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Milk fat globule membrane-enriched milk improves episodic memory: A randomized, parallel, double-blind, placebo-controlled trial in older adults

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Abstract:	Cognitive decline is one of the most important consequences of aging and pharmacological therapies had been largely unsuccessful. Other strategies include the use of functional foods to reduce the burden of cognitive decline. The MFGM is an important source of polar lipids and glycoproteins that decline in the aging brain. We have developed a milk drink fortified with MFGM (MFGM-M) as a dietary supplement in a CRT pilot study. Forty-four > 65-year-old healthy or mildly cognitively impaired subjects received MFGM-M or control milk for 14 weeks, during which they underwent a battery of cognitive tests. Lipidomic analyzes were also performed. The female participants showed improvement in episodic memory, the ability to recall events in our lives. It is conceivable that any intervention should be started before clinical symptoms manifest, as a preventive measure against cognitive decline. Future long-term studies may shed further light on this point.
Suggested Reviewers:	Rafael Jimenez-Flores, PhD Endowed Professor, The Ohio State University jimenez-flores.1@osu.edu Dr. Rafael Jimenez-Flores is an expert scientist related to the studies of MFGM for more than 20 years
	Ronan Lordan University of Pennsylvania ronan.lordan@pennmedicine.upenn.edu Dr. Lordan is an expert in phospholipids in dairy products including MFGM
	Christopher Gardner, PhD Professor-Research, Stanford University cgardner@stanford.edu Expert in health benefits of dietary components or food patterns in the context of randomized controlled trials in free-living adult populations

DECLARATION OF INTEREST STATEMENT

Manuscript: “Milk fat globule membrane-enriched milk improves episodic memory: A randomized, parallel, double-blind, placebo-controlled trial in older adults” By María V. Calvo1, Viviana Loria Kohen2,3, Carmen Díaz-Mardomingo4, Sara García-Herranz4, Shishir Baliyan4, Joao Tomé-Carneiro2, Gonzalo Colmenarejo2, Francesco Visioli2,5, César Venero4*, Javier Fontecha1*.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

COVER LETTER

Dear Editor in-Chief of Journal of Functional Foods,

Dear Professor Vincenzo Fogliano,

Please find submitted our research manuscript titled "Milk fat globule membrane-enriched milk improves episodic memory: A randomized, parallel, double-blind, placebo-controlled trial in older adults" By María V. Calvo[1], Viviana Loria Kohen[2,3], Carmen Díaz-Mardomingo[4], Sara García-Herranz[4], Shishir Baliyan[4], Joao Tomé-Carneiro[2], Gonzalo Colmenarejo[2], Francesco Visioli[2,5], César Venero[4]*, Javier Fontecha[1]*

This manuscript has been transfer from Food Research International by advice of the Editor in Chief Dr. Anderson de Souza Sant'Ana,

This manuscript it is part of a large investigation project of milk fat globule membranes, which are attracting attention worldwide.

Following the excellent experience, i.e. knowledgeable and insightful reviews that we had with our pre-clinical paper (https://doi.org/10.1016/j.foodres.2022.112163), which is already being cited, we would like to publish the human study in Journal of Functional Foods because we believe it will attract even more attention.

In addition to the reviewers who evaluated our previous manuscript, may we humbly suggest the following experts?

Dr. Rafael Jimenez-Flores, Ohio State University, jimenez-flores.1@osu.edu
Dr. Ronan Lordan, University of Pennsylvania, ronan.lordan@pennmedicine.upenn.edu
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We are looking forward to hearing from you and we thank you for your assistance.

Kind regards

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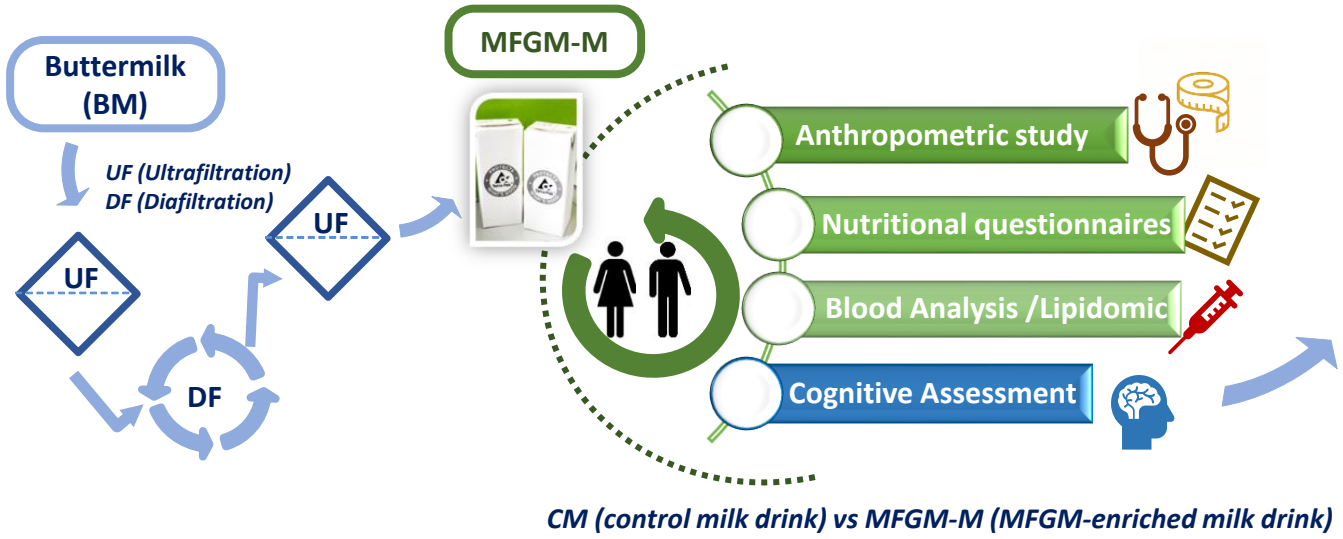
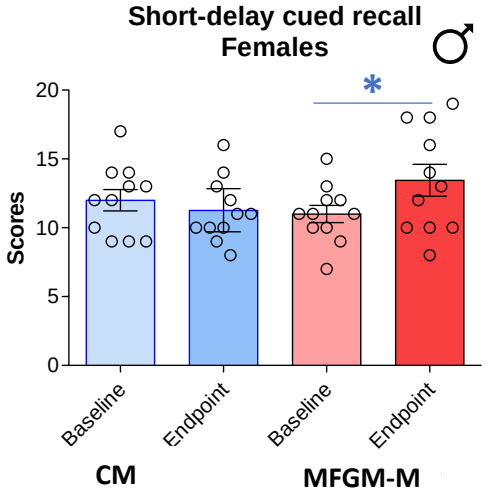
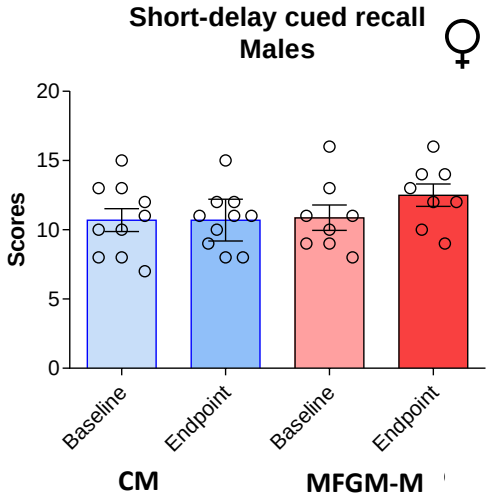
Ethical Statements:

The study protocol was approved by the local Ethics Committee of the IMDEA Food Foundation (IMD PI037) and carried out in accordance with The Code of Ethics of The World Medical Association (Declaration of Helsinki (World Medical, 2013). Participants were recruited from several centers of the Senior UNED University for older adults, located in the Autonomous Community of Madrid, Spain. Written informed consent was obtained from all subjects prior to starting the trial.

Graphical Abstract







Highlights

- MFGM is a source of polar lipids and glycoproteins that could be exploited for nutraceutical.
- Dairy polar lipids of MFGM could prevent age-associated mild cognitive impairment.
- MFGM supplementation augments short-term memory in the elderly.
- An increasing body of evidence suggests that MFGM has nootropic effects.

Milk fat globule membrane-enriched milk improves episodic memory: A randomized, parallel, double-blind, placebo-controlled trial in older adults

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Running Title: MFGM improves episodic memory in older adults.

Abstract

Cognitive decline is one of the most important consequences of aging and pharmacological therapies had been largely unsuccessful. Other strategies include the use of functional foods to reduce the burden of cognitive decline. The MFGM is an important source of polar lipids and glycoproteins that decline in the aging brain. We have developed a milk drink fortified with MFGM (MFGM-M) as a dietary supplement in a CRT pilot study. Forty-four > 65-year-old healthy or mildly cognitively impaired subjects received MFGM-M or control milk for 14 weeks, during which they underwent a battery of cognitive tests. Lipidomic analyzes were also performed. The female participants showed improvement in episodic memory, the ability to recall events in our lives. It is conceivable that any intervention should be started before clinical symptoms manifest, as a preventive measure against cognitive decline. Future long-term studies may shed further light on this point.

Keywords: milk fat globule membrane (MFGM), Cognitive decline, Memory, Aging, Phospholipids, Sphingolipids, Randomized controlled trial.

1 Introduction

Cognitive decline is one of the major consequences of aging and, when this decline progresses to some form of dementia, it has extensive socioeconomic repercussions (Long & Holtzman, 2019). By some estimates, 1.7% of 65- to 69-year-olds have dementia and the risk of developing it doubles every five years after that (Arvanitakis, Shah, & Bennett, 2019) (Collaborators, 2022). As life expectancy increases, so is the prevalence of cognitive decline, ranging from mild impairment to dementia to Alzheimer's disease (AD) (GBD 2019 Dementia Forecasting Collaborators, 2022). At present, about 50 M people around the world have dementia (an umbrella term that encompasses a range of conditions from mild cognitive decline to frank AD), a number expected to rise to 82 M by 2030 and 150 M by 2050 (Arvanitakis et al., 2019; GBD 2019 Dementia Forecasting Collaborators, 2022).

While pharmacological research is actively yet unsuccessfully searching for therapies (with the exception of the recently FDA-approved lecanemab (Walsh, Merrick, Richard, Nurock, & Brayne, 2022), some preventive measures are being proposed and tackle the initial steps of cognitive decline. Among such strategies, a healthy lifestyle that includes regular physical exercise and intellectual activity is of primary importance (Kulmala et al., 2021; Rajah Kumaran et al., 2022). In addition, the use of supplements/functional foods is being actively studied to lessen the burden of cognitive decline and dementia (Dorman et al., 2021; Visioli & Burgos-Ramos, 2016). It is worth noting that, during aging, the central nervous system becomes diminished of phospholipids (PLs) and, in particular, of the polyunsaturated fatty acid (PUFA) docosahexaenoic acid (DHA) (Castro-Gomez, Garcia-Serrano, Visioli, & Fontecha, 2015). This loss has been associated with an increased prevalence of dementia and, specifically, AD. Therefore, PLs supplementation to individuals at risk of cognitive decline could prevent its onset or lessen its consequences (Perez-Galvez et al., 2018).

One nutritionally-relevant source of polar lipids and glycoproteins is the milk fat globule membrane (MFGM) (Fontecha et al., 2020; Pawar, Zabetakis, Gavankar, & Lordan, 2023). Milk fat globules are made of a core, mainly composed of triacylglycerides (TAG; 98–99%), and different concentrations of other compounds such as diacylglycerides (DAG), monoacylglycerides (MAG), free fatty acids (FFAs), and cholesterol (Chol) (Fontecha et al., 2020). This core is surrounded by the MFGM, which is composed of membrane-specific proteins but also of different PLs and sphingolipids potentially useful in neurological pathologies (Fontecha et al., 2020). Buttermilk (BM) is a by-product of butter manufacturing particularly rich in polar lipids from MFGM (up to 20% of total fat). Although BM downstream processing often consists of evaporation and spray drying, membrane separation processes can be also applied to ultrafiltration or microfiltration to further increase the PLs content of the MFGM-enriched powder (Fontecha et al., 2020). Alternatively, PLs-rich fractions could be obtained from BM lipids by using food-grade solvents (Perez-Galvez et al., 2018). There are some interesting approaches to preparing MFGM-enriched ingredients developed at laboratory or pilot scale (Señorans et al., 2023; Calvo et al., 2020), however, their scale-up is often limited by technological or economic considerations.

Currently, there are two areas where MFGM-enriched products could be conceivably exploited: infant nutrition and prevention of age-associated cognitive decline (Fontecha et al., 2020; Pawar et al., 2023). In line with this, in previous pre-clinical studies in a rat model of aging, we demonstrated that an MFGM-rich concentrate, obtained from BM fat by pressurized liquid extraction, alone or in combination with a krill oil concentrate, modulated the miRNA expression (Crespo et al., 2018), improved hippocampal insulin resistance and synaptic signaling (Tome-Carneiro et al., 2018), improved spatial working memory abilities (Baliyan et al., 2023), and reduced emotional memory (contextual fear conditioning) of aged

rats (Garcia-Serrano et al., 2020). Dietary supplementation with a BM concentrate enriched in PLs by ultrafiltration/diafiltration processing was also able to improve the spatial working memory of aged rats and to cause important changes in synaptosomal membrane lipid composition from the hippocampus and the frontal cortex (Baliyan et al., 2023).

By applying membrane processes, we developed an MFGM/PLs-enriched dairy drink from BM to be used as a dietary supplement in a double-blind, randomized, placebo-control pilot study with a cohort of older adults.

The primary goal was to assess whether the intake of a MFGM-enriched product could be beneficial in counteracting the age-associated decline in cognitive functions. Additionally, secondary objective outcomes were to investigate the plasma and erythrocytes concentrations of lipid biomarkers of older adults after milk fat globule membrane enriched milk (MFGM-M) intake as a diet supplementation.

2 Materials and Methods

2.1 Elaboration of the functional milk drink

A milk fat globule membrane-enriched milk product (MFGM-M) has been developed from BM, a dairy whey by-product from the production of butter, through various membrane processes (ultrafiltration and concentration). This process was carried out in collaboration with INNOLACT S.L. (Lugo, Spain) and the Aula de Productos Lácteos (APLTA, USC, Spain). After production, the MFGM-M was heat-treated via UHT to achieve an optimal microbiological quality and was packaged in 200 mL tetra bricks. A UHT skim milk was used as the control (CM). The global composition, the fatty acid (FA) composition, and the lipid classes profile of both products are shown in **Supplementary Table 1**.

2.2 Subjects and intervention

The study protocol was approved by the local Ethics Committee of the IMDEA Food Foundation (IMD PI037) and carried out in accordance with The Code of Ethics of The World Medical Association (Declaration of Helsinki (World Medical, 2013). Participants were recruited from several centers of the Senior UNED University for older adults, located in the Autonomous Community of Madrid, Spain. Written informed consent was obtained from all subjects prior to starting the trial.

We ran a 14-week randomized, parallel, double-blind, placebo-controlled nutritional clinical study with the two groups. One group consumed 200 mL/day of MFGM-M and the control group consumed 200 mL/day of CM. All study personnel and participants were masked to treatment assignment.

Besides receiving general recommendations on physical activity and being informed to follow a low-calorie diet, participants were instructed to consume either CM or MFGM-M, at any time of the day, daily for 14 weeks. Both products were provided in 200 mL bricks of identical appearance and taste. Participants who habitually consumed dairy products were requested to replace one daily dairy ration with the corresponding study product. Since deviations in the dietary intake or physical activity could affect the results obtained, volunteers were asked to fill out a complete nutritional survey (Three-day Regular Food Registry) and a questionnaire concerning physical activity.

The inclusion criteria were: age over 60; ability to perform common activities of daily living independently; cognitively healthy or with mild cognitive impairment (MCI) after conducting a comprehensive semi-structured interview. Exclusion criteria included having any of the following: a Mini-Mental State Examination (MMSE-Spanish version MEC-35) score ≤ 24 (Lobo, Ezquerra, GómezBurgada, Sala, & SevaDíaz, 1979); a neurodegenerative

disorder; chronic disabling disorder, severe cognitive impairment; severe sensory deficit (severe hearing loss, blindness, etc.); any metabolic disease diabetes or stroke. Nutritional-related criteria for exclusion included allergy to fish or milk, lactose intolerance, dietary restrictions impeding the consumption of the nutritional supplement, unwillingness to consume the nutritional supplement with the indicated frequency, consumption of fatty fish more than twice a week, supplements rich in omega 3 FA or psychotropic drugs capable of reducing cognitive function outcomes, such as benzodiazepines or antipsychotics. Finally, volunteers who were not able or willing to reach the study site were also excluded.

2.3 Study products: composition, appearance, compliance, and tolerance

Both products (CM and MFGM-M) had the same packaging and appearance and had to be consumed during daily meals (before, during, or after breakfast, lunch, or dinner). At visit 1 (V1) all volunteers took home the totality of units necessary to complete the entire intervention period. For monitoring purposes, each volunteer was instructed to answer questionnaires regarding intolerance and sensory perception and to daily register study product consumption.

2.4 Study design

Eighty-six volunteers, out of the 130 initially available for participation, were excluded before randomization for not meeting the inclusion/exclusion criteria, not attending the screening visits (V0), or because they decided not to participate due to distance or personal reasons (**Figure 1**). Four volunteers decided to stop participating during the intervention period owing to intolerance to the product, difficulties with attending the last visit, or unrelated illnesses. At endpoint, the 40 volunteers who finished the study reported 100% compliance and were all included in the statistical analyses.

2.5 Analytical methods

2.5.1 Sample size calculation

Due to the lack of previous studies carried out with the functional food we studied, this pilot study was designed to be conducted with a sample size of 40 subjects (men and women). This study was intended to serve as a basis for fine-tuning the sample size in subsequent studies, considering the main efficacy variable.

2.5.2 Cognitive assessment

Volunteers were recruited in cultural and educational centers of several municipalities of the Autonomous Community of Madrid, and cognitively assessed by the Cogni-UNED research group.

The assessment of cognitive function was carried out by psychologists, both at the beginning and at the end of the intervention period (14 weeks), in educational centers and cultural centers frequented by the participants of the study.

After conducting a semi-structured interview, the following evaluation protocol was applied to assess the participants' cognitive abilities: 1) episodic verbal memory: The Spanish version of the CVLT, the TAVEC (Benedet & Alejandre, 2014) in visit 1 (V1) and the Verbal Selective Reminding Rest (VSRT) (Buschke, 1984) in visit 2 (V2); 2) visuospatial memory: copy and immediate recall of Rey–Osterrieth Complex Figure test (Osterrieth, 1944; Rey, 1941) in V1 and Taylor Complex Figure test (Taylor, 1969) an alternate form for the Rey-Osterrieth Complex Figure, in V2 (del Pino, Pena, Ibarretxe-Bilbao, Schretlen, & Ojeda, 2015; Groth-Marnat, 2000; Schaefer, Thakur, & Meager, 2021); 3) Verbal fluency production: i) Phonemic fluency test, in which participants are asked to provide as many words as possible in 1 min beginning with the letter /p/, in visit 1 and with letter /m/ in visit

2 (Peña-Casanova, 1991); ii) Semantic fluency (category animals), in which participants should provide as many names of animals as possible in 1 min in V1 and names of kitchen tools in visit 2 (Peña-Casanova, 1991); 4) Processing speed, attention, and executive function: the Trail Making Test (TMT-A and-B) were used in V1. TMT-A was used to evaluate attention and psychomotor processing speed, and TMT-B to assess attention-switching and executive function performance (Reitan & Wolfson, 1993). The Letter-number sequencing subtest of the Wechsler Adult Intelligence Scale (WAIS) (Wechsler, 1997) was used in V2; 5) Working memory: The Digit Span Forward subtest of the Barcelona test (Peña-Casanova, 1991) was used in V1. Digit Span Forward was used as a measure of the attention and memory span component of working memory. The Digit Span Backward subtest of the Barcelona test (Peña-Casanova, 1991) was used in V1 and of the Wechsler Adult Intelligence Scale (Wechsler, 1997) at the endpoint. This test was used to evaluate the executive part of working memory (Conklin, Curtis, Katsanis, & Iacono, 2000).

Raw scores of all cognitive tests were corrected according to the normative data available for the Spanish population (Campo, Morales, & Martinez-Castillo, 2003; Peña-Casanova, 1991).

2.6 Nutrition assessment and anthropometric and biochemical and physical activity

We performed the following analyses and measurements at baseline and at the end of the study:

Dietetic assessment. All food and beverages consumed by the subjects were recorded using a "Three-day food and drink record" validated for the Spanish population (Ortega, Pérez-Rodrigo, & López-Sobaler, 2015), at the beginning and end of the intervention. Participants received training before the intervention period and reinforcement training during it, in order

to record the weight of all foods consumed when possible and to record household measurements (tablespoons, cups, etc., portion sizes [training reinforced using photographs]) when not. The energy and nutritional contents of the foods consumed were then calculated using DIAL software (Salas-Gonzalez, Aparicio, Loria-Kohen, Ortega, & Lopez-Sobaler, 2022).

Anthropometric variables. Height was measured using a Leicester stadiometer (Biological Medical Technology SL, Barcelona, Spain). Weight, body mass index (BMI), total fat mass (TFM%) and total muscular mass (TMM%) were measured using a BF511 Body Composition Monitor (Omron Healthcare Co. Ltd., Kyoto, Japan). Waist circumference (WC) was measured using a Seca 201 non-elastic tape (Quirumed, Valencia, Spain).

Blood pressure. Systolic (SBP) and diastolic blood pressure (DBP) were measured using a Model M3 Automatic Digital Blood Pressure Monitor (Omron Healthcare Co. Ltd., Kyoto, Japan). Measurements were made at baseline and at the end of the study with the subjects comfortably seated and having neither eaten nor exercised in the previous 30 minutes. A minimum of three readings were taken at intervals of at least 1 min, and the mean SBP and DBP calculated.

Physical Activity. The International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003) was administered at the beginning and end of the intervention to quantify physical activity.

Blood analysis. Subjects were instructed to fast overnight before each blood collection. Blood samples were collected in K_3EDTA tubes (BD Vacutainer, Franklin Lakes, NJ, USA) at each visit between 08:00 and 10:00 to minimize circadian variations and were processed within 48 h of collection. Total cholesterol (TC), HDLc, LDLc, glucose, and TAG levels were determined by enzymatic spectrophotometric assays using an Architect CI8200 instrument

(Abbott Laboratories, IL, USA). Apolipoprotein A-I and B (ApoA-1 and ApoB) levels were determined by immunonephelometry using an Image instrument (Beckman Coulter Inc, CA, USA); insulin was determined by a luminescent immunoassay using the above mentioned Architect CI8200 device.

2.7 Lipidomic analyses

Blood tubes for the collection of the plasma and erythrocyte fractions were centrifuged at 1500x*g*, for 15 min (RT). Plasma and erythrocytes lipids were extracted using the method used by García-Serrano et al. (Garcia-Serrano et al., 2020). Once evaporated under a nitrogen stream and weighted, the lipid extracts were stored at −35 °C until further lipidomic analysis. Briefly, lipid classes were analyzed by HPLC-ELSD, FA methyl esters (FAMEs) by GC-MS and TAGs molecular species, and Chol by GC-FID as described previously (Castro-Gomez, Montero, & Fontecha, 2017). All assays were carried out in triplicate.

2.8 Statistical Analyses

Statistical analysis was performed using the R Statistical Software v.2.15. Continuous descriptive variables were expressed as means and 95% confidence intervals (95% CI) or as mean ± standard error of the mean (SEM). Two-way repeated measures analysis of variance (ANOVA) was used to examine the effect of time and treatment (MFGM-M or CM) on the measured variables with Bonferroni *post hoc* tests. A significant time x treatment interaction indicated that the effects of the groups were different. Data were adjusted for covariates (sex, age, and energy intake). Values of $p < 0.05$ were considered significant for all statistical tests.

3. Results

At baseline, no significant differences were found between groups for any of the anthropometric and plasma variables analyzed, with the exception for the plasma concentrations of phosphatidylserine (PS), or between adjusted cognitive scores in the different cognitive test used, indicating that randomization was effective (**Tables 1, 2A, and 2B**).

3.1 Safety, tolerance, and organoleptic properties of the study products

The safety of the study products was supported by the minimal changes observed in the levels of liver enzymes (GOT, GPT, GGT, AP), total bilirubin, creatinine, and blood pressure (**Table 1**).

During the intervention period, volunteers were asked to register any side effects (severe gastrointestinal symptoms, nausea, heartburn, diarrhea, constipation, bloated belly, bad breath, etc.) that could be related to the consumption of the study products. Constipation and bloated belly were reported by 43% and 33% of volunteers, respectively, whereas other side effects were noted by less than 3% of subjects. The organoleptic characteristics of the study products were positively rated by 80% of volunteers.

3.2 Evolution of anthropometric and biochemical variables, and physical activity

The changes in weight, BMI, TFM (%), and TMM (%) were not statistically different between groups (**Table 1**). At endpoint, the control group showed a mild increase in the levels of LDLc and, particularly, TC, which slightly overpassed the desirable cardiovascular risk threshold (<200 mg/dL). Both groups, especially the control group, showed near optimal HDLc and TAG levels, e.g. HDLc> 60mg/dL. A cut-off value of 0.82 in the Apo B/Apo A-I ratio, a suitable tool for acute risk assessment in cardiac ischemic patients with was reported

to predict multi-vessel coronary artery disease with complex lesion morphology (Galal, Samir, & Shehata, 2020). Here, levels of this ratio were well below this cut-off value in both groups.

3.3 Cognitive assessment

Evolutions of cognition variables are shown in **Tables 2A and 2B.** No significant baseline differences between groups were observed in the cognitive test scores (all $p < 0.300$ **Tables 2A and 2B**). A significant improvement was observed in phonetic fluency between baseline and endpoint in older females ($p<0.002$), although there was no significant effect of treatment ($p < 0.257$) treatment x interaction ($p< 0.559$). Interestingly, the MFGM-M group showed an improvement compared to the control group in short-delay cued-recall episodic verbal memory although this increase was only significantly observed in females ($p<0.044$), but not in males (**see Table 2A, 2B and Supplementary Figure 1**). Neither male nor female older adults experienced any major beneficial effects from dietary supplementation with an MFGM concentrate in the other studied cognitive domains, including; attention, processing speed, visuospatial abilities, verbal fluency, executive function, or working memory.

3.4 Lipid classes distribution and fatty acid composition in plasma and erythrocytes

Lipidomic analyses revealed that lipid classes distribution in plasma and erythrocytes of volunteers was not affected by the consumption of MFGM-M (**Table 3**), with both groups exhibiting a similar evolution after 14 weeks of intervention. Plasma lipids are used as short-term indicators of dietary intake whereas erythrocyte lipids are a reliable indicator of overall FA status (Harris, 2010). The FA composition of plasma and erythrocytes from volunteers are shown in **Table 4 and Table 5,** respectively. Overall, consumption of MFGM-M did not significantly alter concentrations of circulating FA, which did not statistically differ between

groups, after 14 weeks. Particularly the ω3 LC-PUFAs did not increased, which could largely explain the modest effects on studied cognitive variables.

4 Discussion

Cognitive decline and its sequelae, e.g. learning and memory impairment are becoming one of the most prominent undesirable consequences of aging (Long & Holtzman, 2019). Given the continuous increase in lifespan that the world is witnessing, the prevalence of mild cognitive impairment and dementia is very likely to greatly affect healthcare systems worldwide (Long & Holtzman, 2019; Massot Mesquida, Folkvord, Seda, Lupianez-Villanueva, & Toran Monserrat, 2021) and pharma-nutritional interventions are urgently needed (Loughman et al., 2021). Some human trials do suggest that cognitive decline rate might be controlled via the provision of various nutrients, including PUFAs (Davies, Frampton, Fuad, & Slykerman, 2023; Scholey et al., 2013; Soininen et al., 2017).

In this pilot study, we studied the effects of a 14-weeks daily intake of a functional milk drink enriched in PLs from MFGM (6.8 *vs* 0.29% in CM), namely PS and PI on cognitive function in non-demented older adults. The main finding in this intervention study is that diet supplementation improved short-delay cued recall of verbal episodic memory in female, but not male, older adults. Other cognitive domains including attention, working memory of visuospatial abilities were not significantly affected by this treatment. Anthropometric measurements or lipid plasma and erythrocyte biomarkers were not significantly altered by supplementation of MFGM-M as indicated by lipidomic analyses.

Here we observed that female, but not male, older adults that received a diet supplementation with a MFGM-M enriched in PLs, mainly PS and PI, showed an

improvement in verbal episodic memory, which is the ability to remember what happened in our lives (Gallagher & Koh, 2011). This result is in line with earlier clinical trials that have found administering PS resulted in clear gains in memory capabilities within a cognitively impaired population (Crook et al., 1991; Zhang, Yang, & Guo, 2015; Zheng, Fleith, Giuffrida, O'Neill, & Schneider, 2019), as well as with studies that showed the positive effects of PS enriched with ω3 LC-PUFA (Vaisman et al., 2008; Vaisman & Pelled, 2009). However, other authors (Soininen et al., 2017) did not observe any improvement in cognitive abilities when tested PL-based products to counteract the cognitive decline in people with prodromal Alzheimer's disease. The effect of MFGM supplementation on enhancing episodic memory in older adults might be of relevance as this type of memory is one of the earliest cognitive domains impaired in amnestic mild cognitive impairment and in AD (Langbaum et al., 2014; Petersen et al., 1999). Preclinical studies indicated that the administration of milk polar lipids to aged rats was able to modulate the miRNA expression (Crespo et al., 2018), to improve hippocampal insulin resistance and synaptic signaling (Tome-Carneiro et al., 2018), and lower emotional memory (contextual fear conditioning) in aged rats (Garcia-Serrano et al., 2020). Additionally, dietary supplementation with an MFGM concentrate from buttermilk caused the increase of PS (18:1/18:1) level in synaptosomes from the hippocampus and the frontal cortex along with an enhancement of the spatial working memory in aged rats (Baliyan et al., 2023).

Recently, Zhou et al. (2023) reported that MFGM oral administration not only improved spatial memory in male mice, but also increased the number of neurons in the dentate gyrus of the hippocampus, and modulated the expression of proteins that may promote synapse formation and signaling pathways that are related to cognitive processes.

Short-delay cued recall of verbal episodic memory requires both storage of learned words from the target list along with executive processes of working memory, since the information from the interference list has to be inhibited, while the target list is required to be recalled (Hasher & Zacks, 1988; Lange & Oberauer, 2005). The correct functioning of the prefrontal cortex is critical to avoid the retroactive interference that may occur when learning verbal information interferes with the recall of words previously learned (Dewar, Cowan, & Sala, 2007; Sakai & Passingham, 2004). In this sense, older adults are particularly prone to retroactive interference, as they show inefficient inhibitory control mechanisms in reducing interference of irrelevant stimuli that increase their working memory load (Hedden & Park, 2001; Sakai & Passingham, 2004). However, we did not observe any significant improvement in working memory as assessed in the Digit Span Backward test. In contrast, we recently reported that aged rats supplemented with MFGM in the diet, performed better at a spatial orientation/memory-based task (spatial working memory task in the Morris water maze) (Baliyan et al., 2023).

Concerning the sex-specific effect of the diet on episodic memory, it may be hypothesized that the higher levels of PS and PI present in MFGM-M supplementation may have led to higher plasma levels of PS and PI in females rather than in males. However, according to our lipidomic analyses, no significant changes were observed in the plasma and erythrocytes lipid composition of females at the endpoint.

Previous research studies indicated an improvement in working memory in humans after diet supplementation by PLs such as PS (Hellhammer, Waladkhani, Hero, & Buss, 2010). Interestingly, dietary lipids supplementation with soy-derived PS for two weeks in young adults improved performance in a serial subtraction test (Parker et al., 2011), that depends on working memory (Hittmair-Delazer, Semenza, & Denes, 1994). In contrast, other

authors indicated that under psychosocial stress conditions, dietary intake of bovine milk PLs improved reaction time performance on an attention-switching task, but did not improve working memory in men (Boyle et al., 2019). Demographic characteristics of the cohort sample may also account for the differences observed in our study compared to previous studies. Thus, most previous research though has been done on male participants whereas the current study design included both sexes. However, overall, due to the reduced sample size of our pilot study we can only speculate that a larger study would indeed confirm the positive effects of MFGM-M on verbal episodic memory, which is frequently impaired during mild cognitive impairment and associated cognitive disorders (Garcia-Herranz, Diaz-Mardomingo, & Peraita, 2016; Melrose, Harwood, Khoo, Mandelkern, & Sultzer, 2013; Petersen et al., 1999).

Epidemiological data are strongly suggestive of an association between dairy food intake and cognitive function (Duplantier & Gardner, 2021), although the mechanisms underlying such association are still elusive (Visioli & Burgos-Ramos, 2016). Dairy products are rich in polar and complex lipids, similar to those found in the brain, yet their concentrations in the brain and body have been reported to decline with age (Camfield, Owen, Scholey, Pipingas, & Stough, 2011; Perez-Galvez et al., 2018; Scholey et al., 2013; Soininen et al., 2017). Specifically, PS is a major component of the brain, and a decrease in major PS species contents was reported to occur during aging in rodents (Lin et al., 2016; Smidak, Kofeler, Hoeger, & Lubec, 2017). Furthermore, Wackerlig et al. (2020) observed that compared to aged cognitively unimpaired rats, aged animals with impaired spatial memory have significantly lower brain PS contents, thereby providing further evidence of a strong connection between PS contents and the decline in neuronal function over time. PS plays important cellular roles: for example, it participates in mitochondrial membrane integrity, the

release of presynaptic neurotransmitters, the activity of postsynaptic receptors, and the activation of Protein Kinase C in memory formation (Glade & Smith, 2015; Kim, Huang, & Spector, 2014). In animals, PS has been shown to attenuate many neuronal effects of aging, and to improve or restore memory on a variety of tasks (Lee et al., 2015; Ye, Han, Kim, Kim, & Shim, 2020; Zhang et al., 2015). A decrease of SM in cerebral myelin has been related to the slowing in the processing speed associated with aging (Huo et al., 2020; Mielke et al., 2010). For older adults with subjective memory complaints, several studies reported that the intake of PS containing omega-3 FA for 15 weeks improve verbal episodic immediate recall (Richter, Herzog, Lifshitz, Hayun, & Zchut, 2013; Vakhapova, Cohen, Richter, Herzog, & Korczyn, 2010). Further, PI and its phosphorylated derivatives (phosphoinositides) are key secondary messengers in the cell that play an active role as regulators in membrane trafficking and cellular signaling (Ashlin, Blunsom, & Cockcroft, 2021; Morita & Ikeda, 2022). These molecules are abundant in brain tissue where they have been suggested to exert key functions in intracellular signal transduction and to be effective in improving brain function (Perez-Galvez et al., 2018). Additionally, there is evidence that the oral administration of PI stimulates the development and proliferation of nerve cells and improves spatial memory and learning ability in rats (Shin et al., 2020). More recently, their involvement as modulators in the microglial actin remodeling and phagocytosis in Alzheimer's disease has been reported (Desale & Chinnathambi, 2021).

In conclusion, we show that provision of an MFGM-based drink is safe and well tolerated. In terms of effectiveness, it appears that MFGM-M may improve cognitive performance in volunteers as indicated by the results obtained in the short-delay cued-recall episodic subtest. It is conceivable and agreed on by the majority of cognitive impairment research studies that any intervention should be started early in life before clinical symptoms manifest (Frank et

al., 2021). Future, long-term studies, albeit expensive and time-consuming might shed further light on this point. The issue of whether the challenging and quite young field of nutritional psychiatry (Jacka, 2017) could greatly contribute to the prevention and/or treatment of age-associated cognitive impairment (Marx, Moseley, Berk, & Jacka, 2017) requires further research.

5 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6 Author Contributions

JF, CV and FV contributed to conception and design of the study. MVC and JF provided dietary supplement and performed lipidomic analyses, VLC organized the nutritional study and supervised the anthropometric assays, CDM, SGH, SB and CV recruited and selected the participants, performed and supervised the cognitive assessment, MVC, JF, CV and GC organized the database, performed statistical analysis and designed figures. MVC, JF, FV and CV wrote the first draft of the manuscript. JTC wrote sections of the manuscript. JF supervised the project. All authors contributed to manuscript revision, read, and approved the submitted version.

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Figure 1. Study flow.

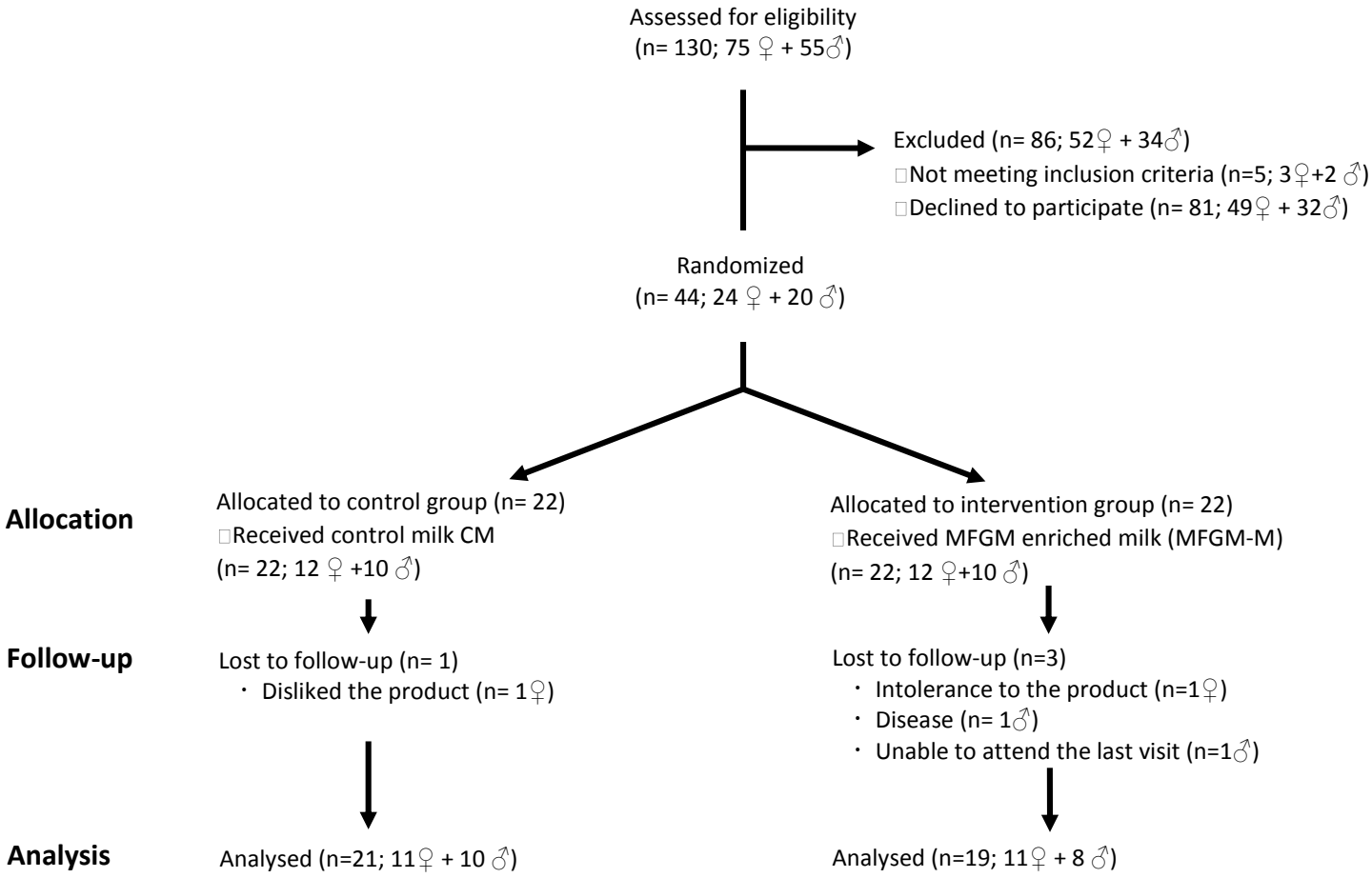

Table 1: Evolution of anthropometric and blood biochemical variables, and physical activity before and after 14 weeks of intervention. Data are means and 95% confidence intervals (IC 95%) .CM, control milk; MFGM-M, MFGM-enriched milk.

Variable	CM		MFGM-M		*Baseline (p)*	*Effect (p)*		
	Baseline	14 weeks	Baseline	14 weeks		time	treatment	time xtreatment
Weight (kg)	72.2 (67.1-77.2)	74.0 (66.9-81.1)	76.7 (68.1-85.2)	72.6 (66.9-78.2)	*0.7120*	*0.0441*	*0.6483*	*0.9108*
BMI (kg/m^2)	27.5 (25.7-29.3)	27.5 (25.4-29.5)	27.5 (25.4-29.6)	28.2 (25.7-30.6)	*0.9970*	*0.0418*	*0.9583*	*0.6518*
TFM (%)	35.4 (31.1-39.7)	35.1 (30.6-39.7)	35.3 (31.4-39.3)	36.0 (31.6-40.5)	*0.9989*	*0.0054*	*0.9321*	*0.4407*
TMM (%)	27.0 (25.0-29.0)	27.0 (24.9-29.1)	26.8 (24.9-28.6)	26.6 (24.5-28.7)	*0.9227*	*0.0141*	*0.7966*	*0.8668*
WC (cm)	95.1 (89.8-100.4)	94.7 (88.1-101.3)	96.7 (90.7-102.8)	98.6 (92.0-105.2)	*0.7354*	*0.0655*	*0.7152*	*0.4383*
TC (mg/dl)	199.1 (185.5-212.7)	208.1 (196.6-219.7)	183.2 (167.7-198.7)	185.2 (166.9-203.5)	*0.1830*	*0.0719*	*0.052*	*0.3416*
LDLc	115.6 (105.6-125.6)	122.3 (112.1-132.5)	103.1 (90.13-116.2)	107.1 (92.3-121.9)	*0.1969*	*0.1431*	*0.0828*	*0.6055*
HDLc	65.58 (58.97-72.19)	67.56 (59.96-75.16)	59.18 (53.1-65.26)	56.54 (50.11-62.97)	*0.2599*	*0.6684*	*0.051*	*0.3224*
TAG	89.6 (79.7-99.5)	91.28 (79.85-102.7)	104.3 (89.22-119.4)	107.9 (91.1-124.7)	*0.1642*	*0.492*	*0.078*	*0.9573*
Apo A1	153.2 (141.1-165.4)	196.1 (177.9-214.2)	143.5 (134.4-152.5)	180.3 (161.3-199.3)	*0.5543*	*0.0000*	*0.1202*	*0.6672*
Apo B	123.8 (118.8-128.7)	115.9 (109.4-122.5)	118.9 (111.8-126.1)	112.3 (99.7-124.8)	*0.5445*	*0.0011*	*0.3784*	*0.8910*
Apo B/Apo A1	0.83 (0.75-0.91)	0.62 (0.54-0.7)	0.84 (0.78-0.9)	0.64 (0.56-0.72)	*0.9754*	*0.0000*	*0.7587*	*0.8800*
Creatinine	0.8 (0.74-0.86)	0.79 (0.73-0.85)	0.85 (0.75-0.95)	0.88 (0.78-0.98)	*0.5055*	*0.9063*	*0.1129*	*0.3364*
GOT	22.1 (18.92-25.28)	17.9 (15.98-19.82)	19.95 (18.23-21.67)	19.11 (16.37-21.85)	*0.3682*	*0.0098*	*0.6645*	*0.0918*
GPT	23.48 (16.42-30.54)	16.8 (13.33-20.27)	17.23 (15.11-19.35)	16.94 (13.59-20.29)	*0.0825*	*0.0491*	*0.1723*	*0.0721*
SBP	138.6 (129.9-147.3)	141.8 (134.1-149.5)	132.9 (122.7-143.1)	134.9 (127.8-142.1)	*0.4938*	*0.4125*	*0.1977*	*0.5021*
DBP	81.3 (76.1-86.5)	78.5 (73.6-83.4)	81.6 (76.0-87.3)	81.2 (74.7-87.7)	*0.9932*	*0.0991*	*0.8351*	*0.7027*
Urea	35.3 (32.3-38.3)	39.2 (36.1-42.3)	38.3 (34.0-42.6)	40.1 (36.0-44.3)	*0.5401*	*0.0556*	*0.6468*	*0.2337*
Glucose (mg/dl)	100.2 (76.1-86.5)	99.6 (90.2-108.9)	96.1 (90.3-101.9)	95.4 (87.3-103.5)	*0.6512*	*0.6129*	*0.4023*	*0.7206*
Total physical activity	1788 (1427-2149)	1900 (1516-2284)	1968 (1484-2452)	1636 (1339-1933)	*0.7051*	*0.3907*	*0.9082*	*0.0559*

Apo, apolipoprotein; BMI, body mass index;; DBP, diastolic blood pressure; GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase; HDLc, high density lipoprotein cholesterol; LDLc, low density lipoprotein cholesterol; TFM, total fat mass; TMM, total muscular mass; WC, waist circumference; SBP, systolic blood pressure; TAG, triglycerides; TC, total cholesterol.

Table 2A. Evolution of cognitive scores after 14 weeks of intervention in older females. Data are means and 95% confidence intervals (IC 95%). CM, control milk drink; MFGM-M, MFGM-enriched milk .

	CM		**MFGM-M**		***Baseline (p)***	***Effect (p)***		
	Baseline	Endpoint	Baseline	Endpoint		time	treatment	Time x treatment
Visuospatial abilities								
Direct copying	13.27 (11.33-15.21)	12.18 (10.37-13.15)	11.73 (9.40-14.06)	10.18 (8.26-12.10)	*0.4440*	*0.1898*	*0.0879*	*0.8173*
Immediate recall	10.64 (9.31-11.97)	12.36 (10.28-14.44)	9.91 (8.05-11.77)	11.09 (9.50-12.68)	*0.7860*	*0.0930*	*0.3110*	*0.7443*
Attention, Processing speed	11.27 (9.66-12.88)	11.45 (10.18-12.72)	12.64 (10.92-14.36)	11.45 (10.53-12.37)	*0.6004*	*0.4477*	*0.3076*	*0.3035*
Verbal Fluency								
Semantic fluency	10.18 (8.81-11.55)	11.27 (9.94-12.60)	11.64 (10.29-12.99)	9.64 (8.42-10.86)	*0.9817*	*0.2055*	*0.8957*	*0.0347*
Phonetic fluency	11.45(10.57-12.33)	13.73 (12.55-14.91)	10.91 (9.17-12.65)	12.55 (11.39-13.71)	*0.7690*	*0.002*	*0.2572*	*0.5596*
Episodic verbal memory								
Short-delay free recall	10.45 (8.44-12.06)	11.27 (9.19-13.35)	9.27 (7.92-10.62)	11.55 (9.81-13.29)	*0.5252*	*0.0684*	*0.6053*	*0.3755*
Short-delay cued recall	12.00 (10.49-13.51)	11.27 (9.90-12.64)	11.00 (9.77-12.23)	13.45 (11.20-15.70)	*0.6004*	*0.2257*	*0.5281*	*0.0440*
Long-delay free recall	11.10 (8.84-13.16)	11.36 (9.93-12.79)	10.82 (9.68-11.96)	11.73 (10.10-12.36)	*0.4135*	*0.4468*	*0.9139*	*0.7429*
Long-delay cued recall	12.73 (9.57-11.77)	13.91 (9.03-11.35)	10.82 (9.51-12.13)	14.73 (12.06-17.40)	*0.5677*	*0.0251*	*0.6114*	*0.2094*
Executive function								
TMT-B	11.64 (10.05-13.23)	11.45 (10.18-12.72)	10.00 (7.94-12.06)	11.45 (10.53-12.37)	*0.2219*	*0.3898*	*0.2762*	*0.2718*
Working memory								
Digit span Forward	12.36 (11.30-13.42)	10.18 (8.57-11.79)	11.91 (10.83-12.99)	10.45 (9.10-11.80)	*0.8298*	*0.0018*	*0.9099*	*0.4802*
Digit span Backward	13.09 (11.87-14.31)	11.91 (10.48-13.34)	12.00 (10.29-13.71)	12.64 (10.39-14.89)	*0.5640*	*0.7172*	*0.8187*	*0.2350*

Table 2B. Evolution of cognitive scores after 14 weeks of intervention in older males. Data are means and 95% confidence intervals (IC 95%). CM, control milk; MFGM-M, MFGM-enriched milk.

	CM		**MFGM-M**		***Baseline (p)***	***Effect (p)***		
	Baseline	Endpoint	Baseline	Endpoint		time	treatment	Time x treatment
Visuospatial abilities								
Direct copying	9.70 (7.88-11.52)	11.30 (8.97-13.63)	11.78 (8.90-14.66)	11.75 (9.44-14.06)	*0.3390*	*0.5084*	*0.3604*	*0.4622*
Immediate recall	12.40 (10.54-14.26)	12.90 (11.14-14.66)	12.22 (10.53-13.91)	13.12 (10.65-15.59)	*0.9858*	*0.3878*	*0.9698*	*0.9010*
Attention, Processing speed	9.30 (9.66-12.88)	9.85 (7.59-11.01)	11.56 (10.52-12.06)	11.38 (9.58-13.18)	*0.0716*	*0.8784*	*0.0426*	*0.6556*
Verbal Fluency								
Semantic fluency	11.40 (9.81-13.99)	10.50 (9.25-11.75)	11.33 (9.94-12.72)	10.00 (8.67-11.33)	*0.9980*	*0.2161*	*0.7506*	*0.8398*
Phonetic fluency	10.80 (9.72-11.88)	11.40 (9.73-13.07)	9.44 (7.79-11.09)	12.00 (10.67-13.33)	*0.3052*	*0.0663*	*0.6042*	*0.2305*
Episodic verbal memory								
Short-delay free recall	9.76 (8.43-11.09)	10.33 (8.92-11.74)	9.10 (8.26-9.94)	11.21 (10.03-12.39)	*0.6771*	*0.0237*	*0.8789*	*0.1791*
Short-delay cued recall	10.30 (8.65-11.95)	10.90 (9.29-12.51)	11.22 (9.85-12.59)	13.00 (10.94-15.06)	*0.6556*	*0.2444*	*0.1271*	*0.5049*
Long-delay free recall	9.60 (8.25-10.95)	8.90 (7.37-10.43)	10.00 (8.22-11.78)	10.25 (8.94-11.56)	*0.7193*	*0.7315*	*0.3217*	*0.5671*
Long-delay cued recall	9.80 (7.84-11.76)	10.20 (7.20-11.20)	10.22 (8.93-11.51)	12.50 (9.07-15.93)	*0.9612*	*0.3741*	*0.3405*	*0.4846*
Executive function								
TMT-B	10.50 (8.54-12.46)	9.80 (8.09-11.51)	10.78 (8.92-12.64)	11.38 (9.58-13.18)	*0.9068*	*0.8971*	*0.3852*	*0.5166*
Working memory								
Digit span Forward	12.30 (10.52-13.82)	12.70 (10.82-14.58)	13.56 (11.68-15.44)	13.00 (10.90-15.10)	*0.5411*	*0.9440*	*0.4705*	*0.6388*
Digit span Backward	12.00 (10.28-13.82)	13.00 (11.28-14.72)	11.67 (9.85-13.49)	12.62 (10.56-14.68)	*0.9954*	*0.3047*	*0.7128*	*0.8930*

Table 3. Evolution of lipid classes in plasma and erythrocytes before and after 14 weeks of intervention. Data are means and 95% confidence intervals (IC 95%). CM, control milk drink; MFGM-M, MFGM-enriched milk drink.

Plasma								
Lipid classes (g/100g fat)	**CM**		**MFGM-M**		***Baseline (p)***	***Effect (p)***		
	Baseline	14 weeks	Baseline	14 weeks		time	treatment	time xtreatment
CE	43.09 (41.48-44.7)	43.28 (41.14-45.42)	41.93 (38.85-45.01)	40.44 (37.4-43.48)	*0.6766*	*0.4625*	*0.2699*	*0.2943*
TAG	14.67 (13.67-15.67)	14.61 (13.57-15.65)	15.7 (13.64-17.76)	16.2 (14.69-17.71)	*0.4754*	*0.6558*	*0.1566*	*0.5526*
FFA+Chol	6.84 (5.17-8.51)	6.93 (4.91-8.95)	9.02 (6.59-11.45)	9.7 (6.98-12.42)	*0.2189*	*0.775*	*0.1221*	*0.8617*
ΣPL	35.4 (34.11-36.69)	35.17 (33.8-36.54)	33.35 (31.76-34.94)	33.66 (32.11-35.21)	*0.062*	*0.8112*	*0.0567*	*0.4135*
g/100g of PL								
PE	29.12 (27.79-30.45)	29.59 (28.2-30.98)	28.87 (27.36-30.38)	29.19 (27.43-30.95)	*0.9475*	*0.373*	*0.7151*	*0.7446*
PS	0.95 (0.79-1.11)	1.21 (1.01-1.41)	1.01 (0.87-1.15)	1.21 (1.03-1.39)	*0.8399*	*0.0004*	*0.8532*	*0.4847*
PC	62.52 (60.66-64.38)	61.86 (60.21-63.51)	62.99 (61.28-64.7)	62.29 (60.33-64.25)	*0.8781*	*0.1863*	*0.6947*	*0.9903*
SM	7.41 (6.8-8.02)	7.33 (6.8-7.86)	7.12 (6.63-7.61)	7.3 (6.73-7.87)	*0.6417*	*0.7396*	*0.7019*	*0.3441*
Erythrocytes								
Lipid classes (g/100g fat)	**CM**		**MFGM-M**		***Baseline (p)***	***Effect (p)***		
	Baseline	14 weeks	Baseline	14 weeks		time	treatment	time xtreatment
CE	4.58 (3.85-5.31)	7.23 (6.13-8.33)	4.81 (3.71-5.91)	6.62 (5.13-8.11)	*0.9411*	*0.0000*	*0.7103*	*0.1597*
TAG	1.30 (1.06-1.54)	2.03 (1.66-2.4)	1.62 (1.23-2.01)	2.34 (1.81-2.87)	*0.3681*	*0.0000*	*0.2486*	*0.6889*
FFA+Chol	52.17 (50.99-53.35)	49.88 (48.59-51.17)	50.24 (48.89-51.59)	49.66 (48.01-51.31)	*0.0743*	*0.0001*	*0.2468*	*0.0176*
ΣPL	41.96 (40.96-42.96)	40.86 (40.21-41.51)	43.33 (42.57-44.09)	41.38 (40.15-42.61)	*0.0556*	*0.0004*	*0.0644*	*0.3415*
g/100g of PL								
PE	37.94 (36.71-39.17)	37.6 (36.86-38.34)	38.23 (37.5-38.96)	36.53 (33.98-39.08)	*0.9539*	*0.0975*	*0.6454*	*0.2634*
PS	0.80 (0.62-0.98)	0.56 (0.44-0.68)	0.55 (0.39-0.71)	0.47 (0.35-0.59)	*0.0139*	*0.0019*	*0.0374*	*0.1076*
PC	43.96 (42.63-45.29)	46.05 (45.07-47.03)	43.04 (41.79-44.29)	46.15 (44.52-47.78)	*0.5094*	*0.0000*	*0.5174*	*0.4136*
SM	17.25 (15.76-18.74)	15.79 (15.34-16.24)	18.19 (16.76-19.62)	16.85 (14.65-19.05)	*0.5407*	*0.0538*	*0.2069*	*0.8898*

CE, cholesterol ester; FFA+Chol, free fatty acids+ cholesterol;PC, phosphatidylcholine; PE, phosphatidylethanolamine; PL, polar lipids; PS, phosphatidylserine; SM, sphingomyelin; TAG, triacylglycerols.

Table 4. Evolution of FAME in plasma before and after 14 weeks of intervention. Data are means and 95% confidence intervals (IC 95%). CM, control milk; MFGM-M, MFGM enriched milk.

Fatty acid	CM		MFGM-M		*Baseline (p)*	*Effect (p)*		
	Baseline	14 weeks	Baseline	14 weeks		time	treatment	time xtreatment
C14:0	0.32 (0.26-0.38)	0.35 (0.29-0.41)	0.36 (0.3-0.42)	0.38 (0.28-0.48)	0.6363	0.3889	0.4582	0.8036
C15:0	0.04 (0.04-0.04)	0.05 (0.05-0.05)	0.04 (0.04-0.04)	0.04 (0.04-0.04)	0.9993	0.6166	0.8687	0.7417
C12:0 DMA	0.19 (0.17-0.21)	0.19 (0.15-0.23)	0.16 (0.14-0.18)	0.18 (0.16-0.2)	0.1743	0.0673	0.1851	0.3244
C16:0	22.09 (21.29-22.89)	22.52 (21.76-23.28)	22.07 (21.23-22.91)	21.78 (20.86-22.7)	0.9986	0.8384	0.4712	0.1307
C16:1 *cis*7	0.04 (0.04-0.04)	0.04 (0.02-0.06)	0.04 (0.02-0.06)	0.04 (0.04-0.04)	0.5501	0.7917	0.3764	0.936
C16:1 *cis* 9	0.61 (0.41-0.81)	0.62 (0.44-0.8)	0.47 (0.35-0.59)	0.48 (0.34-0.62)	0.296	0.7333	0.2078	0.9259
C18:0 *anteiso*	0.05 (0.05-0.05)	0.05 (0.05-0.05)	0.05 (0.05-0.05)	0.05 (0.05-0.05)	0.9953	0.0561	0.7929	0.5897
C18:0 DMA	0.08 (0.06-0.1)	0.09 (0.07-0.11)	0.06 (0.06-0.06)	0.08 (0.06-0.1)	0.1988	0.2702	0.1537	0.4572
C18:0	5.91 (5.69-6.13)	5.9 (5.66-6.14)	6.05 (5.78-6.32)	6.13 (5.89-6.37)	0.595	0.5867	0.1966	0.453
C18:1 *cis* 9	20.93 (19.54-22.32)	20.78 (19.07-22.49)	22.69 (20.49-24.89)	22.92 (20.92-24.92)	0.263	0.9869	0.1306	0.7967
C18:1 *cis*11	0.56 (0.5-0.62)	0.57 (0.51-0.63)	0.56 (0.48-0.64)	0.56 (0.5-0.62)	0.9998	0.6467	0.9938	0.9658
C18:2 (LA)	36.35 (33.74-38.96)	35.48 (32.7-38.26)	35.14 (32.44-37.84)	34.68 (32.29-37.07)	0.6592	0.1948	0.5442	0.8486
C20:3	1.1 (0.83-1.37)	1.19 (0.92-1.46)	0.86 (0.72-1)	1.06 (0.96-1.16)	0.1442	0.0222	0.1793	0.3498
C20:4 (AA)	10 (8.65-11.35)	10.56 (8.8-12.32)	9.57 (8.39-10.75)	10.3 (9.08-11.52)	0.8159	0.014	0.818	0.4669
C20:5n3 (EPA)	0.47 (0.22-0.72)	0.49 (0.14-0.84)	0.59 (0.28-0.9)	0.26 (0.16-0.36)	0.7684	0.0953	0.7051	0.0672
C22:5n3 (DPA)	0.02 (0-0.04)	0.02 (0-0.04)	0.01 (0.01-0.03)	0.01 (0.01-0.01)	0.6105	0.5887	0.2010	0.8410
C22:6n3 (DHA)	1.24 (0.93-1.55)	1.1 (0.81-1.39)	1.27 (0.98-1.56)	1.05 (0.83-1.27)	0.9667	0.0327	0.9572	0.716
ΣDMA	0.27 (0.23-0.31)	0.28 (0.22-0.34)	0.22 (0.18-0.26)	0.26 (0.24-0.28)	0.1581	0.1111	0.1575	0.3461
ΣSFA	28.42 (27.56-29.28)	28.87 (28.07-29.67)	28.57 (27.69-29.45)	28.38 (27.4-29.36)	0.9445	0.6471	0.7848	0.2306
ΣMUFA	22.13 (20.62-23.64)	22.01 (20.17-23.85)	23.76 (21.45-26.07)	24 (21.9-26.1)	0.347	0.9706	0.1841	0.8012
ΣPUFA	49.18 (47.16-51.2)	48.84 (46.55-51.13)	47.44 (44.68-50.2)	47.36 (44.93-49.79)	0.4184	0.7751	0.3319	0.7498
ΣMCFA	0.37 (0.31-0.43)	0.4 (0.32-0.48)	0.41 (0.35-0.47)	0.43 (0.33-0.53)	0.6611	0.3967	0.4862	0.7949
ΣLCFA	86.53 (84.86-88.2)	85.96 (83.9-88.02)	87.07 (85.74-88.4)	86.64 (85.39-87.89)	0.777	0.0399	0.6833	0.7908
ΣVLCFA	12.83 (11.18-14.48)	13.36 (11.32-15.4)	12.3 (10.99-13.61)	12.68 (11.43-13.93)	0.7775	0.0568	0.6794	0.8054

AA, Arachidonic acid; DHA, docosahexaenoic acid; DMA: dimethyl acetals; DPA docosapentaenoic acid; EPA, eicosapentaenoic acid;LA, linoleic acid;LCFA, long chain fatty acids; MCFA, medium chain fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFA, saturated fatty acids; VLCFA, very long chain fatty acids.

Table 5. Evolution of FAME in erythrocytes before and after 14 weeks of intervention. Data are means and 95% confidence intervals (IC 95%). CM, control milk drink; MFGM-M, MFGM enriched milk.

Fatty acid	CM		MFGM-M		*Baseline (p)*	*Effect (p)*		
	Baseline	14 weeks	Baseline	14 weeks		time	treatment	time xtreatment
C14:0	0.10 (0.08-0.12)	0.12 (0.1-0.14)	0.08 (0.06-0.1)	0.11 (0.07-0.15)	0.133	0.0133	0.1901	0.2594
C16:0 DMA	1.6 (1.5-1.7)	1.47 (1.37-1.57)	1.48 (1.38-1.58)	1.45 (1.31-1.59)	0.1911	0.0119	0.2243	0.3286
C16:0	20.53 (19.9-21.16)	20.63 (20.08-21.18)	20.2 (19.67-20.73)	20.26 (19.57-20.95)	0.604	0.528	0.4352	0.811
C16:1 cis9	0.01 (0.01-0.01)	0.03 (0.01-0.05)	0.02 (0-0.04)	0.03 (0.01-0.05)	0.9466	0.0003	0.8529	0.8252
C17:0	0.02 (0-0.04)	0.04 (0.02-0.06)	0.01 (0.01-0.01)	0.04 (0.02-0.06)	0.1041	0.0001	0.2854	0.1005
C18:0 DMA	2.82 (2.62-3.02)	2.48 (2.36-2.6)	2.74 (2.49-2.99)	2.68 (2.44-2.92)	0.7375	0.0002	0.8998	0.2001
C17:1	0.26 (0.24-0.28)	0.22 (0.2-0.24)	0.26 (0.24-0.28)	0.23 (0.19-0.27)	0.977	0.0000	0.8415	0.9168
C18:0	16.25 (15.84-16.66)	15.26 (14.81-15.71)	16.86 (16.06-17.66)	16.47 (15.55-17.39)	0.3037	0.0015	0.0591	0.2371
C18:1 *cis*9	12.02 (11.43-12.61)	12.48 (11.79-13.17)	12.7 (11.62-13.78)	13.08 (11.88-14.28)	0.374	0.0325	0.2698	0.9848
C18:1 *cis*11	0.19 (0.17-0.21)	0.22 (0.2-0.24)	0.17 (0.15-0.19)	0.2 (0.16-0.24)	0.4413	0.004	0.1801	0.8718
C18:2n6 (LA)	10.94 (9.86-12.02)	13.16 (11.3-15.02)	10.51 (9.33-11.69)	11.77 (9.91-13.63)	0.8706	0.0009	0.3225	0.2083
C20:3	1.16 (1.02-1.3)	1.24 (1.06-1.42)	1.05 (0.93-1.17)	1.21 (1.05-1.37)	0.4343	0.0264	0.4638	0.3785
C20:4 (AA)	26.25 (25.09-27.41)	25.63 (24.16-27.1)	26.09 (24.56-27.62)	25.49 (23.73-27.25)	0.9749	0.1364	0.9535	0.4721
C20:5n3 (EPA)	0.4 (0.22-0.58)	0.35 (0.17-0.53)	0.46 (0.28-0.64)	0.34 (0.14-0.54)	0.8082	0.0637	0.826	0.4033
C22:5n3 (DPA)	0.82 (0.72-0.92)	0.8 (0.7-0.9)	0.69 (0.61-0.77)	0.7 (0.66-0.74)	0.0608	0.6754	0.0295	0.995
C22:6n3 (DHA)	4.88 (4.14-5.62)	4.28 (3.67-4.89)	5.16 (4.49-5.83)	4.35 (3.84-4.86)	0.7085	0.0004	0.6817	0.5056
ni	1.73 (1.42-2.04)	1.58 (1.31-1.85)	1.52 (1.3-1.74)	1.59 (1.34-1.84)	0.3061	0.0662	0.3897	0.1089
ΣDMA	4.42 (4.15-4.69)	3.95 (3.75-4.15)	4.22 (3.93-4.51)	4.13 (3.78-4.48)	0.4686	0.0006	0.6061	0.2085
ΣSFA	36.91 (36.01-37.81)	36.05 (35.19-36.91)	37.15 (36.15-38.15)	36.88 (35.43-38.33)	0.9213	0.1109	0.4397	0.3737
ΣMUFA	12.49 (11.88-13.1)	12.96 (12.25-13.67)	13.15 (12.05-14.25)	13.54 (12.31-14.77)	0.4029	0.0349	0.2945	0.9708
ΣPUFA	44.45 (43.53-45.37)	45.47 (44.45-46.49)	43.97 (42.42-45.52)	43.86 (42.57-45.15)	0.7799	0.1332	0.2688	0.1792
ΣMCFA	0.11 (0.09-0.13)	0.12 (0.1-0.14)	0.08 (0.06-0.1)	0.11 (0.07-0.15)	0.0583	0.0214	0.087	0.2669
ΣLCFA	60.23 (59-61.46)	62.04 (60.39-63.69)	60.73 (58.73-62.73)	62.07 (60.09-64.05)	0.8502	0.0022	0.8481	0.4992
ΣVLCFA	32.35 (31.23-33.47)	31.07 (29.6-32.54)	32.4 (30.5-34.3)	30.88 (29.23-32.53)	0.9973	0.0046	0.9035	0.8347

AA, arachidonic acid; DHA, docosahexaenoic acid; DMA, dimethyl acetals; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid;LCFA, long chain fatty acids; MCFA, medium chain fatty acids; MUFA, monounsaturated fatty acids; ni, non-identified; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; VLCFA, very long chain fatty acids.

Supplementary Table 1: Composition of MFGM-enriched milk (MFGM-M) and control milk (CM)

Global composition	CM	MFGM-M
Fat (%, w/w)	1.55	2.79
Total protein (%, w/w)	11.29	10.91
Whey proteins (%, w/w)	0.70	0.64
Caseins (%, w/w)	10.33	9.95
Ash (%, w/w)	1.36	1.26
Lactose (%,w/w)	4.59	4.79
Calcium (mg/100g)	331.31	287.85
Phosphorus (mg/100g)	333.52	321.95
Magnesium (mg/100g)	23.80	21.81
Cholesterol (mg/ 100g)	9.69	32.61

Lipid classes(g/100g fat)	CM	MFGM-M
TAG	98.05 ± 0.006	85.05 ± 0.063
DAG	1.49 ± 0.010	7.11 ± 0.253
FFA+Chol	0.16 ± 0.002	0.96 ± 0.030
MAG		0.04 ± 0.004
Glucer		0.02 ± 0.004
Lacer		0.04 ± 0.020
∑PL	0.29 ± 0.015	6.77 ± 0.317
g/100g PL		
PE	55.17 ± 1.520	48.89 ± 1.103
PI		3.33 ± 0.557
PS	1.09 ± 0.619	8.76 ± 0.031
PC	37.42 ± 1.778	32.32 ± 0.737
SM	6.32 ± 0.877	6.7 ± 1.251

Fatty acid (g/100g fat)	CM	MFGM-M
ΣSFA	68.84 ± 0.30	63.12 ± 0.26
C4:0	3.85 ± 0.14	3.29 ± 0.01
C6:0	2.72 ± 0.09	2.31 ± 0.05
C8:0	1.43 ± 0.004	1.21 ± 0.03
C10:0	2.93 ± 0.02	2.51 ± 0.03
C12:0	3.27 ± 0.04	2.84 ± 0.01
C14:0	10.58 ± 0.16	9.22 ± 0.09
C15:0 *anteiso*	0.19 ± 0.003	0.14 ± 0.001
C15:0 *iso*	0.4 ± 0.002	0.36 ± 0.011
C15:0	1.05 ± 0.001	1.03 ± 0.018
C16:0 *iso*	0.21 ± 0.004	0.17 ± 0.003
C16:0	30.99 ± 0.37	28.83 ± 0.31
C17:0 *anteiso*	0.3 ± 0.005	0.26 ± 0.01
C17:0	0.57 ± 0.002	0.55 ± 0.01
C18:0	9.78 ± 0.05	9.8 ± 0.26
ΣMUFA	26,55 ± 0,23	28.78 ± 0.17
C10:1	0.25 ± 0.003	0.20 ± 0.001
C14:1*cis*9	0.86 ± 0.01	0.71 ± 0.01
C16:1*cis9*	1.7 ± 0.01	1.7 ± 0.03
C18:1 *cis9*	20.13 ± 0.13	22.2 ± 0.08
ΣC18:1*trans*	2.02 ± 0.002	1.93 ± 0.04
ΣC18:1 *cis*	21.52 ± 0.12	24 ± 0.13
ΣPUFA	3.99 ± 0.05	7.34 ± 0.1
C18:2 *transtrans*	0.27 ± 0.001	0.31 ± 0.01
C18:2 (LA)	2.34 ± 0.02	4.77 ± 0.03
C18:3α (ALA)	0.6 ± 0.02	0.71 ± 0.01
C18:2 *cis*9, *trans*11 (RA)	1.02 ± 0.02	0.87 ± 0.003
C20:3n6	0.13 ± 0.01	0.60 ± 0.03
C20:4 (AA)	**0.23 ± 0.001**	**0.79 ± 0.05**

Data are means ± SD. AA, arachidonic acid; ALA, α-linolenic acid; CMD, control milk drink; DAG,diacylglycerols;DHA, docosahexaenoic acid; FFA+Chol,free fatty acids+cholesterol;FMD, functional milk drink; Glucer, glucosylceramides; LA, linoleic acid;Lacer, lactosylceramides;LCFA, long chain fatty acids; MAG,monoacylglycerols;MCFA, medium chain fatty acids; MUFA, monounsaturated fatty acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidyl inositol; PL; polar lipids; PS, phosphatidylserine;PUFA, polyunsaturated fatty acids; RA, rumenic acid; SFA, saturated fatty acids; SM, sphingomyelin; TAG,triacylglycerols; VLCFA, very long chain fatty acids.

Supplementary Figure 1: Evolution of short-delay cued recall (an episodic verbal memory test) after 14 weeks of intervention in older females and males. CM: control milk drink; MFGM-M: MFGM-enriched milk. Asterisk (*) indicates significant differences between treatment ($p<0.05$).