health Statistics reference percentiles for anthropometry measures) and that the protein content of the formula is of sufficient biological quality (US Department of Health & Human Services et al. 2014). However, surprisingly little research has focused on the effects of compositional differences among the varying types of formulas (CMF vs. soy formula vs. protein hydrolysates) on development parameters other than growth, especially since these formulas differ substantially in their type and form of protein. Recent evidence does suggest that infants fed soy-based formula had growth patterns and developmental/intelligence quotient scores similar to those of children fed human milk or CMF (Andres et al. 2012; Malloy and Berendes 1998; Vandenplas et al. 2014).

Our prior work, which focused on flavor learning and satiation, randomized healthy 0.5-month-old infants to CMF, the most commonly consumed infant formula in the United States (Oliveira et al. 2010), or extensively protein hydrolyzed formulas (EHF), a type of cow-milk-based formula often given to infants with allergies to proteins, which have substantially higher concentrations, and the most diverse profile, of free amino acids (FAAs)

(Ventura et al. 2012b). We found that 1–3 months of exposure to EHF, before the infant reached 3.5 months of age, was sufficient to result in greater preference for the flavor of this formula compared with CMF-fed infants who never experienced EHF during their first 8.5 months (Mennella and Castor 2012). However, these infants' preferences were less pronounced than those of infants exposed to EHF for the entire 8-month period, thus suggesting a dosing effect. The “window” for early acceptance and long-term influences began to close around 3.5–4.5 months.

Because the CMF and EHF used in our previous trials (Mennella et al. 2011a) were made by the same formula manufacturer, which used the same fat blend in the two types of formula (Product information received from Mead Johnson Nutrition on 7/16/2014), in this study we probed whether differential effects on other aspects of development resulted from being randomized to formulas that drastically differ in amounts (>90-fold difference) of FAAs (Ventura et al. 2012b). Objective broad assessments of the behavioral domains of fine and gross motor control (FM, GM), receptive and expressive language (RL, EL), and visual reception (VR) were made each month to explore whether randomization to one of two formulas that differ substantially in amount and form of its protein content, yet share similar fat blends and LCPUFA content, would result in differential development.

Subjects and Methods

Study Design

Subjects were healthy, term, formula-fed infants and their mothers (n=79 dyads) who participated in a clinical trial on the effect of formula on flavor preferences (see (Mennella et al. 2011a) for details). Upon enrollment (at ~0.5 months of age), dyads were randomized either to a control group who fed only CMF during the first 8 months (CMF8 group) or to one of two experimental groups: one group fed EHF for 1-3 months during first 4.5 months and CMF otherwise (EHF1-3 group); the other fed EHF for 8 months (EHF8 group). Mothers were not aware of the hypotheses or which of the formulas they were provided to feed their infants. Formula was provided free of charge at the time of the monthly procedures throughout the study. Procedures were approved by the Office of Regulatory Affairs at the University of Pennsylvania, and informed written consent was obtained from each mother prior to study entry. This trial was registered at clinicaltrials.gov (no. NCT00994747).

Study Formulas

Table 1 lists some of the key ingredients and nutrient composition of the two infant formulas (Mead Johnson Nutrition, Evansville, IN). The formulas were isocaloric and contained a similar fat blend but differed in the structure of the protein. The CMF contains two sources of protein, nonfat milk and whey protein concentrate, which provide amino acids in the form of intact casein and whey proteins. The sole source of protein in EHF is casein hydrolysate, which is a casein protein that has been enzymatically treated (cleaving the peptide bonds), resulting in higher levels of free amino acids (FAA) (Ventura et al. 2012b) and small peptides (Trabulsi and Mennella 2012). Per FDA guidelines for nutrient composition of infant formulas (United States Food and Drug Administration 2004), the vitamin and

mineral composition of the two study formulas are similar; they are the same for 12 of 15 vitamins and 8 of 12 minerals; the differences in the remainder are considered minimal (Mead Johnson Nutrition/Enfamil (2015); Mead Johnson Nutrition/Nutramigen (2015)).

Study Procedures

After enrollment in the overall study at 0.5 months, beginning at the 1.5-month visit and then at the start of each 1-month cycle until the infant reached 8.5 months of age, dyads came to the Monell Center where infants were weighed and measured in lightweight clothing and diapers, and cognitive and motor development was assessed using the Mullen Scales of Early Learning: AGS Edition (Mullen 1995), an individually administered, norm-referenced test intended to assess mental and motor ability of infants of varying ages. All assessments were completed in the same laboratory within approximately 1 hour after feeding to ensure the child was alert and not hungry. The child's mother sat in a chair during the assessment and was instructed to remain neutral in facial expressions and not to talk during the testing.

The Mullen Scales of Early Learning measure five distinct skills: gross motor skills (GM) and the four "cognitive" skills fine motor (FM), visual reception (VR), receptive language (RL), and expressive language (EL). Examples of the abilities assessed at the 5.5-month visit are holds head steady, tracks objects, holds an object, localizes sounds, and vocalizes a variety of sounds. The raw scores for each of the five subscales (GM, FM, VR, RL, and EL) were converted to age-adjusted normalized scores (T-scores); as point of reference, T-scores have a mean of 50 and a standard deviation (SD) of 10, and scores between 40 and 60 are considered average. The Early Learning Composite (ELC), a composite score that represents general intelligence, was generated by summing the T-scores for VR, FM, RL, and EL and then converted into an age-adjusted normalized score. ELC scores have a mean of 100 and an SD of 15, with scores between 85 and 115 considered average.

Statistical Analyses

Descriptive statistics were used to examine the distribution of infant gender, and race/ethnicity, as well as maternal age (years), body mass index (BMI), parity, and education. Anthropometric measures were normalized to z-scores relative to growth standards of the World Health Organization (2006). Chi-square analyses for categorical variables (infant race/ethnicity, maternal parity, and education) and analyses of variance for continuous variables (maternal age and BMI) determined if there were differences by diet group (CMF8, EHF1-3, EHF8) in subject characteristics and each Mullen subscale at baseline (1.5 months), as well as the start (5.5 months) and the end (8.5 months) of the observation period.

From 5.5 to 8.5 months, differences among the three diet groups were determined using generalized estimating equations (GEE) that included diet group, time, and diet group $\times$ time interactions. Essentially, this model examines whether the slopes of the line computed by the Mullen scores over time differ for the diet groups (Sousa et al. 2005). A statistically significant diet group $\times$ time interaction is interpreted as a significant diet group effect over time, meaning that the slope of the lines (trajectory of change) differ by diet group. The

GEE model used the time period of 5.5 to 8.5 months because each infant in the experimental groups (EHF1-3, EHF8) had at least one month of exposure to EHF by 5.5 months and because one month of exposure to EHF before this age period was sufficient to shift preferences for flavor (Mennella and Castor 2012; Mennella et al. 2011a).

All variables with skewed distributions (as determined by the Shapiro-Wilk test) were normalized using a logarithmic transformation. Results for continuous variables are presented as means and standard errors of the mean (SEM), and percentages are used for categorical variables. For all analyses, the statistical significance level was set at p-value 0.05 (two tailed). Data were analyzed using Stata version 12 (StataCorp, LP, College Station, TX). When the GEE model indicated a significant diet group $\times$ time interaction, the Bonferroni post hoc multiple comparison method was used to determine differences between the three groups.

Results

Subject Characteristics and Completion of Task

The majority (89.9%) of infants completed the 8.5-month study: 14 of 16 in the CMF8 group, 45 of 46 in the EHF1-3 group, and 12 of 17 in the EHF8 group. Table 2 lists the characteristics of the final study population of 71 dyads, who reflect the diversity of race/ethnicity and educational levels of the city of Philadelphia (Pew Charitable Trust 2014). The three groups did not statistically significantly differ in infant sex ratio, race/ethnicity, maternal age, maternal BMI, parity, or income levels. Neither did they differ in any anthropometric measures: weight-for-age z-score (WAZ), length-for-age z-score (LAZ), weight-for-length z-(WLZ) scores were within one standard deviation of the WHO growth reference standard.

Mullens Scales of Early Learning

On average, Mullen scores were as expected for a general population sample of infants (mean subscale scores for all time points, all groups: FM, 44.9 ± 3.3 ; EL, 44.2 ± 2.9 ; ELC, 89.8 ± 5.5 ; GM, 51.2 ± 4.7 ; VR, 46.0 ± 5.0 ; RL, 43.6 ± 4.1). At baseline (1.5 months) and at beginning of the observation period under study (5.5 months), GM, VR, FM, RL, EL, and ELC scores were not significantly different among the three diet groups.

As shown in Figure 1, between the ages of 5.5 and 8.5 months, no significant differences were observed among the diet groups in the slopes of the lines (trajectory of change) for FM (Figure 1d), EL (Figure 1e), or ELC (Figure 1f). However, significant differences were observed in GM (Figure 1a), VR (Figure 1b), and RL (Figure 1c) trajectories during this time period. Compared to the CMF8 group, both EHF groups had higher overall GM and VR scores. As shown in Table 3, the EHF1-3 group's GM scores averaged 1.5 points higher (95% CI: 0.1, 3.0, $p < 0.05$), and the EHF8 group's GM scores averaged 2.2 points higher (95% CI: 0.3, 4.0, $p < 0.05$). VR scores for the EHF1-3 group averaged 1.9 points higher (95% CI: 0.1, 3.8, $P < 0.05$) and for the EHF8 group averaged 2.2 points higher (95% CI: -0.2, 4.5; $p = 0.07$). In contrast, EHF8 (but not EHF1-3) infants' RL scores averaged 1.8 points lower (95% CI: 0.1, 3.6, $p < 0.05$) than CMF group RL scores during this time period.

As an exploratory analysis, the EHF groups were combined and compared with the CMF group. Interestingly, at the end of the observation period, the only difference in the Mullen scores between the control and experimental diet groups was for VR; scores were significantly higher for EPH infants than CMF control infants at 8.5 months (46.5 vs 42.0; $p<0.03$).

Discussion

This small RCT is the first to suggest that variation in the protein form in infant formula fed during the first months of life may relate to variations in developmental outcomes. Healthy infants randomized to feed EHF during the first months of life differed in cognitive (VR, RL) and motor (GM) development compared with infants randomized to CMF, which differs from EHF primarily in the form of protein (e.g., lower FAA and small peptide concentrations); all other macronutrients were comparable between the formulas (Table 1).

While the majority of Mullen scores were within normal ranges and consistent with prior research on breast- and formula-fed infants of this age range (Keim et al. 2012), the average VR scores (a measurement of a child's ability to process information using patterns, memory, and sequencing) and GM scores (a measurement of central motor control and mobility) were higher among infants who had at least one month of exposure to EHF. The differences in magnitude of the GM and VR scores between EHF1-3 and EHF8 groups suggest a dosing effect, similar to the pattern observed for flavor learning (Mennella and Castor 2012; Mennella et al. 2011a). Moreover, such differences may persist beyond 8.5 months since those infants who had fed EHF had higher VR scores than infants fed only CMF at 8.5 months.

The average RL score, a measurement of ability to process linguistic input, were higher in the CMF8 group than in the EHF8 (but not the EHF1-3) group. This measure is highly dependent on auditory processing, an unexplored area in the effect of diet composition compared to visual and motor development. Nevertheless, by the end of the observation period when infants were 8.5 months, there were no differences in Mullen scores (with the exception of VR scores mentioned above) between EHF and CMF fed infants. Such transience in developmental outcomes has been observed in other studies as a function of early breastfeeding (Auestad et al. 2003). Further, the effects of early diet on such outcomes as growth (Dewey 1998; Koletzko et al. 2009; Mennella et al. 2011b; Rzehak et al. 2009) and visual development (Deoni et al. 2013) are evident early and then disappear, although the time course differs among studies, as do the lengths of the trials and the types of developmental outcome measures.

While the strength of this study lies in the fact that it is an RCT of healthy infants with no familial history of atopic disease or health conditions, we acknowledge that this was an exploratory study and not the primary aim of the RCT, which was to examine early flavor learning and satiation in formula-fed infants during the first 4 months of life (Mennella et al. 2011a). Although some recent studies have used the Mullen Scales of Early Learning to assess the impact of breastfeeding and LCPUFA intake on infant cognitive development (Keim et al. 2012), most have used other measures, such as the Bayley Scales of Infant

Development (Andres et al. 2012; Luttikhuis dos Santos et al. 2013; Rose et al. 2013; Timby et al. 2014). Nevertheless, the findings we present here indicate one potential factor affecting early cognitive development in relation to formula feeding and highlight how little information is available on the impact of current formulas on motor and cognitive development. Clearly, more research is needed to determine the effects of duration and timing of exposure using a wider range of cognitive measurements.

The infant formulas used here had a similar fat blend but differed substantially in the form and amount of protein (see Table 1), in particular, in the concentrations of FAAs (Ventura et al. 2012c). Because of their unbound nature, FAAs such as free glutamate can be sensed by receptors in the oral cavity (Chaudhuri et al. 1989), intestinal and gastric walls (San Gabriel and Uneyama 2013), and, perhaps most important, microglial cells in the developing and mature brain (Murugan et al. 2013). Exposure to increased amounts of free glutamate added to CMF results in greater satiation in human infants (Mennella et al. 2011a; Ventura et al. 2012a); whether the site of action is the gastrointestinal tract, brain, or both is unknown. We note, however, that differing levels in FAA levels other than glutamatic acid/glutamate, as presented in Table 1, could contribute to observed differences in cognitive development between CMF and EHF-fed infants. For example, tryptophan, an essential amino acid, is a metabolic precursor of serotonin which plays crucial roles in the development of neural circuits (e.g., retinotectal) and plasticity (Gonzalez et al. 2008; Serfaty et al. 2008) and has been shown to reduce sleep latencies in infants fed formulas supplemented with this amino acid (Steinberg et al. 1992). Cysteine, which is higher in EHF than CMF, can be converted to taurine, a conditionally essential amino acid in infants (Brosnan and Brosnan 2006). In addition to playing a role in bile salt synthesis, taurine is proposed to have a role in neurotransmitter and retinal function (Young and Cepko 2004).

If FAAs contribute to differences in cognitive or motor outcome in infants, the recent findings of no differences in Bayley Mental Developmental or Psychomotor Indices (Andres et al. 2012) between infants fed soy formula and those fed CMF would not be surprising, because FAA concentrations in soy formulas are more similar to those in CMF than to those in EHF (Ventura et al. 2012b). On the other hand, the substantial differences in FAAs between CMF and breast milk (Agostoni et al. 2000) may contribute to differences in cognitive development between breastfed and never breastfed infants (Oddy et al. 2003), but one would expect variability among breastfed infants since the FAA content of breast milk varies due to stage of lactation as well as the individual mother (Baldéon et al. 2014).

Conclusions

The findings from this RCT trial are the first to suggest that variation in the form of the protein content in formula fed to infants may relate to variations in cognitive development scores during the early stages of infancy. Although much work has focused on the effects of LCPUFAs on cognitive development, and RCT trials have shown that fat and bioactive peptide contents may explain some differences in cognitive development between breastfed and formula-fed infants or among formula-fed infants, the findings we present here suggest that FAAs may also be contributing factors. Concentrations of FAAs in human breast milk are 2- to 5-fold higher than in CMF (Agostoni et al. 2000), the most commonly consumed

infant formula (accounting for 80% of all U.S. infant formula sales (Oliveira et al. 2010)) which may account for reported differential cognitive development between breastfed and CMF-fed infants (Oddy et al. 2003). The novel results presented here highlight how little information is available on the impact of current formulas on motor and cognitive development, indicating the need for further research on the effects of these and other compositional differences among the varying types of formulas (CMF vs. soy formula vs. partial hydrolysate vs. EHF) on infant developmental outcomes other than growth during the first 4 months of life.

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Figure 1.

Mean and SEM Mullen Scale scores by diet group (CMF8, EHF1-3, EHF8) and time point (5.5–8.5 months): gross motor (a), visual reception (b), receptive language (c), fine motor (d), expressive language (e), and early learning composite (f). The gray shading signifies the normal range for a given scale. Generalized estimating equations (GEE) determined whether the slopes of the lines differed between the diet groups (CMF8, EHF1-3, EHF8) and over

time (5.5 to 8.5). The red line signifies a significant difference between EHF8 and CMF8; the blue line signifies a significant difference between EHF1-3 and CMF8.

Table 1

Nutritional composition of the test formulas

Characteristic	CMF (Enfamil ^a)	EHF (Nutramigen ^b)
Calories, kcal/100 ml	67.7	67.7
Protein, g/100 ml	1.4	1.9
Sources of protein	Nonfat milk, whey protein concentrate	Hydrolyzed casein protein
Total FAAs (μmol/100 ml) ^c	86.4	8037.5
Essential FAA:		
Histidine	0.9	188.0
Isoleucine	tr	532.7
Leucine	nd	1188.6
Lysine	2.2	825.4
Methionine	nd	285.4
Phenylalanine	1.1	428.3
Threonine	0.5	465.3
Tryptophan	nd	134.8
Valine	1.0	703.8
Semi-essential FAA:		
Arginine	1.0	348.9
Cystine	nd	54.2
Taurine	52.9	49.6
Tyrosine	0.9	117.0
Nonessential FAA:		
Alanine	3.1	490.5
Asparagine	tr	370.5
Aspartic acid	0.9	153.5
Glutamic Acid/Glutamate ^d	10.9	747.2
Glutamine	nd	nd
Glycine	3.8	165.8
Proline	6.3	266.7
Serine	0.9	521.3
Total FAAs	864	80,375
Carbohydrate, g/100 ml	7.4	7.0
Sources of carbohydrate	Lactose, galacto-oligosaccharides, polydextrose	Corn syrup solids, modified corn starch
Fat, g/100 ml	3.6	3.6
Sources of fat	4% palm olein, 19.5% coconut, 19.5% soy, 14.5% high-oleic sunflower oil	44% palm olein, 19.5% coconut, 19.5% soy, 14.5% high-oleic sunflower oil
DHA, mg/100 ml	0.23	0.23
ARA, mg/100 ml	0.11	0.11

Abbreviations: CMF, cow milk formula; EHF, extensive protein hydrolysate formula; DHA, docosahexaenoic acid; ARA, arachidonic acid; FAA, free amino acid; tr: trace; nd: not detected.

^aMacronutrient data obtained from the manufacturer's website (Enfamil: <http://www.meadjohnson.com/pediatrics/us-en/product-information/products/infants/enfamil-infant>, accessed on 19 August 2015), except as noted.

^bMacronutrient data obtained from the manufacturer's website (Nutramigen: <http://www.meadjohnson.com/pediatrics/us-en/product-information/products/infants/nutramigen-with-enflora>, accessed on 19 August 2015), except as noted.

^cFree amino acid content from Ventura et al. (2012).

^dAnalysis of the formula by automatic amino acid analyzer (model L-8900; Hitachi, Tokyo, Japan) cannot distinguish between glutamic acid and glutamate; from Ventura et al. (2012).

Table 2

Infant and maternal characteristics at baseline (0.5 months of age) for all subjects and by diet group, % [n] or mean (SEM)

Characteristics	All infants and mothers (n=71)	Diet group		
		CMF8 (Control group) (n=14)	EHF1–3 (Experimental group) (n=45)	EHF8 (Experimental group) (n=12)
<u>Infants</u>				
Sex (% male)	55% [39]	50% [7]	55% [25]	58% [7]
Race				
African American	48% [34]	42% [6]	51% [23]	41% [5]
Caucasian	20% [14]	21% [3]	17% [8]	25% [3]
Other/more than one	32% [23]	35% [5]	31% [14]	33% [4]
Ethnicity (% Hispanic)	25% [18]	42% [6]	22% [10]	16% [2]
Baseline anthropometric measures				
WAZ	0.22 [1.0]	0.56 [0.7]	0.16 [0.94]	0.09 [1.5]
LAZ	−0.41 [1.4]	−0.28 [0.8]	−0.42 [1.5]	−0.51 [1.3]
WLZ	0.65 [1.4]	1.02 [1.0]	0.58 [1.37]	0.52 [1.9]
<u>Mothers</u>				
Age (years)	25.9 (0.8)	25.6 (7.4)	25.6 (6.0)	27.0 (7.9)
BMI (kg/m2)	31.0 (1.1)	35.8 (12.8)	29.9 (7.8)	30.0 (5.6)
Parity (% multiparous)	45% [32]	57% [8]	48% [22]	16% [2]
Education (% college or more)	34% [24]	14% [2]	37% [17]	41% [5]

No significant differences among groups as determined by chi-square analyses for categorical variables and analyses of variance for continuous variables.

Abbreviations: WAZ: weight-for-age zscore, LAZ: Length-for-age zscore; WLZ: weight-for-length zscore, BMI: body mass index

Table 3

Associations between diet group and Mullen Scale of Early Learning score from 5.5 to 8.5 months of age (Model 2): β -coefficients and (95% confidence intervals) from generalized estimating equations.

Model ^a	Motor subscale: GM	FM	Cognitive subscales:			Composite of cognitive subscales:		
			VR	RL	EL	ELC		
Main effect of time	-0.89 (-2.2, 0.4)	2.9* (1.1, 4.8)	-1.5 (-3.2, 0.1)	-1.9* (-3.1, -0.7)	0.1 (-1.2, 1.4)	-0.1 (-1.9, 1.6)		
Main effect of diet group: EHF1-3	-12.6* (-22.7, -2.4)	-8.0 (-21.9, 5.9)	-11.8 (-24.3, 0.7)	6.1 (-3.1, 15.2)	-3.1 (-12.9, 6.7)	-7.9 (-21.5, 5.7)		
Main effect of diet group: EHF8	-14.9* (-27.8, -1.89)	0.2 (-17.6, 17.9)	-12.7 (-28.7, 3.3)	12.1* (0.4, 23.9)	-6.5 (-19.0, 6.1)	-2.9 (-20.3, 14.5)		
Time $\times$ diet group: EHF1-3	1.5* (0.1, 3.0)	1.1 (-0.9, 3.2)	1.9* (0.1, 3.8)	-1.0 (-2.3, 0.4)	0.4 (-1.1, 1.9)	1.1 (-0.9, 3.1)		
Time $\times$ diet group: EHF8	2.2* (0.3, 4.0)	0.3 (-2.3, 2.9)	2.2 (-0.2, 4.5)	-1.8* (-3.6, -0.1)	1.3 (-0.6, 3.1)	0.9 (-1.7, 3.4)		

* p<0.05;

EHF vs CMF group.