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ASSOCIATIONS OF SHORT AND MEDIUM CHAIN SATURATED FATTY ACIDS AND DAIRY WITH COGNITIVE FUNCTIONING IN THE BOSTON PUERTO RICAN HEALTH STUDY

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ABSTRACT

ASSOCIATIONS OF SHORT AND MEDIUM CHAIN SATURATED FATTY ACIDS AND DAIRY WITH COGNITIVE FUNCTIONING IN THE BOSTON PUERTO RICAN HEALTH STUDY

By

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Cognitive decline is a major public health concern. Evidence suggests that Hispanic/Latino adults, specifically Puerto Rican adults, in the US are at a higher risk of developing dementia or cognitive decline due to the high prevalence of risk factors, including cardiovascular disease and diabetes. Emerging evidence suggests that specific dietary fatty acids, short and medium-chain-length saturated fatty acids, that are found in dairy may be beneficial for cognitive function. However, there are limited observational studies examining the effects of SMCSFA and dairy on cognitive function in Hispanic/Latino adults. We examined the cross-sectional and prospective associations between SMCSFA and dairy consumption with cognitive function among Puerto Rican adults.

Data were from the Boston Puerto Rican Health Study (57 yrs, 71% female), an ongoing prospective cohort study. Diet was assessed using a validated food frequency questionnaire. Dairy products included milk, cheese, yogurt, cream, and butter. Our primary exposures were the sum of SMCSFA(%TE), a dietary fatty acid pattern consistent with high SMCSFA, total dairy (s/d), regular & reduced fat dairy (s/d), and nonfat dairy products (s/d). A battery of neurocognitive tests was administered by trained staff in the language of preference. Global cognitive function score (GCS) was calculated as the mean z-scores of the individual tests. A subset of BPRHS participants returned for neurocognitive testing at 13-yr follow-up. We

analyzed SMCSFA in a substitution analysis at the expense of *trans* fats and added sugar. Dairy models were adjusted for total energy, age, sex, physical activity, smoking status, and education. We also conducted a substation analysis of dairy at the expense of red and processed meats. Our cross-sectional analysis was conducted using multivariate linear regression. Our prospective analysis assessed the change over baseline, 2 and 13 years using a mixed effects model with time-covarying covariates.

In the final sample, participants consumed 2.42 s/day of dairy and less than 1% of their diet was derived from SMCSFA. Most dairy was consumed through 2% and whole milk (37%). Most SMCSFA in the diet were derived from cheese (30%) and whole milk (22%). Butter was significantly related to GCS over 13 years of follow-up. There were no significant associations between SMCSFA, total dairy, regular & reduced fat dairy, or nonfat dairy with cognitive function cross-sectionally or prospectively.

In this cohort of Puerto Rican adults, our findings suggest there were no associations between SMCSFA and dairy with cognitive function. Future prospective studies should examine this relationship in a similar population with larger sample sizes and over a longer duration.

INTRODUCTION

Dementia is a major public health concern.[a] In the U.S., it is one of the top five leading causes of premature mortality.[2] An estimated one in nine adults suffer from cognitive decline.[3] Cognitive impairment is costly to the individual with medical costs ranging from $6,000 to over $100,000 a year and upwards of $305 billion annually to the United States.[4-7] Beyond financial concerns, the World Health Organization (WHO) recognizes that those living with dementia have an impaired quality of life, as well as an increased risk of other cardiometabolic diseases.[8-10] There is strong interest in dietary fat intake as a modifiable risk factor for cognitive decline because of documented effects on related chronic health conditions, including cardiovascular disease (CVD).[11] It is widely recommended to moderate the intake of saturated fat to reduce the risk for heart disease and stroke, however, current evidence on the impact of saturated fat consumption in relation to cognitive function remains inconclusive with reports of adverse and null associations.[12] These results may be partly because previous investigations of saturated fat and cognitive function or dementia, examined total saturated fat consumption rather than considering the individual species that make up the saturated fat class.[12]

Saturated fatty acids (SFA) can be differentiated by the number of carbons that make up the hydrocarbon tail, which include short (<6 carbons), medium (7 to <12 carbons), long (12 to 20 carbons), and very-long (>20 carbons) chain SFA species.[13] Building evidence from epidemiological and animal studies suggests that short and medium-chain-length SFA may benefit metabolic pathways implicated in cognitive decline, including inflammation and insulin resistance.[14-17] In a recent randomized control trial (RCT), researchers observed improvements

[a] Dementia is defined by the Alzheimer's Association as "a group of symptoms associated with a decline in memory, reasoning or other thinking skills" whereas Alzheimer's disease is "a degenerative brain disease that is caused by complex brain changes following cell damage". [1]

in insulin resistance among participants when consuming medium-chain SFAs (MCSFA), as compared to long-chain SFAs (LCSFA).[18] In the European Prospective Investigation into Cancer and Nutrition-Netherlands Cohort, investigators found that over 12 years, there was an association between lower ischemic heart disease and short-chain SFAs (SCSFA) and MCSFA consumption.[19] These included butyric acid (C4), capric acid (C10), pentadecanoic acid (C15), margaric acid (C17), and myristic acid (C14), which are SFAs found in varying amounts in dairy products.[20] There is a vast knowledge base of research conducted regarding fat consumption and CVD suggesting there may be an association between CVD and cognitive function. [21, 22] However, there is a paucity of data available on the potential impact of SCSFA and MCSFA dietary consumption on cognitive function.

Current recommendations and studies regarding fat consumption compare total SFAs to unsaturated fatty acids. Similar to that CVD recommendations, available systematic reviews and meta-analyses have reported adverse and null associations between total SFA and cognitive function.[5,23] This may be due to most previous studies not examining the specific chain length groups of SFAs. Although SFAs as a class of fats have been investigated concerning cognitive function and dementia, the results remain inconclusive. Our preliminary data in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL) suggests that SCSFA and MCSFAs may benefit cognitive function, as they were associated with a higher global cognitive function score. [24] Of note, dairy products were the main contributor to SCSFA and MCSFA consumption among the study participants. Studies of food SCSFA and MCSFA in food sources, including dairy, have shown some benefits with cognitive outcomes, but further investigation is needed.[25] To our knowledge, our previous work in the HCHS/SOL is the first epidemiological study to investigate SCSFA and MCSFA in relation to cognitive function. However, these findings were

cross-sectional in nature, and a prospective study design with repeated measures of diet and cognitive function was needed to replicate this novel finding.

Although few data are available, some evidence suggests that Hispanics/Latinos may be disproportionately burdened by dementia and risk factors of cognitive decline. Current research estimates that Hispanics/Latinos experience a 47% higher prevalence of dementia as compared to non-Hispanic Whites.[26] It has been reported that Puerto Ricans have a greater prevalence of CVD compared to Mexicans, and are just below in type 2 diabetes as compared to Mexicans, the largest group of Hispanics/Latinos living in the United States.[27,28] Moreover, a limited number of studies have been conducted in Hispanic/Latino cohorts examining the relationship between diet and cognitive function. [29, 30] Additional studies examining these associations are necessary to inform culturally specific dietary recommendations and interventions to reduce the burden of cognitive decline. [31,32]

Considering the current evidence, we hypothesized that SMCSFA and their food source, regular and reduced-fat dairy products, would be beneficially related to cognitive functioning in Puerto Rican adults. The primary objective of this study was to examine the prospective relationship between dairy fat and dairy products with cognitive functioning over 13 years. Secondarily, we examined the associations of SMCSFA and dairy products with cognitive function cross-sectionally at baseline.

BACKGROUND

Public Health Relevance

According to Behavioral Risk Factor Surveillance System data collected between 2015 to 2017, 11.1% of Americans reported having a form of cognitive decline.[3] Furthermore, dementia disproportionally affects Hispanic/Latinos residing in the United States.[3] Chen and Zissimopoulous [26] used the Health and Retirement Study cohort to examine associations between race and cognition. The investigators found that as of 2012, the prevalence of dementia in the Hispanic/Latino population was 16.7%, compared to that of the non-Hispanic White population with a 7.4% prevalence. From 2000 to 2012, a 25% decrease in cognitive decline prevalence was seen in both African American and non-Hispanic White respondents, but this decrease was not observed in the Hispanic/Latino population. Instead, it remained stagnant for the majority of the study.

Short and Medium Chain Saturated Fat and Risk Factors of Cognitive Decline

Dietary fat intake and its relationship with CVD have been widely investigated. According to the Guidelines set by the US Department of Agriculture (USDA) it is recommended to consume less than 10% of calories from dietary saturated fat.[11] In 2017, the American Heart Association (AHA) released a Presidential Advisory regarding saturated fat intake and the risk of CVD.[33] The advisory was consistent with other recommendations in that saturated fat as a whole should be replaced with unsaturated fats. The advisory addressed three specific saturated fats, lauric acid (C12), myristic acid (C14), and palmitic acid (C16). A 2017 systematic review and regression analysis published by the WHO, found that these SFA were associated with raising HDL and LDL cholesterol.[34] This evidence suggests that these specific

SFA may have negative health impacts, but the study did not look at the SFAs with less than 12 carbons.

Emerging evidence suggests SCSFA and MCSFA may have positive impacts on cardiometabolic risk factors, including insulin resistance and inflammation.[18,35,36] The most abundant SCSFA in the diet is butyrate (C4), which is found in milk, cheese, and yogurt.[37] In a recent animal RCT, sodium butyrate (NaB) was administered to mice to examine its implications on endothelial dysfunction, a major contributor to atherosclerosis.[35] Researchers found that mice receiving NaB decreased reactive oxygen species (ROS) and inhibitors of nitric oxide. Furthermore, mice were treated with interleukin-1 beta (IL1-β**)** to induce a state of inflammation and the administration of NaB downregulated ROS production in the IL1-β treated mice. An RCT out of the University of Copenhagen, analyzed the effects of MCSFA on insulin resistance in 17 men.[18] The subjects either consumed a control diet (63% carbohydrate, 14% protein, and 34% fat) or one of the two experimental diets, one with a 75% increase in energy and 82% of calories derived from LCSFA. The second experimental group was similar to the first, but 5% of the LCSFA were replaced with MCSFA. The LCSFA group reduced insulin sensitivity and insulin-stimulated glucose disposal, whereas the group with 5% MCSFA did not see the same impairments. In a recent animal RCT study, it was found that a ketogenic diet (KD) containing MCSFA was negatively associated with mTOR, a regulatory pathway of insulin and insulin-like growth factors.[36] Research suggests that reduced mTOR activity may improve insulin sensitivity.[36] The KD high in MCSFA was also associated with lower levels of serum tumor necrosis factor-alpha (TNF-α), an indicator of inflammation, compared to the diet groups with lower dietary MCSFA. These studies suggest the potential benefits of MCSFA as a replacement for LCSFA.

Evidence suggests there is a link between CVD and cardiometabolic risk factors with cognitive decline.[38] A 2017 recent systematic review and meta-analysis examined the effects of cognitive impairment and heart failure.[39] It was found that among the heart failure patients that were included in observational studies, approximately 40% had cognitive impairment. A separate systematic review and meta-analysis found that coronary heart disease was prospectively associated with cognitive decline or dementia.[40] A prospective study conducted in Australia, (n=77) assessed cognitive function in adults over 45 years old who had chronic heart failure class I, II, or III.[41] Significant cognitive decline was observed over the two years. It is hypothesized that the link between CVD and cognitive impairment may be due to the nitric oxide (NO) pathway. NO is necessary for brain signaling and functioning. Inflammation and insulin resistance decrease endothelial cell function for these brain processes by downregulating the production of NO.[42] Furthermore, CVD can cause detriments to the structure of the brain, including alterations to the hippocampus, as well as increased white matter lesions, cerebral infarcts, micro-bleeds, and gray matter atrophy, thus leading to cognitive impairment.[41,43]

The relationship between CVD and cognitive function suggests there may be an association between dietary fat intake and cognitive function. At this point, the data are inconclusive due to substantial variation and heterogeneity in methodology when comparing SFAs and cognitive function.[12] A recent systematic review and meta-analysis comparing total fat, SFA, MUFA, and PUFA, found that SFAs as a whole may be associated with lower cognitive function.[12] Evidence regarding specific chain lengths of SFA implies that there may be a positive association between SCSFA and MCSFA and cognitive function.

The data are limited regarding the effects of specific chain length dietary SFA, but emerging evidence is suggesting SCSFA may be associated with improved cognition. One

proposed biological mechanism of SCSFA improving cognitive function is through the inhibition of histone deacetylase. When SCSFA are absorbed into the colon, they may inhibit histone deacetylase.[44,45] SCSFA can bind to a G protein-coupled receptor and act as a ligand, inhibiting histone deacetylase.[45] Histone acetylation is a part of gene expression during normal cognitive function, and histone deacetylase disrupts this. Histone deacetylase causes a buildup of amyloid-β plaques in the brain, which is a diagnostic criterion for Alzheimer's disease (AD).[44] The inhibition of histone deacetylase may decrease amyloid-β plaque formation and therefore may improve cognitive function. SCSFA's ability to promote histone acetylation has also been linked to anti-inflammatory and neuroprotective effects.[45]

In a recent animal RCT, mice underwent a 12-week intervention of sodium butyrate (NaB) administration, an SCFSA, either being assigned to the control group or receiving 5mg NaB/kg/day or 15mg NaB/kg/day.[41] The mice included were in an early stage of AD, characterized by the concentration of amyloid-β peptides. At the end of the study, a 40% decrease in amyloid-β peptide was observed in both experimental groups compared to the control, suggesting potential benefits of NaB on cognitive function. An earlier study in 2011 also found NaB inhibited histone deacetylase and improved cognitive decline when administered at a later stage of AD in mice.[46] Interestingly, an RCT examined mice who underwent cecal ligation and perforation surgery, which increased histone deacetylase, impairing memory and cognitive function.[47] NaB was administered and an improvement in cognition was observed. More research is needed to determine the effects of SCSFA in humans.

MCSFAs have been examined in in-vitro, animal, and experimental research. It is hypothesized that MCSFA may reduce oxidative stress, which is associated with decreased endothelial and brain cell function and cell death.[48]A recent in-vitro study examined the effects

of MCSFA, capric acid (C10) versus a LCSFA, eicosanoid acid (C20), on oxidative stress measured through Reactive Oxygen Species (ROS), superoxide radical anion ($O2^{\bullet-}$), and hydrogen peroxide (H_2O_2). Researchers found that cells that received C10 significantly decreased intracellular $O2^{\bullet-}$ and H_2O_2 released, compared to cells receiving C20. $O2^{\bullet-}$ is an ROS that is released from the mitochondrial electron transport chain. The enzyme superoxide dismutase (SOD1) is responsible for converting $O2^{\bullet-}$ to H_2O_2, which is associated with cell death. Authors suggest that C10 downregulates the conversion of $O2^{\bullet}$ through inhibiting SOD1 and stimulates two enzymes that detoxify H_2O_2 into water and oxygen. This would decrease the amount of ROS in cells, therefore decreasing the amount of cell death, and improving endothelial cell function. A recent RCT examined the effects of an MCSFA supplement, containing both caprylic acid (C8) and C10 in older adults (n=64) over 3 months.[49] The data suggested the MCSFA had positive impacts on cognitive function, measured by the Mini-Mental State Examination (MMSE). Also of note, MMSE scores decreased in the participants who took an LCSFA supplement. In an earlier RCT in elderly adults (n=38), investigators found that MCSFA in combination with the amino acid leucine and cholecalciferol, cognitive function scores measured by MMSE improved by 10.6%, compared to the diet with leucine and LCSFA, which decreased by 11.2%.[50]

Some evidence suggests that MCSFAs may have benefits on cognitive function due to the availability of ketones, created through ketosis. Ketones are considered the preferred energy source for the brain during times of low glucose availability, such as during a prolonged fasting period or with insulin resistance.[51] In an RCT examining individuals with Mild Cognitive Impairment (MCI) (n=52), participants were blindly randomized into either a control group, who received a placebo, versus an experimental group who received 30 grams of a ketogenic

medium-chain triglyceride (kMCT) for 6 months.[51] There was an association between the increases in plasma ketones, derived from the kMCT, and improved scores in the Trail Making Test, Verbal Fluency, and Boston Naming Test, all three tests of cognitive function. This study hypothesized that MCI may be associated with lower energy status in the brain. KMCT increased the prevalence of serum ketones, increasing the energy available to the brain in the form of ketones, as opposed to glucose. In a similar study conducted in a Japanese cohort (n=20) the participants consumed a ketogenic formula containing 20g of MSCFA, composed of both caprylic acid (C8) and capric acid (C10).[52] Participants completed cognitive tests 120 minutes following consumption. Over 2-3 months, there were positive associations between the ketogenic MCSFA formula in working memory, short-term memory, and processing speed. In 2016, a prospective clinical intervention in a Japanese cohort (n=22) examined the impacts of Anoxa, a medical food that contains 20g of C8.[53] Improvements in cognitive measures were observed in patients who did not have the Apolipoprotein, a genetic trait associated with Alzheimer's disease, and a low MMSE score (>14). In 2017, this study was reanalyzed to determine which aspects of cognitive function Anoxa did impact.[53] Researchers found that the MCSFA supplement improved memory and orientation, but only in patients in the early stages of MCI.

Animal studies were also conducted to explore the relationship between MCSFA and cognitive function. A study was conducted on a sample of dogs because cognitive dysfunction in dogs parallels that of dementia in humans.[54] Similar to studies conducted in humans, it was found that dog's cognitive function significantly improved when given an MCSFA supplement over 90 days, measured by owners' reports of different cognitive measures, including disorientation, altered social interaction, anxiety, sleep-wake cycle disturbance, house training, learning and memory, and altered activity. In an RCT conducted on mice, C10 was associated

with improved novel object recognition.[55] From the research, MCSFA may be beneficial to cognitive function.

Dairy and Cognitive Function

Dairy is a food source containing both SCSFA and MCSFA. In one cup of whole milk, there is roughly 1 g of SCSFA and 4 g of MCSFA.[20] Current research on dairy intake and cognitive function is inconclusive due to varying methodology and dairy types examined. A cross-sectional study conducted using the Maine-Syracuse Longitudinal Study cohort (n=972) found that there was a linear trend between the frequency of dairy consumption and cognitive function, measured through a battery of neurocognitive tests.[56] Similarly, in an RCT comparing diets with high quantities of dairy to low quantities of dairy, investigators found that working memory via the Spatial Span Backwards test was significantly lower in those consuming the lower quantities of dairy as compared to those consuming the high dairy diet.[57] Although it seems dairy may be beneficial for cognitive function, it remains inconclusive because these studies compared total dairy to cognitive function, as opposed to low-fat dairy and high-fat dairy. In a recent cross-sectional study consisting of older Dutch adults (n=619), dietary data was collected via a 190-item Food Frequency Questionnaire (FFQ).[58] Global cognitive function was measured through a battery of cognitive function tests and a z-score was used to calculate a compound score. It was found that there was an association between higher levels of fermented dairy products, as well as skimmed dairy with higher executive functioning scores. There were no associations observed between total dairy consumption, full-fat dairy, or non-fermented dairy. The investigators did find that fermented dairy products, including buttermilk, may be associated with higher cognitive functioning due to the lactic acid bacteria and the probiotic benefits they provide to the gut-brain axis. The skimmed dairy may have shown benefits for cognitive function

because of nonfat components, including vitamin D, vitamin B-12, and calcium.[59] A recent prospective study in an elderly Japanese population (n=237) found that lower baseline intake of milk was associated with cognitive decline in men.[60] This study did not specify which fat types of milk. In a separate prospective study in a similar population of elderly Japanese men (n=1,081), the researchers found a significant inverse relationship between milk and dairy intake and cognitive function, but did not differentiate between dairy fat type.[61] Over the course of 20 years, a prospective study using the Atherosclerosis Risk in Communities (ARIC) Cohort (n=13,751), an association between milk intake and cognitive decline was observed, but this study specifically examined low-fat milk and skim milk.[62] However, a cross-sectional study conducted in a Chinese population (n=3670) found that low-fat milk was associated with higher cognitive function in women.[63] At this time, the data is conflicting. More research is needed to determine the effects specifically of full-fat dairy and cognitive function.

The data on cognitive function and dietary SCSFA and MCSFA are limited, especially in the Hispanic/Latino population. Our preliminary work investigated dietary fatty acids and cognitive function in the HCHS/SOL population in a cross-sectional study.[24] Karazurna et al found that SCSFA and MSCFA were associated with higher global cognitive function.[24] To our knowledge, the current study is the first to prospectively examine the impacts of SCSFA and MCSFA and specific dairy fat types on cognition in an older Puerto Rican population. Our findings present novel evidence to inform future dietary guidelines regarding dairy and specific saturated fatty acids in Hispanic/Latino adults living in the US

METHODS

Participants

Data were derived from the Boston Puerto Rican Health Study (BPRHS), an ongoing cohort study conducted out of the Greater Boston Area. The study aims to examine the relationship between psychosocial stress and related health outcomes, including cognitive function and depression.[64] Diet and cognitive function were measured at baseline, 2 years, and 8 years. As previously reported, participants were an average of 55 years of age and mostly female 75-78% at baseline and 13 years, respectively.[64] Participants were recruited through the 2000 US census tracker, flier distribution at local events such as fairs, or festivals, television, and radio advertisements, or by personal referral to the study. Inclusion criteria for this study included self-identification as Puerto Rican descent in the census, the ability to answer study questions in either English or Spanish, between 45 and 75 years old, and living in the metropolitan area of Boston. Exclusion criteria included missing dietary, cognitive, or covariate data, serious health conditions in which the individual could not answer questions, the plan to move from the area within 2 years, or a score below 10 on the MMSE.[64] All participants provided written informed consent. The BPRHS was approved by the Institutional Review Board at Tufts Medical Center, Northeastern University, and the University of Massachusetts Lowell.[64]

Assessment of Dietary Intake

Dietary intake was measured at baseline, 2, and 13 years by a 126-item validated semi-quantitative FFQ, which included frequency and portion size questions for an extensive list of foods.[64] The questionnaire was based on the National Cancer Institute-Block FFQ and was

adapted for a more accurate representation of the Puerto Rican population.[64] Food amounts were linked to the Nutrition Data System for Research (NDSR), to estimate the nutrient intakes.[65]

SCSFA and MCSFA were defined as those with <6 carbons and 7 to ≤12 carbons, respectively **(Supplementary Table 1).** Dietary fatty acids were expressed as a percent of total energy. Our primary fatty acid exposure was total short and medium-chain SFA, calculated as the sum of these fatty acids per day.

Dairy products of interest included milk, yogurt, cheese, cream, and butter. One serving of milk, yogurt, cheese, cream, and butter were 1 cup, 1 cup, 2 oz, 1 tbsp, and 1 tsp, respectively. Dairy intake was estimated by merging BPRHS diet data with the NDSR nutrient database to extract servings per day of each individual food item per person. Nine food items were not available in NDSR, including vegetarian lasagna, pasta with pesto sauce, and pasta with clam sauce, amongst others. Furthermore, dairy desserts, including ice cream, frozen yogurt, pudding and others, loaded onto dairy-based dessert categories, as opposed to dairy categories. To estimate dairy in these food items, we utilized the USDA nutrient database. The USDA nutrient database provided servings/100g of milk, cheese, and yogurt per food item, which was consistent with the NDSR. However, the USDA did not provide servings of butter or cream, so this was estimated by calculating the gram amount of cream and/or butter through recipes and extracting this value from the solid fat servings from the USDA database. This gram amount was then multiplied by grams per serving to identify the number of servings of butter and cream included in each food item.

The primary dairy exposures were total dairy (milk, yogurt, cheese, butter, and cream), regular and reduced-fat dairy, and nonfat dairy. They were expressed as energy-adjusted servings

per day. Regular and reduced-fat dairy was defined as any of the previously mentioned dairy products that contained fat, including whole fat, 2%, 1%, or labeled as reduced fat. Nonfat dairy was defined as products with 0% fat or labeled as skim. Dairy exposures were expressed as energy-adjusted servings per day by dividing the total servings by total energy (kcal) and multiplying by 2000 (kcal).

Dietary Fatty Acid Pattern

We utilized the factor loading score that was consistent with high SMCSFA intake, previously derived in Karazurna et al.[24] This factor loading score was developed using principal component analysis with varimax rotation (proc factor in SAS). This analysis is used commonly in nutritional epidemiology to identify patterns of nutrient intake.[66] The input variables included were dietary fat represented as total fat, 26 specific fatty acids, and MUFAs from plant and animal sources, which were calculated similarly to previous studies.[67] Plant MUFAs were determined to be from fruit, vegetables, grains, and legumes. Animal MUFAs were from meat, animal fat products, and dairy products. MUFAs from food items that had both plant and animal sources, such as pizza, were calculated through recipes by calculating gram amounts of each MUFA source and determining the proportion from plants versus animals.

Neurocognitive Testing

Neurocognitive tests were administered at baseline, 2 and 13-year follow-ups. Cognitive function was measured through a battery of tests including two Word List Learning tests, Digit Span Forward and Backwards, three Stroop tests, Verbal Fluency, Clock Drawing, and Figure Copying.[29] Cognitive tests were conducted by trained interviewers in the language of the participant's preference (Spanish or English). The Word List Learning tests included an

immediate recall, where participants were asked to repeat a list of words that were read to them immediately prior and were given five attempts to do so. This test aimed to measure verbal memory.[68] The second component of Word List Learning was to measure retention and recognition, in which the participant identified words that were included in a previous list. Digit Span forward and backward were administered to measure attention and working memory.[68] These tests required the participant to repeat a list of numbers that were read to them in the order they were given or in reverse, respectively. There were three Stroop Tests to quantify processing speed, cognitive flexibility, and response to inhibition.[68] Stroop Tests 1 and 2 required the participant to read a list of words as fast as possible within a 45-second time frame. Stroop test 3 requires the participant to name the color of a word as fast as they can in a 45-seconds.[69] Verbal fluency tested English language fluency and executive functioning by asking the participants to name as many words as they can think of with the letter C and was repeated two more times with the letters F and L.[68] Clock drawing and figure copying both were assessing visual-spatial organization by requiring the participant to draw a clock with a specific time, and eight different figures that were described by the administrator of the test.[70,71]

Consistent with previous studies of diet and cognitive function, we calculated a global cognitive function including the aforementioned cognitive tests.[72] The composite score was calculated by standardizing each individual test through a *z* score transformation, and the average of these scores created the global score.[69,72]

Covariates

Sociodemographic covariates that were collected at baseline, 2 years, and 13 years included sex (male/female)[53,59,68,73], age (years)[50,59,61,64,75,], education level (< high school or high

school graduate)[53,64], smoking status (yes/no)[53,59,75], and physical activity level score[59,68], which was measured by a modified version of the Paffenbarger questionnaire, which calculates a score based off of all activities within the past 24 hours.[75] Clinical covariates included self-reported diabetes status (yes/no)[53,65,66] and CVD status (yes/no). [52,74]

Dietary covariates were considered confounding variables and to hold constant for substitution models. Potential confounding dietary covariates included total energy (kcal/day), fruit (s/day), vegetable (s/day) added sugar (%TE), MUFA (%TE), PUFA (%TE), VLCSFA (%TE) and LCSFA (%TE).[73] Substitution model covariates further included total carbohydrates (%TE), total fat (%TE), total protein (%TE), total alcohol (%TE), *trans* fatty acids (%TE), carbohydrates without added sugar (%TE), summed servings of grains, fruit, vegetables, animal proteins without red meat, dairy, legumes, oils, and alcohol. [76]

Statistical Analyses

Data were examined by histograms and transformation was applied when necessary. Means and proportions were used to report baseline dietary, cognitive, and demographic data. Our primary analyses were repeated-measures linear mixed-effects models fitted to assess SMCSFA and dairy intakes at baseline with 2-year and 13-year changes in overall cognitive function and individual neurocognitive tests. (n=345). We examined the prospective associations between SMCSFA and cognitive outcomes using a substitution analysis at the expense of *trans* fatty acids, holding total energy (kcal), fat(%TE), carbohydrate (%TE), protein (%TE), alcohol(%TE), LCSFA(%TE), VLCSFA(%TE), MUFA (%TE), and PUFA (%TE) intake constant, as well as adjusting for age, sex, education level, smoking status, and physical activity level. We also conducted a substitution analysis at the expense of added sugar, adjusting for all

the *trans* fatty acid model covariates plus total carbohydrates minus those from added sugar, and *trans* fats. We also conducted a prospective model with the factor score loading onto SMCSFA derived from Karazurna et al.[24.] In this model, we adjusted for age, sex, education level, and time covarying total energy, physical activity, and smoking status.

Dairy exposures were also analyzed with repeated-measured linear mixed-effects models assessing the changes from baseline to 2 and 13 years with overall cognitive function and individual neurocognitive tests. Model 1 was adjusted for age, sex, and time covarying total energy, physical activity level, smoking status, and physical activity level. Model 2 was adjusted for model 1 covariates plus time covarying fruit, vegetable, and added sugar consumption. Because of the documented effects of SMCSFA and CVD and insulin resistance on cognitive function[39,74] we conducted a third model adjusting for CVD and diabetes status to identify potential mediation.

Secondary analyses included cross-sectional linear regression models at baseline. Similar to our prospective analyses, we conducted substitution models with SMCSFA and cognitive outcomes at the expense of *trans* fats and added sugar, as well as the SMCSFA factor loading score. We adjusted for the baseline measures of the covariates mentioned previously (n=1,292). We also examined our dairy exposures cross-sectionally, adjusting for the baseline measures for each model mentioned previously.

We conducted a variety of additional analyses. Dairy has previously been defined as milk, cheese, and yogurt, excluding dairy desserts.[58,59,] We repeated each of our analyses with dairy defined as the sum of milk, cheese, and yogurt energy-adjusted servings per day. We also conducted a prospective substitution analysis with dairy at the expense of red and processed

meats. This was done by holding total energy, and the sum of total servings of fruit, vegetables, legumes, and animal proteins without red and processed meat, grains, and oils constant at each time point, as well as adjusting for age, sex, education level, and time covarying physical activity level and smoking status.

To adjust for multiple hypothesis testing and reduce the risk of Type 1 errors, we utilized a False Discovery Rate (FDR) to identify statistical significance. FDR corrected p-values were considered statically significant if <0.05. All analyses were conducted in SAS 9.

RESULTS

Baseline demographic and dietary data

Individuals consuming greater amounts of total, regular and nonfat dairy products were significantly older than those in the first quantile of dairy intake. Greater intakes of nonfat dairy products had significantly more female participants, fewer smokers, lower education, and lower total energy intake. **(Table 1)** Greater consumption of all three dairy exposures had greater intakes of SMCSFA and LCSFA. Higher consumption of regular/reduced fat dairy products was also consumed less VLCSFA and PUFA consumption. Participants who consumed greater amounts of SMCSFA had significantly greater total energy intake, LCSFA, and MUFA consumption. However, they had significantly less PUFA intake **(Table 2).**

Participants with greater regular/reduced fat dairy consumption consumed significantly more milk, cheese, yogurt, and cream compared to the lowest group. Participants in the highest quartile for nonfat dairy consumed significantly less butter, compared to the lowest group. **(Table 3).** Whole and 2% milk products accounted for more than 1/3 of total dairy intake in this cohort. Other top contributors to dairy intake were cheese, 1% milk products, and frozen dairy dessert products **(Table 4).** Most SMCSFA were consumed through cheese and whole milk **(Table 5).**

SMCSFA Intake & Cognitive Function

SMCSFA were trending towards beneficial associations when replacing both trans fats and added sugar related to GCS. [β (95% CI) =0.06(-0.01,0.12) ,p=0.08; β (95% CL)=0.05 (-0.01,0.19, p=0.09] cross-sectionally at baseline. FDR indicated these results were no longer

significant; however, the relationship was trending toward significance (FDR=0.07) **(Table 7).** Prospectively, there were no significant observations observed.

Dairy Intake & Cognitive Function

Total dairy and regular/reduced fat dairy were normally distributed but nonfat dairy distribution was skewed toward 0. The nonfat dairy variable was log-transformed to normalize the data; however, this did not change the results, so the original skim values were reported. Dairy components were also skewed. A log transformation was applied to milk, cheese, cream, and butter. Yogurt contained >50% of participants with 0 values, therefore yogurt was examined categorically 1) 0 s/day/2000kcal, 2) Less than the median (0.10s/day/2000kcal) or 3) greater than the median value. The mean baseline total dairy intake was 2.42 s/day, with 2.2 s/day from regular and reduced fat products and 0.20 servings from nonfat products.

Total, regular/reduced, and nonfat dairy were not significantly related to global cognitive score cross-sectionally at baseline (Table 7). However, retention was beneficially related to regular/reduced fat dairy [β (95 % CI) = 0.04 (0.002,0.08), p=0.04] but was no longer significant after FDR adjustment (FDR=0.70) Retention was also trending toward a positive significant relationship from total dairy [β (95 % CI) =0.03 (-0.004,0.07), p=0.06] **(Supplemental Table 3)**. Baseline milk, cheese, cream, and butter were not significantly related to GCS at baseline. **(Table 8)** However, individuals who were consuming some yogurt, but less than the median had significant higher GCS (mean=0.13 ± 0.2) compared to group 1 (mean=0.007 ± 0.12, p<0.001) and group 3 (mean=0.03 ± 0.02,p=0.003).

When examined prospectively, no significant relationships were observed between total, regular/reduced, nonfat dairy or SMCSFA and GCS **(Table 9)** or any of the individual

neurocognitive tests **(Supplemental Table 4**). Butter was significantly related to higher GCS [β (95 % CI) =0.008 (0.008,0.02, p=0.04] and yogurt was trending toward a significant relationship [β (95 % CI)=0.01 (-0.002,0.03), p=0.07]. This was no longer significant when adjusted for multiple hypothesis testing (Table 4). We tested yogurt further as a categorical variable because of the number of participants who consumed 0 servings per day, however, these results were null. All dairy models were examined with potential mediators, CVD, and diabetes, but the results did not change.

Additional Analyses

We conducted multiple sensitivity analyses to ensure the accuracy of our results. Cross-sectional analyses were run with the final sample (n=345) and total dairy was trending toward there were no significant differences from the larger sample. Results did not differ when analyzed with a standard definition of dairy, including only milk, cheese, and yogurt, and excluding dairy desserts. We examined substitution analysis for servings of dairy at the expense of servings of red and processed meat and the results were null (**Supplemental Table 5**)

DISCUSSION

In contrast to our hypothesis, SMCSFA total dairy, regular and reduced fat dairy, and nonfat dairy were not significantly associated with cognitive function over 13 years of follow-up in Puerto Rican adults living in the Boston metro area. When examining the individual dairy components prospectively, butter was significantly related to GCS, however, this relationship was attenuated when adjusted for multiple hypothesis testing. Cross-sectionally at baseline, SMCSFA were positively trending toward a significant beneficial relationship with GCS. Total, regular & reduced, and nonfat dairy were not significantly related to GCS, however, yogurt was significantly related to GCS when expressed categorically.

In the current study, results suggested that SMCSFA were positively associated with global cognitive function when substituted for an equal caloric amount of *trans* fatty acids. However, these associations were attenuated in prospective analyses with 13 years of follow-up. In a prior study conducted by Karazurna et al [24] in the HCHS/SOL study, a dietary fatty acid pattern consistent with high SMCSFA consumption was beneficially associated with global cognitive function in a cross-sectional analysis amongst 8,942 Hispanic/Latino adults living in the US. A prospective analysis was not conducted in that study. Although our results are consistent with the cross-sectional analysis in the HCHS/SOL study, our longitudinal analysis does not support the benefit of dietary SMCSFA on cognitive function.

To our knowledge, this is the first prospective cohort study to examine the associations between SMCSFA and cognitive function or dementia. Our literature review identified that previous studies examining this relationship have been conducted in animals or control trials, most of which have observed positive associations between SMCSFA and cognitive function.[24,36,46-53] We built upon this emerging evidence in examining the associations

longitudinally. The cross-sectional associations observed in our study were consistent with previous research, but the null prospective relationships may be due to reverse causation. It is possible that individuals with higher cognitive function scores would be more likely to choose foods based on their nutritional health components, such as dairy, a major source of SMCSFA. Our results highlight the importance of conducting prospective studies to clarify the directionality of associations.

Regardless, there is building evidence to suggest that short and MCSFA may benefit cognitive functioning. An RCT in rats with early-stage dementia found that the administration of 15 mg/kg of body weight NaB per day for 12 weeks, compared to the control group who did not receive this, improved cognitive measures including decreased amyloid-β peptides in the brain and increased fear response.[44] A separate RCT conducted in rats with advanced dementia found significant improvements in memory when NaB was administered.[50] Similar findings have been observed in human RCTs. Ota et al [52] examined the effects of a 20g combination of caprylic acid and capric acid, two SFAs, on cognitive function in Japanese adults (n=20) with mild to moderate AD. Investigators found that after 8 weeks, participants improved both immediate and longer-term memory scores. After 12 weeks, investigators continued to see improvements in neurocognitive testing, including digit testing. To our knowledge, the current study is the first to examine SMCSFA prospectively.

The relationship between SMCSFA may be due to anti-inflammatory effects, as well as MCSFA's ability to synthesize ketones for energy Studies have found that MCSFA downregulates the receptor responsible for stimulating the release of inflammatory cytokines, decreasing systemic inflammation.[77] Inflammatory markers, such as IL-6 and TNF-α promote

oxidative stress and decreased endothelial cell function of the blood-brain barrier, decreasing cognitive function in the brain.[78] Furthermore, research suggests that MCSFA can improve cardiometabolic risk factors, including insulin resistance.[79,] It is known that the brain requires energy to function, primarily glucose. During a state of insulin resistance and low glucose availability to the brain, MCSFA are absorbed into the intestines and are able to cross the blood-brain barrier, and become metabolized into ketones, an alternative source of energy for the brain.[79] It is hypothesized that the availability of the ketones from the MCSFAs to the brain may be beneficial for cognitive functioning as an energy source for cognitive functioning, especially during times of insulin resistance in the brain.[79] The documented effects of SMCSFA on the cardiometabolic risk factors may improve cognitive function.

Considering the prior evidence suggesting that SMCSFA consumption may improve cardiometabolic risk factors of cognitive decline,[18,35,36] we additionally examined dairy consumption. Karazurna et al[24] reported that dairy products were the main source of SMCSFA in the diets of Hispanic/Latino adults. Prospectively, butter was beneficially related to GCS. Cross-sectionally, yogurt was significantly beneficial to GCS. While FDR corrections attenuated the relationship, these results remain suggestive of a potential benefit. When compared by fat type of dairy product, there were no significant relationships observed with GCS.

Our findings contrast other observational studies that found dairy was associated with better cognitive functioning. In 2003, Yamanda et al[80] examined potential risk factors for dementia in Japanese older adults who had survived the atomic bombings in 1945 (n=1,774). Researchers reported a significant reduction in risk of Japanese older adults for dementia in participants who reported consuming milk at least 4 times per week, as compared to those who consumed less.

Similar results were observed in a separate cohort study examining milk and dairy with dementia in older Japanese adults (n=1,081) over 17 years of follow-up.[61] There was a significant inverse association between milk and dairy consumption and risk of dementia. De Gogi et al [58] found that skim dairy, fermented dairy, and buttermilk were significantly associated with higher executive functioning. Furthermore, this study found that 30g of cheese decreased the probability of low processing speeds.

Studies have also found adverse associations between dairy intake and cognitive functioning. Petruski-Ivelva et al [62] found that US adults who consumed greater amounts of milk were at greater risk of cognitive decline over 20 years. However, the authors note that 75% of reported milk intake was from skim milk products. Furthermore, the authors analyzed total dairy intake by race and there were no significant differences between quartiles of dairy intake in white or black Americans. A cross-sectional study examining dairy intake and cognitive function in Australian men found that whole fat cream, whole fat cheese and low-fat cheese were associated with poorer memory and an increase in cognitive failures.[57] This study was subject to confounding and recall bias. Dairy was expressed as crude servings per day, which does not account for different energy needs between different people. Non-energy-adjusted dairy variables may contribute to the deleterious effects of dairy, as higher total energy intake has previously been associated with poorer health outcomes.[81] Furthermore, this study also collected cognitive data using the Cognitive Failures Questionnaire, in which participants self-reported their cognitive abilities. These methodological components may have contributed to the negative associations observed between dairy and cognitive function.

Our results are in support of recent meta-analyses and other prospectively designed studies that have found null associations between total dairy and global cognitive functioning.[82] Kesse-Guyot et al[73] reported null associations between total dairy intake or individual dairy components and cognitive functioning in a cohort study of French adults (n=3,076). However, authors did identify that in women, individuals who consumed less than or greater than French Dietary Guidelines significantly decreased both working and verbal memory, as compared to individuals who are meeting the recommendations. We built upon prior studies examining the impact of dairy on cognitive function by examining these associations in a Puerto Rican adult cohort. Evidence suggests that Puerto Ricans, as compared to other Hispanic/Latino Groups, experience a higher prevalence of cognitive impairment and cardiometabolic health conditions. Collectively, our findings suggest that total dairy consumption may not affect older Puerto Rican adults.

Total dairy was not significantly related to cognitive functioning; however, butter was significantly beneficial to GCS prospectively. Although when adjusted for multiple hypotheses testing the significance was attenuating, this is still suggestive of a beneficial relationship. Butter has been controversial in its relationship to CVD, however, recent findings suggest that butter may not be detrimental to cardiovascular health. Recent evidence has found that total dairy intake, including butter, decreased risk of CVD death, cardiovascular diseases, and stroke.[83] Butter consumption was low, and no statistical significance was observed with butter on its own. There are very limited studies examining butter and cognitive function. In a recent prospective analysis, Granic et al[84] found that participants who consumed high amounts of butter were significantly more likely to be considered impaired, compared to those who consumed low meat, but less likely compared to those who consumed high amounts of red meat, determined through

cluster analysis. The authors did not disclose specific quantities of each food product consumed. Butter intake was not significantly associated with cognitive decline over 5 years of follow-up.

There are several reasons that may explain the null associations of SMCSFA and dairy with cognitive functioning. Cognitive decline takes time to develop and may require a longer study duration to observe the cognitive decline progression. The Alzheimer's Association suggests that the onset of dementia can vary drastically between individuals, ranging from months to years from the identification of symptoms to death from some form of cognitive impairment.[85] Some studies that have found beneficial associations have been conducted over a longer duration compared to the current study. In 2019, McEvoy et al [86] examined the effects of the adherence to the Dietary Approach to Stop Hypertension, the Mediterranean Diet, or the A Priori Diet Quality Score diet at baseline age of 25 and cognitive functioning at 25- and 30-year follow-ups, utilizing the Coronary Artery Risk Development in Young Adults cohort study. Higher dietary scores were significantly associated with cognitive outcomes at 25 years. However, the cognitive change over 5 years from years 25-30 was not significant. This may indicate that a longer follow-up period is necessary to observe changes in cognitive function. A separate prospective analysis followed Finish adults over a mean of 22 years and observed a 28% reduction of risk for dementia associated with the top quartile of cheese consumption compared to the lowest.[87] This study also suggests the need for a longer follow up. In the current study, participants were followed for a mean of 12.6 ± 1.13 years. Evidence suggests that longer follow-up may be necessary to observe significant changes on cognitive function.

There may be the potential for loss to follow-up bias within our final sample. At the 13-year visit, a sub-sample of BPRHS participants (517 of 1491 at baseline) returned for dietary

assessment and cognitive testing. It is possible that individuals who were experiencing cognitive decline or developed cognitive impairment did not return for the 13-yr study visit due to the cognitive burden of the study-related activities. Participants in who did not return to the study had significantly lower GCS compared to those who did (p=0.006). Participants were significantly older (p <0.003) and had a greater prevalence of diabetes (p=0.01) **(Supplementary Table 2)**. Thus, the analytical sample in the prospective analysis were healthier both with a lower prevalence of diabetes and significantly higher global cognitive score, more likely to be female, and younger as compared to those who elected to not return because of these significant differences in those who dropped out of the study. These differences may play a significant role in why null observations were observed.

There was low overall reported consumption of SMCSFA and dairy, which also may have contributed to the null associations. SMCSFA consumption in the current study was similar to the reported intake in the HCHS/SOL but may not meet a threshold amount needed to see benefits longitudinally.[24] In a recent RCT in young adults, participants were administered 12g or 18g of MCT for 4 weeks.[77] Cognitive function was measured through a battery of tests once per week. There was a significant improvement in both experimental groups, as compared to the control group who did not consume the MCT supplement. Participants in the current study were consuming an average of less than 1g per day of MCT. Similarly, dairy intake may have been too low to be associated with changes in cognitive function. Previous prospective studies have suggested that consuming less than the recommended daily intakes may be adversely related to cognitive functioning.[74] Furthermore, De Goeij et al[58] reported median intake of 30g/day for milk, 90g per day of yogurt, and 31g per day of total cheese when examining the cross-sectional

associations of dairy with cognitive performance. Researchers found a significant relationship between a 30g increase of cheese per day and improved processing speeds. The current study reported consuming median intakes of 0.95 servings of milk/day, 0.39 servings of cheese per day, and 0.03 servings of yogurt per day. This would be equivalent to about 232 g/day of milk, 11g/day of cheese, and about 7g/day of yogurt per day. The lower intakes of cheese and yogurt within this population may be contributing to the null results. As mentioned previously, a prospective study in Finish adults over a 22-year follow-up found that cheese intake in the top quartile (31g/day) was at a significantly decreased risk of dementia compared to the bottom quartile (0.7g/day).[87] In the current study, average dairy intake fell below the US Dietary Guidelines Recommendation of 3cups/day/2000kcal. These studies may suggest that nutrients specifically found in cheese may be beneficial for cognitive functioning, and the current cohort did not meet the threshold amount.

Although our study is one of the first to examine SMCSFA and dairy consumption and cognitive function among Hispanic/Latino adults, our sample is relatively small. Our prospective analysis was conducted in 345 Puerto Rican adult participants but may not be large enough to detect statical power. Other studies that have observed a significant relationship have been conducted in cohorts with greater than 345 participants. A prospective study conducted in 13,751 US adults between 45 and 64 years old identified a significant relationship between total milk intake and cognitive decline.[62] Similar findings were observed in other epidemiological studies with larger samples sizes than the current study.[73,81]

We considered the potential for differential effects among those with diabetes. Insulin resistance has been previously reported to impede cognitive function through endothelial cell

damage and decreased insulin sensitivity in the brain.[87] Previous research conducted in Puerto Ricans has found that the Mediterranean Diet may benefit cognitive function in participants with diabetes.[88] In the current study, there were no associations between SMCSFA or dairy exposures and GCS in participants who had self-reported diabetes (n=267) at any time point. This may suggest that a nondairy component of the Mediterranean diet was beneficial in improving glycemic control.

In addition to the aforementioned limitations of the study, specifically length of follow-up, loss to follow-up bias, the timing of dietary assessment, and sample size, there are a few additional limitations to consider. There is the potential for error in the neurocognitive testing procedure. Participants cognition may not be accurately represented due to measurement error or interviewer bias. Future studies should consider a more precise measure of brain function, such as functional magnetic resonance imaging or brain volume measurements. Diet was self-reported which may lead to measurement error if participants are not accurately representing their actual intake. However, the FFQ was validated for this population, which minimizes the misclassification bias of the exposure. Previous studies have utilized adipose tissue 15:0 and 17:0 as nutritional biomarkers for dairy intake and should be considered for future analyses.[90]

There are several strengths of the current study. This study was conducted amongst Puerto Rican adults, an underrepresented group in current literature. The prospective study assessments and validation of the FFQ minimize the risk of misclassification bias. We also minimized bias by adjusting models for multiple confounding variables.

In conclusion, when accounting for multiple hypothesis testing within our study, there was no associations between SMCSFA and dairy with cognitive functioning in Puerto Rican

adults. This population is at an increased risk of developing some form of cognitive decline, potentially due to their high rates of diabetes and CVD. Longer prospective studies with larger sample sizes should be conducted within this population to determine dietary interventions that may be protective against cognitive decline.

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APPENDIX A

TABLES & FIGURES

Table 1. Demographic data by dairy and SMCSFA quartile at baseline (n=345).

	Q1	Q2	Q3	Q4
Total Dairy (s/day)	1.08±0.06	1.86±0.06	2.54±0.06	4.35±0.03
Age (years)	55.1 ± 0.69	53.8± 0.69	55.6± 0.69	57.3 ± 0.69*
Female (%)	74.8	74.5	78.1	83.6
Smoker (%)	29.9	14.8	22.0	17.2
High School Education (%)	15.6	21.1	17.2	19.8
Physical Activity Level	30.8 ± 0.41	32.6 ± 0.415	31.8 ± 0.41	32.1 ± 0.42
CVD (%)	20.0	11.7	19.8	19.9
Diabetes (%)	31.0	38.2	29.8	30.5
Regular/Reduced Fat Dairy (s/day)	0.98±0.06	1.69±0.06	2.32±0.06	3.85±0.06
Age (years)	55.7 ± 0.69	53.0±0.69	55.0±0.69	57.1 ± 0.69*
Female (%)	77.8	75.9	75.8	81.5
Smoker (%)	25.4	19.5	15.8	16.7
High School Education (%)	18.0	22.0	16.0	17.5
Physical Activity Level	31.1 ± 0.42	32±0.42	31.8±0.41	31.9 ± 0.42
CVD (%)	19.1	11.6	22.8	17.8
Diabetes (%)	29.4	35.5	34.1	30.5
Nonfat Dairy (s/day)	0.01±0.03	0.04±0.03	0.08±0.03	0.68±0.02
Age (years)	53.9 ± 0.70*	55.4±0.70	56.0±0.70	56.7 ± 0.7*
Female (%)	67.8	78.2	79.0	86*
Smoker (%)	33.7	22.9	17.2	10.1**
High School Education (%)	8.34	23.9	19.1	21.9
Physical Activity Level	31.9 ± 0.42	31.8±0.41	31.3 ±0.42	32.2 ± 0.42
CVD (%)	14.8	18.8	23.9	13.8
Diabetes (%)	22.5	33.1	37.2	36.6*
SMCSFA (%TE)	0.31 ± 0.01	0.50±0.01	0.70±0.01	1.1 ± 0.02
Age (years)	55.8 ± 0.70	54.0±0.70	55.9±0.70	56.1 ± 0.70
Female (%)	76.7	80.8	75.8	77.6
Smoker (%)	23.3	22.1	18.9	19.5
High School Education (%)	13.8	21.3	20.0	18.2
Physical Activity Level	31.4±0.42	31.7±0.42	32.2±0.42	31.8±0.42
CVD (%)	19.1	14.8	20.7	16.7
Diabetes (%)	29.4	36.6	31.6	31.8

Trend analyses were conducted to determine statistical differences between quartiles. Total dairy includes milk, cheese, yogurt, cream, and butter. Regular dairy includes full-fat dairy products. Reduced fat includes 1% and 2% dairy products.

Nonfat dairy includes skim or 0% fat products. Dairy variables are energy adjusted servings per day and SMCSFA is expressed as a % TE. SMCSFA= short and medium chain saturated fatty acids, TE= total energy, CVD=cardiovascular disease * indicates p<0.05, ** indicates p <0.001

Table 2. Dietary data by dairy and SMCSFA quartile at baseline (n=345).

	Q1	Q2	Q3	Q4
Total Dairy (s/day)				
TE (kcal/day)	2060 ± 106	2350 ± 106	2240 ± 106	2270 ± 107
SMCSFA (% TE)	0.36 ± 0.02	0.51 ± 0.02	0.73 ± 0.02	0.99 ± 0.02**
LCSFA (% TE)	7.82 ±0.02	8.37±0.02	9.34±0.02	10.1±0.02
VLCSFA (% TE)	9.53±0.01	8.94±0.01	8.73±0.01	8.48±0.01
MUFA (% TE)	11.1±0.02	11.2±0.02	11.2±0.02	10.8±0.02
PUFA (% TE)	9.44±0.02	8.84±0.02	8.56±0.02	7.18±0.02**
Fruit (s/day)	2.23 ± 0.17	2.24 ± 0.17	2.12 ± 0.17	2.07 ± 0.17
Vegetables (s/day)	2.10 ± 0.12	2.31 ± 0.12	2.06 ± 0.12	1.85 ± 0.10
Sugar (% TE)	9.49 ± 0.58	10.5 ± 0.58	10.7 ± 0.58	10.2 ± 0.56
Regular/Reduced Fat (s/day)				
TE (kcal/day)	2040 ± 107	2270 ± 107	2320 ± 106	2290 ± 107
SMCSFA (% TE)	0.33 ± 0.02	0.53 ± 0.02	0.702 ± 0.02	1.04 ± 0.02**
LCSFA (% TE)	7.51±0.02	8.51±0.02	9.23±0.02	10.4±0.02**
VLCSFA (% TE)	9.83±0.01	8.73±0.01	8.76±0.01	8.35±0.01*
MUFA (% TE)	10.9±0.02	11.2±0.02	11.2±0.02	11.0±0.02
PUFA (% TE)	9.15±0.02	8.98±0.02	8.53±0.02	7.36±0.02**
Fruit (s/day)	2.34 ± 0.17	2.24 ± 0.17	2.02 ± 0.17	2.06 ± 0.17
Vegetables (s/day)	2.2 ± 0.12	2.34 ± 0.116	2.03 ± 0.114	1.75 ± 0.12
Sugar (% TE)	9.66 ± 0.57	9.66 ± 0.58	11 ± 0.571	10.6 ± 0.58
Nonfat dairy (s/day)				
TE (kcal/day)	2230 ± 106	2270± 107	2320± 107	2010 ± 106*
SMCSFA (% TE)	0.63 ± 0.03	0.53 ± 0.02	0.702 ± 0.02	0.61 ± 0.03
LCSFA (% TE)	9.07±0.02	8.51±0.02	9.23±0.02	8.20±0.02*
VLCSFA (% TE)	8.41±0.01	8.73±0.01	8.76±0.01	8.48±0.01
MUFA (% TE)	11.3±0.02	11.2±0.02	11.2±0.02	10.3±0.02**
PUFA (% TE)	8.95±0.02	8.98±0.02	8.53±0.02	7.50±0.02**
Fruit (s/day)	2.09 ± 0.17	2.24 ± 0.17	2.02 ± 0.17	2.61 ± 0.17*
Vegetables (s/day)	2.04 ± 0.12	2.34 ± 0.12	2.03 ± 0.11	2.41 ± 0.12*
Sugar (% TE)	9.85 ± 0.58	9.66 ± 0.58	11.0 ± 0.57	9.23 ± 0.58
SMCSFA (%TE)				
TE (kcal/day)	1970±10.5	2140± 10.5	2430 ±10.5	2380±10.5*
LCSFA (% TE)	7.0±0.01	8.46±0.01	9.17±0.01	11.00±0.01**
VLCSFA (% TE)	9.2±0.01	9.26±0.01	8.73±0.01	8.48±0.01

MUFA (% TE)	10.4±0.21	11.2±0.21	11.2±0.21	11.5±0.21*
PUFA (% TE)	8.91±0.02	9.23±0.01	8.24±0.01	764±0.02**
Fruit (s/day)	2.54±0.17	2.0±0.17	2.11±0.17	2.01±0.17
Vegetables (s/day)	2.51±0.11	2.26±0.11	1.87±0.11	1.69±0.11
Sugar (%TE)	9.04±0.57	9.87±0.57	11.2±0.57	10.8±0.57*

Trend analyses were conducted to determine statistical differences. Total dairy includes milk, cheese, yogurt, cream, and butter. Regular dairy includes full fat dairy products. Reduced fat includes 1% and 2% dairy products. Nonfat dairy includes skim or 0% fat products. SMCSFA= short and medium chain saturated fatty acids, TE=total energy, LCSFA=long chain saturated fatty acids, VLCSFA= very-long chain saturated fatty acids, MUFA= monounsaturated fatty acids, PUFA=polyunsaturated fatty acids . * indicates p<0.05, ** indicates p <0.001

Table 3. Baseline dairy component intake by dairy quartile (n=345).

	Dairy (s/day)				
	Total Dairy		**Regular/Reduced Fat Dairy**		**Nonfat dairy**
Dairy components	**Q1**	**Q4**	**Q1**	**Q4**	**Q1**
	1.08±0.03	4.35±0.03	0.98±0.03	3.98±0.03	0.01±0.02
Milk (s/day)	0.43 ± 0.05	2.55 ± 0.05**	0.55±0.05	2.31±0.05**	0.08±0.06
Cheese (s/day)	0.30 ± 0.02	0.64 ± 0.02**	0.29±0.02	0.65±0.02**	0.50±0.02
Yogurt (s/day)	0.05 ± 0.01	0.24 ± 0.01**	0.08±0.01	0.19±0.01**	0.07±0.01
Cream (s/day)	0.10 ± 0.02	0.51 ± 0.03**	0.08±0.03	0.52 ±0.03**	0.29±0.03
Butter (s/day)	0.20 ± 0.02	0.42 ± 0.02**	0.20±0.02	0.44±0.02	0.36±0.02

Trend analyses were conducted to identify statistical significance between quartiles. * Indicates p <0.05, ** indicates p <0.001.

Table 4. Top 10 contributors to total dairy intake at baseline (n=345).

Food	**% Total dairy**
2% Milk	18.78
Whole milk	18.78
Cheese	16.89
1% or skim milk	9.41
Frozen dairy dessert	8.69
Yogurt	4.52
Butter	3.87
Potato dishes	3.68
Cakes and candy	2.77
Breads	1.94

Top foods were estimated by proc rank by groups of foods. Top 10 contributors were included.

Table 5. Top 10 contributors to SMCSFA intake at baseline (n=345).

Food	% Total SMCSFA
Cheese	29.94
Whole milk	22.14
2% milk	13.71
Frozen dairy dessert	7.14
Potato dishes	2.89
Yogurt	2.82
Cakes and candy	2.57
Butter	2.49
Pizza	1.83
Nuts	1.60

Top foods were estimated by proc rank by groups of foods. Top 10 contributors were included. SMCSFA=short and medium chain saturated fatty acids.

Table 6. Individual neurocognitive test means at baseline (n=345)

Test	Mean ± SE
GCS	0.12 ± 0.02
Digit Span Forward	7.60 ± 0.10
Digit Span Backward	3.60±0.07
Clock Drawing	2.21 ±0.05
Word List Learning	38.9±0.50
Retention	30.2±0.27
Recognition	84.1 ±1.37
Stroop	34.12±0.08
Verbal Fluency	25.09±0.50
Figure Copying	11.22±0.36

Individual tests were z-score transformed to be standardized to create global cognitive functioning score (GCS).

Table 7. Cross-sectional associations of dairy and SMCSFA with GCS (n=1292) at baseline

	β-coefficient (95% CI)	P-value
Total Dairy	0.01 (-0.01, 0.03)	0.27
Regular/Reduced Fat Dairy	0.01 (-0.01, 0.03)	0.20
Nonfat Dairy	-0.01(-0.05, 0.04)	0.83
SMCSFA Factor Score	0.02 (-0.01,0.04)	0.23
SMCSFA Substitution Models		
trans fats	0.06(-0.01, 0.10)	0.08
Added sugar	0.105(-0.01, 0.12)	0.09

Proc reg was used to run a cross-sectional multiple linear regression model with dairy and SMCSFA with GCS at baseline. Dairy models were adjusted for age, sex, education, total energy, physical activity and smoking status at baseline and baseline intake of added sugar, fruit, and vegetables. SMCSFA factor score was derived using Karazurna et al factor 2 score[24]. GCS was calculated as the composite score of individual z-score transformed cognitive tests. This model was adjusted for age, sex, education, total energy, physical activity, and smoking status. SMCSFA substitution models were adjusted for the aforementioned covariates. Substitution at the expense of *trans* fats held all other dietary covariates constant, including total carbohydrate, total fat, total protein, total alcohol, long chain saturated fats, very-long chain saturated fats, monounsaturated fats, and polyunsaturated fats. Substitution at the expense of added sugar held the previously mentioned dietary covariates constant as well as *trans* fats and carbohydrates minus those from added sugar. There were not significant findings.

Table 8. Cross-sectional analysis of dairy components with GCS at baseline (n=1292).

	β-coefficient (95% CI)	P-value
Milk	0.004 (-0.02, 0.03)	0.74
Cheese	0.01(-0.01, 0.04)	0.32
Butter	0.01 (-0.01, 0.03)	0.45
Cream	-0.01 (-0.02, 0.01)	0.55

Proc reg was used to run a cross-sectional multiple linear regression model with milk, cheese, butter and cream. Because of many non 0 values, yogurt was analyzed as a categorical variable, described elsewhere. GCS was calculated as the composite score of individual z-score transformed cognitive tests. Models were adjusted for age, sex, education, total energy, physical activity and smoking status at baseline, baseline intake of added sugar, fruit, and vegetables. There were no significant relationships observed.

Table 9. Prospective associations of dairy and SMCSFA with GCS (n=345).

	Model	β-coefficient (95% CI)	P-value
Total Dairy		0.0003 (-0.002, 0.003)	0.83
Regular/Reduced Fat Dairy		0.01 (-0.002, 0.003)	0.71
Nonfat Dairy		-0.002 (-0.01, 0.01)	0.65
SMCSFA Pattern Score		0.002 (-0.002,0.01)	0.26
SMCSFA	*trans* fats	0.001 (-0.001, 0.004)	0.40
	Added sugar	0.001 (-0.001, 0.004)	0.39

Proc mixed was used to conduct mixed-effect models. Dairy components were estimated at baseline, 2 and 13 years. Total dairy includes milk, cheese, yogurt, cream, and butter. Regular dairy includes full fat dairy products. Reduced fat includes 1% and 2% dairy products. Nonfat includes skim or 0% fat products. GCS was calculated as the composite score of individual z-score transformed cognitive tests. Models were adjusted for age, sex, education, and time covarying total energy, physical activity and smoking status, and intake of added sugar, fruit, and vegetables. SMCSFA Pattern score was derived with factor scores derived from Karazurna et al.[24] The dietary pattern score models were adjusted for age, sex, education, time covarying total energy, physical activity, and smoking status. SMCSFA were also analyzed as substitution analyses at the expense of *trans* fats and added sugar. Substitution models were adjusted for total fat, total carbohydrate, and total protein. Substitution model at the expense of added sugar adjusted for *trans*-fat covariates plus *trans* fats, and carbohydrate intake minus added sugar. There were no statistically significant findings.

Table 10. Prospective analysis of dairy components with GCS at baseline (n=345).

	β-coefficient (95% CI)	P-value
Milk	-0.002 (-0.005,0.002)	0.39
Yogurt	0.01 (-0.002,0.03)	0.07
Yogurt [b]	-0.002 (-0.008,0.003)	0.45
Yogurt [c]	0.01 (-0.001, 0.01)	0.59
Cheese	-0.002 (-0.009,0.004)	0.47
Butter	0.008 (0.0002, 0.02)	0.04
Cream	-0.001 (0.005, 0.003)	0.59

Proc mixed was used to conduct mixed-effect models. Dairy components were estimated at baseline, 2 and 13 years. There were no statistically significant findings. Total dairy includes milk, cheese, yogurt, cream, and butter. Regular dairy includes full fat dairy products. Reduced fat includes 1% and 2% dairy products. Nonfat includes skim or 0% fat products. GCS was calculated as the composite score of individual z-score transformed cognitive tests. Model 1 was adjusted for age, sex, education, and time covarying total energy, physical activity and smoking status Model 2 was adjusted for model 1 covariates plus time covarying intake of added sugar, fruit, and vegetables.
[a] analyzed as a continuous variable in using proc mixed. [b] compared high consumers of yogurt to low and non consumers. [c] compared low consumers with high and non consumers.

Supplemental Table 1. Saturated Fatty Acid Chain Length Categorization

Fatty Acid Group	Individual species names (Carbon chain length)
Short chain SFA	Butyric acid (C4)
Medium-chain SFA	Caproid acid (C6), Caprylic acid (C8), Capric acid (C10), Lauric acid (C12)
Long-chain SFA	Myristic (C14), Palmitic acid (C16), Stearic acid (C18)
Very-long chain SFA	Arachidonic acid (C20), Behenic acid (C22)

Supplemental Table 2. Demographic and dietary covariates comparing participants who were in the final sample versus those who dropped out.

Variable	Final Sample(n=345)	Lost to Follow-up (n=947)	P-value
Age	55.5±0.35	57.1±0.26	0.0003*
Sex (%)	77.8	68.3	0.0006*
GCS	0.12±0.02	0.02±0.02	0.006*
Total dairy (s/day)	2.42±0.06	2.52±0.04	0.21
SMCSFA (%TE)	0.65±0.02	0.66 ±0.01	0.57
Physical activity level	31.8±0.21	31.7±0.17	0.79
Education level (%)	18.3	15.7	0.24
Smoking status (%)	20	25.4	0.08
Diabetes (%)	32.4	40	0.01*
CVD (%)	17.8	21.2	0.14

Proc GLM was used to determine statistical significance between group means. * Indicates $p<0.05$. GCS= Global Cognitive Score SMSFA= short and medium chain saturated fatty acid, TE= total energy, CVD=cardiovascular disease. * Indicates $p<0.05$

Supplemental Table 3. Cross-sectional associations of dairy and SMCSFA with individual neurocognitive tests at baseline (1292).

Exposure	**Test**	**β-coefficient (95% CI)**	**P-value**
Total Dairy	Digit Span Forward	0.02 (-0.01, 0.06)	0.13
	Digit Span Backward	0.01 (-0.02, 0.04)	0.58
	Clock Drawing	0.01 (-0.02, 0.05)	0.42
	Word List Learning	0.02 (-0.01, 0.05)	0.31
	Retention	0.03 (-0.004, 0.07)	0.09
	Recognition	-0.003 (-0.04, 0.03)	0.87
	Stroop	0.01 (-0.02, 0.05)	0.55
	Verbal Fluency	-0.004 (-0.04, 0.03)	0.79
	Figure Copying	-0.002 (-0.03, 0.03)	0.90
Regular/Reduced Fat Dairy	Digit Span Forward	0.02 (-0.01, 0.06)	0.18
	Digit Span Backward	0.01 (-0.02, 0.04)	0.64
	Clock Drawing	0.01 (-0.01, 0.05)	0.34
	Word List Learning	0.02 (-0.01, 0.06)	0.23
	Retention	0.04 (0.002, 0.08)	0.04
	Recognition	-0.002 (-0.04, 0.03)	0.89
	Stroop	0.01 (-0.02, 0.05)	0.45
	Verbal Fluency	-0.01 (-0.05, 0.02)	0.47
	Figure Copying	0.006 (-0.03, 0.04)	0.72
Nonfat Dairy	Digit Span Forward	0.02 (-0.06, 0.12)	0.52
	Digit Span Backward	0.01 (-0.07, 0.10)	0.76

	Clock Drawing	-0.01 (-0.01, 0.07)	0.83
	Word List Learning	-0.01 (-0.09, 0.07)	0.78
	Retention	-0.02 (-0.12, 0.06)	0.62
	Recognition	-0.005 (-0.09, 0.08)	0.91
	Stroop	-0.01 (-0.11, 0.08)	0.79
	Verbal Fluency	0.04 (-0.04, 0.13)	0.32
	Figure Copying	-0.04 (-0.13, 0.04)	0.27

Proc reg was used to run a cross-sectional multiple linear regression models. Total dairy includes milk, cheese, yogurt, cream, and butter. Regular dairy includes full fat dairy products. Reduced fat includes 1% and 2% dairy products. Nonfat dairy includes skim or 0% fat products. GCS was calculated as the composite score of individual z-score transformed cognitive tests. Models were adjusted for age, sex, education, total energy, physical activity and smoking status at baseline, and baseline intake of added sugar, fruit, and vegetables. *p <0.05, however; FDR corrections were applied to adjust for multiple hypothesis testing and attenuated significant findings.

Supplemental Table 4. Prospective analysis of dairy and SMCSFA and GCS at baseline, 2 and 13 years (n=345).

	Test	**β-coefficient (95% CI)**	**P-value**
Total Dairy	Digit Span Forward	-0.002 (-0.01, 0.004)	0.6
	Digit Span Backward	-0.003 (-0.009, 0.003)	0.31
	Clock Drawing	-0.002 (-0.01, 0.004)	0.53
	Word List Learning	0.002 (-0.005, 0.01)	0.56
	Retention	0.004 (-0.003, 0.01)	0.22
	Recognition	-0.002 (-0.01, 0.004)	0.52
	Stroop	-0.003 (-0.01, 0.004)	0.41
	Verbal Fluency	0.003 (-0.002, 0.01)	0.31
	Figure Copying	0.004 (-0.001, 0.01)	0.14
Regular/Reduced fat dairy	Digit Span Forward	-0.0001(-0.01, 0.01)	0.98
	Digit Span Backward	-0.002 (-0.01, 0.005)	0.62
	Clock Drawing	-0.002 (-0.01, 0.004)	0.51
	Word List Learning	0.002 (-0.004, 0.01)	0.5
	Retention	0.004 (-0.003, 0.01)	0.31
	Recognition	-0.002 (-0.01, 0.01)	0.57
	Stroop	-0.004 (-0.01, 0.003)	0.31
	Verbal Fluency	0.01 (-0.001, 0.01)	0.1
	Figure Copying	0.01 (-0.001, 0.01)	0.12
Nonfat Dairy	Digit Span Forward	-0.01 (-0.02, 0.01)	0.21
	Digit Span Backward	-0.01 (-0.02, 0.004)	0.14
	Clock Drawing	-0.0003 (-0.01, 0.01)	0.96
	Word List Learning	0.0002 (-0.01, 0.01)	0.98
	Retention	0.005 (-0.01, 0.02)	0.58
	Recognition	-0.002 (-0.02, 0.01)	0.82
	Stroop	0.00272 (-0.01, 0.02)	0.78
	Verbal Fluency	-0.01 (-0.02, 0.01)	0.2

Figure Copying	-0.001 (-0.01, 0.01)	0.85

Proc mixed was used to conduct mixed-effect models. Dairy components were estimated at baseline, 2 and 13 years. Total dairy includes milk, cheese, yogurt, cream, and butter. Regular dairy includes full fat dairy products. Reduced fat includes 1% and 2% dairy products. Nonfat includes skim or 0% fat products. GCS was calculated as the composite score of individual z-score transformed cognitive tests. Models were adjusted for age, sex, education, and time covarying total energy, physical activity and smoking status, and intake of added sugar, fruit, and vegetables. GCS was calculated as the composite score of individual z-score transformed cognitive tests. Models were adjusted for age, sex, education, total energy, physical activity and smoking status at baseline, and baseline intake of added sugar, fruit, and vegetables. *p <0.05, however; FDR corrections were applied to adjust for multiple hypothesis testing and attenuated significant findings.

Supplemental Table 5. Prospective substitution of dairy at the expense of red meat with GCS (n=345)

	β-coefficient (95% CI)	**P-value**
Total Dairy	0.0004 (-0.002, 0.003)	0.78
Regular/Reduced Fat Dairy	0.01 (-0.002, 0.003)	0.66
Nonfat Dairy	-0.002 (-0.01, 0.01)	0.63

Proc mixed was used to conduct mixed-effect models. Total dairy includes milk, cheese, yogurt, cream, and butter. Regular dairy includes full fat dairy products. Reduced fat includes 1% and 2% dairy products. Nonfat includes skim or 0% fat products. GCS was calculated as the composite score of individual z-score transformed cognitive tests. Models were adjusted for age, sex, education level, and time covarying smoking status, physical activity level, total servings of fruit, vegetables, grains, legumes, animal proteins, oils, and alcohol, per day. There were no statistically significant findings.