RESEARCHREPORT

HUMAN NUTRITION

Project Title and NPB project identification number

Investigating the Role of Pork Consumption on Cognition and Brain Health through Innovation in Nutritional Cognitive Neuroscience (21-142)

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Industry Summary

Our research program applied interdisciplinary methods in nutritional cognitive neuroscience to investigate the role of pork consumption on cognitive performance and brain health. We conducted nutrient biomarker analysis to estimate the concentration of nutrients in each participant's blood. We then applied principal components analysis to derive phenotypes that capture dietary patterns of protein consumption. This approach allowed us to perform a fine-grained analysis of protein consumption that distinguished between different protein categories (pork, beef, poultry, and fish) and preparations of protein (processed versus unprocessed). We examined the association between the observed protein phenotypes and state-of-the-art measures of cognitive performance and brain health (as assessed by structural and functional MRI).

Our research was guided by 5 primary objectives: (1) *Discover Nutrient Biomarkers of Pork Consumption* (i.e., to investigate the key nutrients associated with eating different types of pork and non-pork proteins); (2) *Differentiate Pork Consumers from Non-Pork Consumers* (i.e., to compare cognitive performance and brain function in individuals who typically consume pork compared to those who typically consume non-pork protein); (3) *Differentiate Pork Consumers from Consumers of Other Types of Protein* (i.e., to investigate how consumption of pork protein compares to non-pork protein with respect to cognitive performance and brain function); (4) Differentiate Between Different Types of Pork (i.e., to examine how consumption of different types of pork protein relate to cognitive performance and brain function); (5) *Examine the Longitudinal Effects of Pork Consumption* (i.e., to assess whether dietary changes in protein consumption were associated with corresponding changes in cognitive performance and brain function).

The results of our study provide evidence for four primary conclusions. First, we observed that several B vitamins, minerals (selenium and zinc), and omega-6 PUFAs were associated with the consumption of pork (as measured by dietary questionnaires and blood-based biomarkers of nutrition). Notably, prior research suggests that these nutrients play an important role in brain health and are associated with cognitive performance on tests of attention, learning, and memory. Second, our study provides novel evidence that these nutrients may be linked to the function of specific brain networks. Our analysis revealed that consumption of different types of pork, but especially lean pork, is associated with the efficiency of information processing within brain networks for visual and attentional processing (as measured by their small-world propensity). Third, to further investigate the role of pork protein in well-established dietary patterns, we conducted an analysis of the contribution of pork and other food categories in the Mediterranean Diet with respect to measures of brain health. Our analysis demonstrated that the consumption of lean pork was associated with favorable Brain Age, a measure of one's chronological age relative to their estimated brain age, providing an index of slow versus accelerated brain aging. Fourth, we investigated whether changes in pork consumption over time accounted for trajectories of cognitive performance and brain function in late life. We found that pork consumption in our longitudinal cohort did not change significantly over time and therefore was not associated with changes in cognition and brain health.

In closing, we emphasize that our findings do not support conclusions about the causal role of pork consumption on cognitive performance and brain health. However, we believe that the results of our study provide promising evidence to motivate the design of future intervention trials to investigate the potential benefits of lean pork protein on Brain Age and targeted measures of brain network function.

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Key Findings

- We identified pork phenotypes derived from dietary and nutrient biomarker analysis.
- We observed that pork phenotypes were associated with the efficiency of brain function with the visual and attentional networks.
- Our research motivates the design of pork-focused intervention trials to establish and validate the beneficial effects of fresh lean pork consumption of cognitive performance and brain health.

Keywords

- Brain imaging
- Nutrient biomarkers
- Cognitive function
- Brain health
- Protein phenotypes

Scientific Abstract

Accumulating evidence in the nutritional sciences demonstrates that meat consumption may have favorable effects of cognitive and brain health, with recent reports indicating that: (i) increased poultry intake may reduce the risk of cognitive decline; (ii) meat consumption may be associated with higher general intelligence; and (iii) higher red meat intake may be associated with lower risk of memory impairments (2020, Nutrients, Meat Consumption, Cognitive Function and Disorders: A Systematic Review with Narrative Synthesis and Meta-Analysis, Zhang et al.; 2021, Nature Scientific Reports, Diet and general cognitive ability in the UK Biobank dataset, Hepsomali & Groeger). Despite the promise of these findings for the National Pork Board, these studies fail to distinguish lean pork from other dietary sources of protein, and therefore motivate the need for greater precision in characterizing the link between lean pork consumption and measures of cognitive performance and brain health.

The present study examined this issue by applying interdisciplinary methods in nutritional cognitive neuroscience to derive dietary and nutrient biomarker phenotypes of pork consumption and to examine their association with measures of cognitive performance and functional brain network efficiency. Our findings provide novel evidence that lean pork consumption is associated with functional efficiency within the visual and ventral attentional networks, motivating the design of randomized controlled trials to establish the role of lean pork consumption in cognitive performance and brain health.

Introduction

Our research program employed methods in nutritional cognitive neuroscience to examine the relationship between protein phenotypes and measures of cognitive performance and brain function.

Objectives

- Objective 1. *Discover Nutrient Biomarkers of Pork Consumption*
- Objective 2. *Differentiate Pork Consumers from Non-Pork Consumers*
- Objective 3. *Differentiate Pork Consumers from Consumers of Other Types of Protein*
- Objective 4. *Differentiate Between Different Types of Pork*
- Objective 5. *Examine the Longitudinal Effects of Pork Consumption*

Materials & Methods

Our study examined previously collected data from 150 healthy elderly adults from the Illinois Brain Aging Study cohort, a sample of community-dwelling Caucasian men and women aged 65–75 years. Participants were neurologically healthy, with no evidence of cognitive impairment, as defined by a score of lower than 26 on the Mini-Mental State Examination. Exclusion criteria included: diagnosis of mild cognitive impairment, dementia, psychiatric illness within the last three years, stroke within the past twelve months, cancer within the last three years, current chemotherapy or radiation, an inability to complete study activities, prior involvement in cognitive training or dietary intervention studies, and contraindications for magnetic resonance imaging (MRI). All participants were right-handed with normal, or corrected to normal, vision. The enrolled subjects had a complete dataset at time of data analysis, including cognitive testing, resting-state fMRI, nutrient blood biomarkers and survey responses to the Diet History Questionnaire II, or DHQII. Participants had a mean age of 69 years and 63 percent of participants were female.

Our analysis of the data applied multiple statistical methods, including descriptive statistics, linear regression, ANCOVA and data reduction models (e.g., Principal Components Analysis, or PCA). We also used more advanced modeling techniques, including machine learning approaches (https://pubmed.ncbi.nlm.nih.gov/32108865/) that systematically control for dietary covariates (e.g., fruits and vegetables). We also used Structural Equation Modeling (SEM) tools to help asses if there were changes in protein consumption that related to brain and cognitive changes over time. These types of analyses, machine learning and SEM, can identify with greater sensitivity the effect of pork protein sources.

We started with a subset of survey questions from the DHQII (Table 1) to create a dataset that informed the consumption of pork and other types of proteins. These questions spanned multiple types of pork and other protein sources to capture variability in the diet. The DHQII reports frequencies for protein consumption on a scale of 0 to 10, with “0” designated as “Never” consumed during the previous year and “10” designated as a consumption frequency of “2 or more times per day”. The questions were grouped to create five distinct protein phenotypes, four pork only phenotypes (General Pork, Unprocessed Pork, Processed Pork, and Lean Pork) and one animal protein phenotype (Lean Meat) which represents a combination of protein from beef, chicken, and fish.

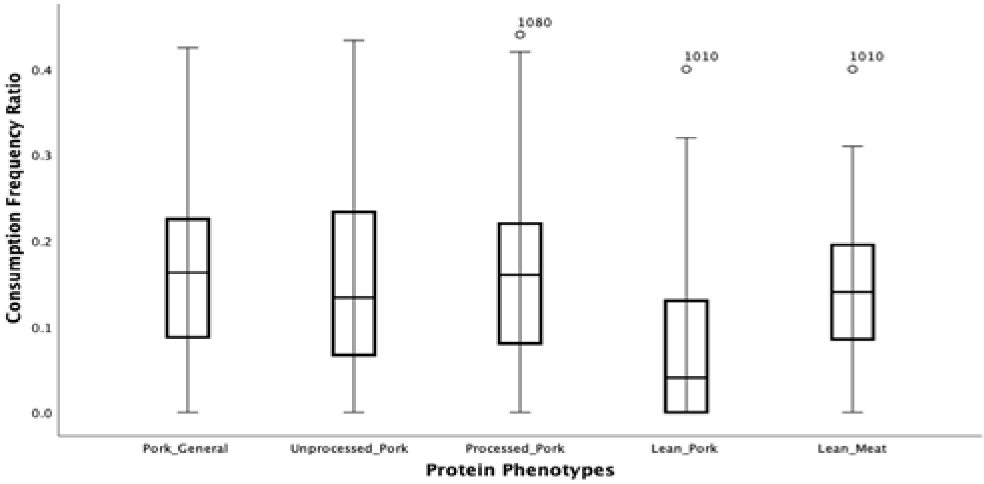
Summing across each participant's score for all questions within a phenotype, and then dividing by the maximum score possible for the phenotype, yielded a Consumption Frequency Ratio scaled between 0 and 1, with 0 representing no consumption and 1 representing high consumption (see Fig. 1).

General Pork	
Q525	How often did you eat luncheon or deli-style ham? (We will ask about other ham later.)
Q529	How often did you eat other cold cuts or luncheon meats (such as bologna, salami, corned beef, pastrami, or others, including low-fat)? (Please do not include ham, turkey, or chicken cold cuts.)
Q533	How often did you eat hot dogs or frankfurters? (Please do not include sausages or vegetarian hot dogs.)
Q578	How often did you eat pork or beef spareribs?
Q595	How often did you eat baked ham or ham steak?
Q606	How often did you eat pork (including chops, roasts, and in mixed dishes)? (Please do not include ham, ham steak, or sausage.)
Q615	How often did you eat bacon (including low-fat)?
Q619	How often did you eat sausage (including low-fat)?
Unprocessed Pork	
Q578	How often did you eat pork or beef spareribs?
Q595	How often did you eat baked ham or ham steak?
Q606	How often did you eat pork (including chops, roasts, and in mixed dishes)? (Please do not include ham, ham steak, or sausage.)
Processed Pork	
Q525	How often did you eat luncheon or deli-style ham? (We will ask about other ham later.)
Q529	How often did you eat other cold cuts or luncheon meats (such as bologna, salami, corned beef, pastrami, or others, including low-fat)? (Please do not include ham, turkey, or chicken cold cuts.)
Q533	How often did you eat hot dogs or frankfurters? (Please do not include sausages or vegetarian hot dogs.)
Q615	How often did you eat bacon (including low-fat)?
Q619	How often did you eat sausage (including low-fat)?
Lean Pork	
Q527	How often was the luncheon or deli-style ham you ate light, low-fat, or fat-free?
Q535	How often were the hot dogs or frankfurters you ate light or low-fat?
Q617	How often was the bacon you ate light, low-fat, or lean?
Q621	How often was the sausage you ate light, low-fat, or lean?

Lean Meat	
Q527	How often was the luncheon or deli-style ham you ate light, low-fat, or fat-free?
Q531	How often were the other cold cuts or luncheon meats you ate light, low-fat, or fat-free? (Please do not include ham, turkey, or chicken cold cuts.)
Q535	How often were the hot dogs or frankfurters you ate light or low-fat?
Q562	How often were these beef hamburgers or cheeseburgers made with lean ground beef?
Q576	How often was the steak you ate lean steak?
Q592	How often was the chicken you ate WHITE meat?
Q617	How often was the bacon you ate light, low-fat, or lean?
Q621	How often was the sausage you ate light, low-fat, or lean?
Q625	How often was the canned tuna you ate water-packed?
Q640	How often did you eat other fish that was NOT FRIED (not including shellfish)?

Table 1. Diet History Questionnaire II (DHQII) questions comprising 4 pork only phenotypes (General Pork, Unprocessed Pork, Processed Pork, and Lean Pork) and 1 meat phenotype comprised of pork, beef, chicken, and fish (Lean Meat).

Fig. 1. Distribution of Protein Phenotypes (x-axis) based on Consumption Frequency Ratio.



Results

Objective 1: Discover Nutrient Biomarkers of Pork Consumption. We investigated nutrients derived from eating pork by augmenting food frequency questionnaires with state-of-the-art blood-based biomarkers. Pork is an important source of multiple vitamins (B1, B2, B3, B6, B12, choline, and phosphatidylcholine), minerals (iron, magnesium, phosphorous, potassium, zinc, and selenium), fatty acids (mono- and poly-unsaturated), protein and amino acids (beta-alanine, taurine, creatine, and glutathione). We assessed the concentrations of these nutrients for each protein phenotype. The nutrient biomarkers were derived from a blood draw with the resulting nutrient concentrations determined by a lab assay whereas DHQII nutrient biomarkers were determined from survey responses. These nutrient concentrations complement each other because some blood-based biomarkers were not determined by the DHQII and while some DHQII determined nutrients did not have an analogous blood biomarker.

Within our food frequency questionnaire data, we parsed different types of pork, including chops, roasts, in mixed dishes, bacon, baked ham or ham steak; luncheon or deli style ham; and sausage. The cut of pork, in addition to whether it is cured or processed, changes the nutrients derived. Consumption of these different types of pork generated five protein phenotypes: General Pork, Unprocessed Pork, Processed Pork, Lean Pork, and non-pork Lean Meat which included lean and unprocessed cuts of beef, poultry, and fish.

Each of the five phenotypes, and the nutrient biomarkers were analyzed with a model that included sex, age, education, income, body mass index, and total energy intake as covariates. The results of the associations between the DHQII nutrient biomarkers and protein phenotypes are provided in Table 2. Many of the pork phenotypes provided several important essential vitamins and minerals, including B1, B3, B12, Selenium and Zinc. Four of these essential vitamins and minerals are unique to the pork only phenotypes: Vitamins B1, B3 and B12 and zinc. Both vitamin B3, or niacin, and zinc are only associated with all four pork phenotypes, but not lean meat. Vitamin B1 was only associated with the General and Unprocessed Pork phenotypes. Vitamin B12 was associated with the General, Unprocessed and Processed Pork phenotypes. Zinc was associated with General, Unprocessed, Processed, and Lean Pork phenotypes. Only selenium was associated with the Lean Meat phenotype derived from non-pork protein sources.

	General Pork	Unprocessed Pork	Processed Pork	Lean Pork	Lean Meat
B1	**0.19**	**0.28**	0.11	0.14	0.06
B3	**0.26**	**0.32**	**0.19**	**0.19**	0.18
B12	**0.30**	**0.27**	**0.27**	0.13	0.16
Selenium	**0.24**	**0.27**	**0.19**	**0.17**	**0.20**
Zinc	**0.31**	**0.36**	**0.24**	**0.17**	0.12

Table 2. Correlation coefficients between DHQII nutrient biomarkers and protein phenotypes. Bolded numbers represent correlations with a p-value < 0.05.

The results of the associations between the blood-based nutrient biomarkers and protein phenotypes are provided in Table 3. Unprocessed Pork was positively associated with two mono-unsaturated fatty acids (MUFAs, C22.1n.9 and trans-C16.1n.9) and three omega-3 poly-unsaturated fatty acids (PUFAs, C20.4n.6, C20.4n.6 (arachidonic acid), and C22.5n.6) Processed Pork was positively associated with choline, the MUFAs C22.1n.9 and PUFAs C20.4n.6, C20.4n.6, C22.5n.6., C18.4n.3 and C18.2.CLA while it was inversely associated with vitamin K. Lean Pork consumption was positively associated with lycopene, vitamins A, E and K, and the omega-6 PUFA gamma linolenic acid (GLA, C18.4n.3). Lean pork is negatively associated with trans fatty acids. Finally, the Lean Meat phenotype, which does not include pork, was associated with lycopene, vitamin A and two omega-6 PUFAs (GLA, C18.4n.3 and C20.4n.6).

	Unprocessed Pork	Processed Pork	Lean Pork	Lean Meat
Lycopene	0.14	-0.01	**0.26**	**0.24**
Vitamin E	0.09	0.05	**0.24**	0.17
Vitamin A	0.15	0.13	**0.34**	**0.41**
Choline	0.15	**0.24**	0.08	0.04
Vitamin K	-0.22	**-0.35**	**0.28**	0.23
Total Trans Fats	0.01	0.14	**-0.27**	-0.15
MUFA (C22.1n.9)	**0.24**	0.22	-0.09	-0.09
MUFA (trans-C16.1n.9)	**0.30**	**0.25**	-0.11	-0.04
Omega-3 (C18.4n.3)	0.09	**0.28**	-0.04	0.02
Omega-6 (C18.3n.6)	0.11	0.08	**0.24**	**0.25**
Omega-6 (C20.4n.6)	**0.30**	**0.35**	0.15	**0.24**
Omega-6 (C22.4n.6)	**0.36**	**0.42**	0.06	0.12
Omega-6 (C22.5n.6)	**0.31**	**0.34**	0.13	0.18
Omega-6 (C18.2.CLA)	0.06	**0.26**	-0.11	-0.10

Table 3. Correlation coefficients between blood based nutrient biomarkers and protein phenotypes. Bolded numbers represent correlations with a p-value < 0.05.

Objective 2: Differentiate Pork Consumers from Non-Pork Consumers. Objective 2 examined whether the Lean Pork phenotype differed from the non-pork Lean Meat phenotype for measure of brain function. The Lean Meat phenotype consisted of beef, poultry, and fish. We controlled for other dietary covariates in the model (e.g., fruits, vegetables, and grains). Our investigation of brain function focused on functional brain networks that reflect the intrinsic organization of the human brain. Functional brain networks support specific cognitive functions and interact to enable complex behavior and decision making. To investigate how efficiently each network is functionally organized, we examined their small world propensity. This measure quantifies the efficiency of information processing within each network, such that a larger value represents more efficient network organization and is typically associated with superior cognitive performance. Table 4 presents the results.

	Lean Pork	Lean Meat

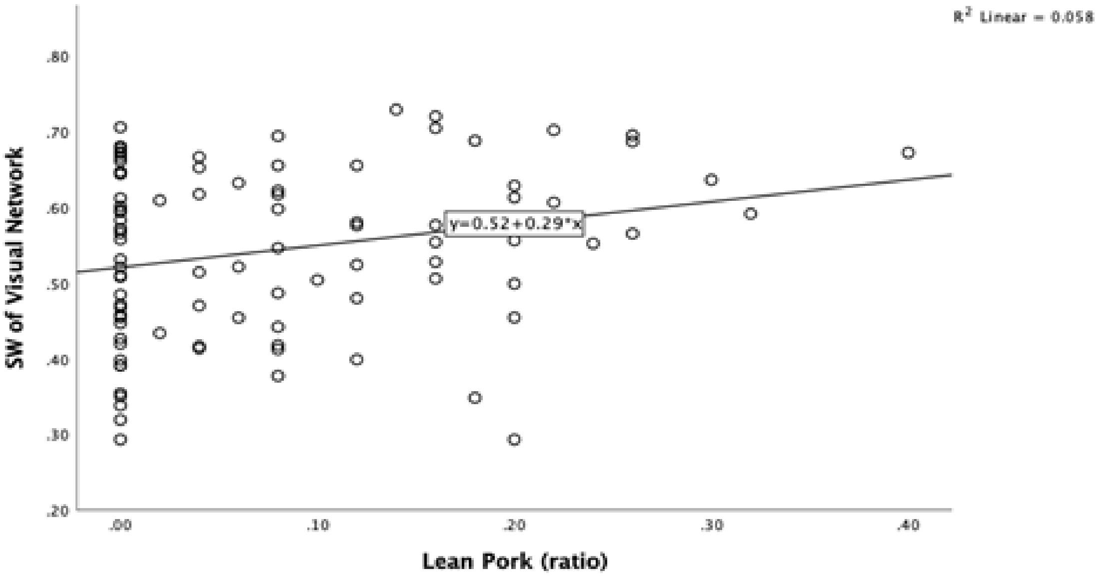
Brain Age	-0.02	-0.15
Visual	**0.24**	0.18
Motor	0.15	**0.27**
Dorsal Attention	-0.08	-0.03
Ventral Attention	**0.28**	**0.23**
Limbic	-0.02	0.00
Fronto Parietal	0.02	0.03
Default	0.14	0.17

Table 4. Correlation coefficients between Brain Age (row 1) or small world propensity functional brain connectivity networks (rows 2-8) and protein phenotypes. Bolded numbers represent correlations with a p-value < 0.05.

Brain Age is a well-established structural MRI measure that reflects the relationship between an individual's chronological and neurobiological age. Individuals whose estimated neurobiological age is less than their chronological age are believed to have good brain health, whereas those whose neurobiological age is greater than their chronological age are believed to have accelerated brain aging, or poorer health. Notably, our findings suggest that Brain Age is not associated with protein phenotypes.

With respect to brain network efficiency, the Lean Pork phenotype was positively associated with two brain networks, the visual and ventral attention networks. Fig. 2 illustrates the relationship between small world propensity of the visual network and the Lean Pork phenotype. The more general Lean Meat phenotype was positively associated with the motor and ventral attention networks. The default mode, fronto-parietal and limbic networks were not associated with either protein phenotype.

Fig. 2. Correlation between the Lean Pork phenotype (x-axis) and the visual brain network characterized by small world propensity.



Objective 3: Differentiate Pork Consumers from Consumers of Other Types of Protein. We compared the consumption of pork protein to non-pork protein for two measures of brain function: Brain Age and small-world propensity of functional brain networks. Pork protein phenotypes included Pork, Lean Pork, and All Pork whereas the non-pork phenotype included Beef, Poultry and Fish.

Brain Age was not associated with any of the protein phenotypes (see Fig. 3). However, a follow-up analysis examining the impact of a Medi Pork diet on Brain Age revealed that the fruit and lean pork components of that diet are primary contributors to a lower Brain Age (Fig. 4).

Finally, relative to several other protein sources, only Lean Pork was associated with brain network efficiency, as evidenced by an association with the small-world propensity of the Default Mode Network (see Fig. 5).

Fig 3. The vertical bars in the figure illustrate the relationship between Brain Age and pork- and non-pork protein sources. If the line crosses the dashed horizontal line (0), then the protein category is not significantly related to Brain Age.

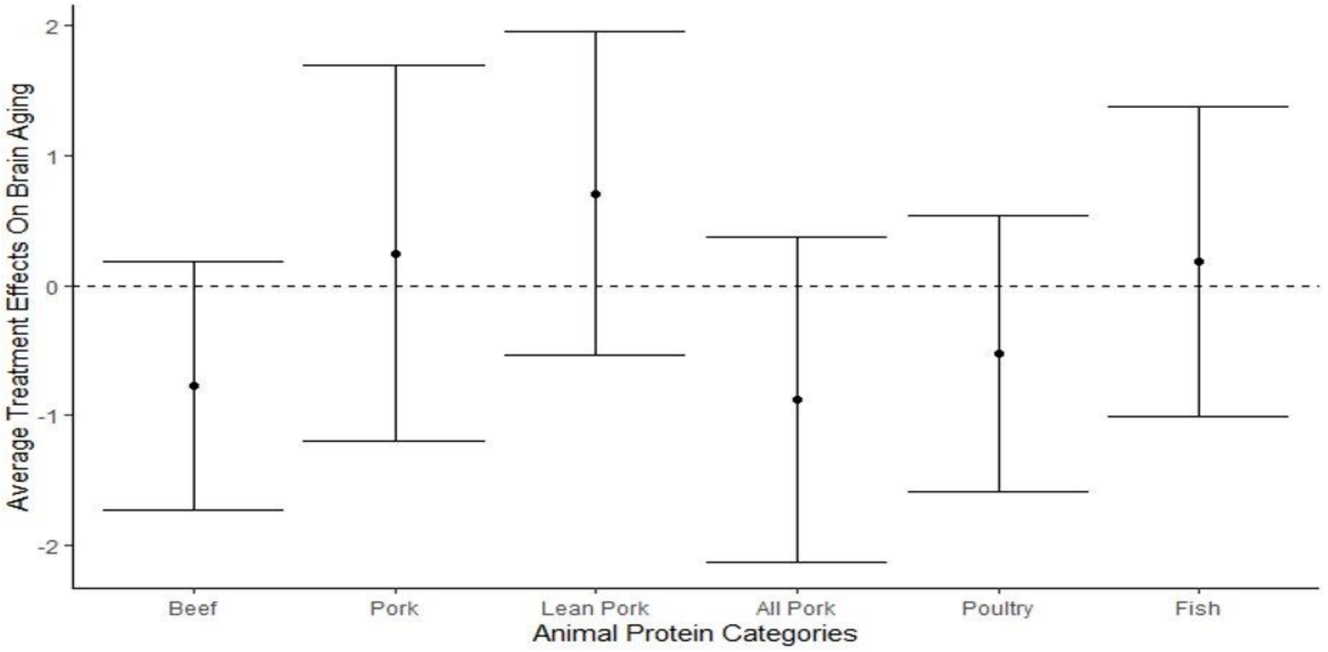


Fig. 4. The vertical bars in the figure illustrate the association between the Medi Pork diet and Brain Age. If the vertical line crosses the dashed horizontal line (0), then the brain networks is not significantly related to consumption of Lean Pork.

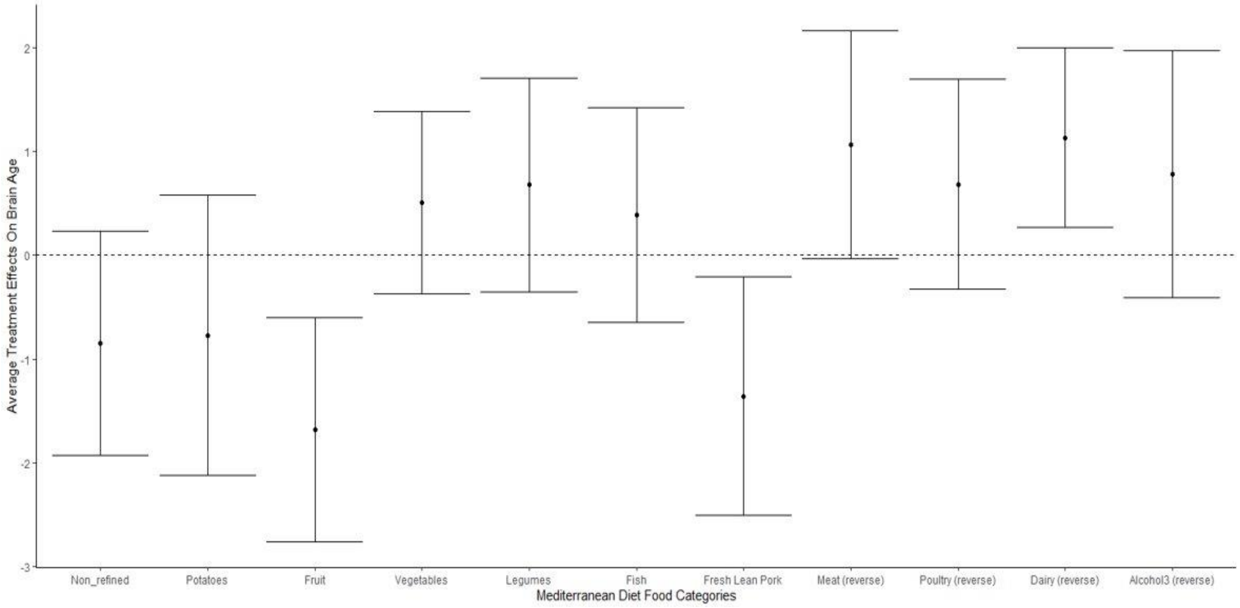
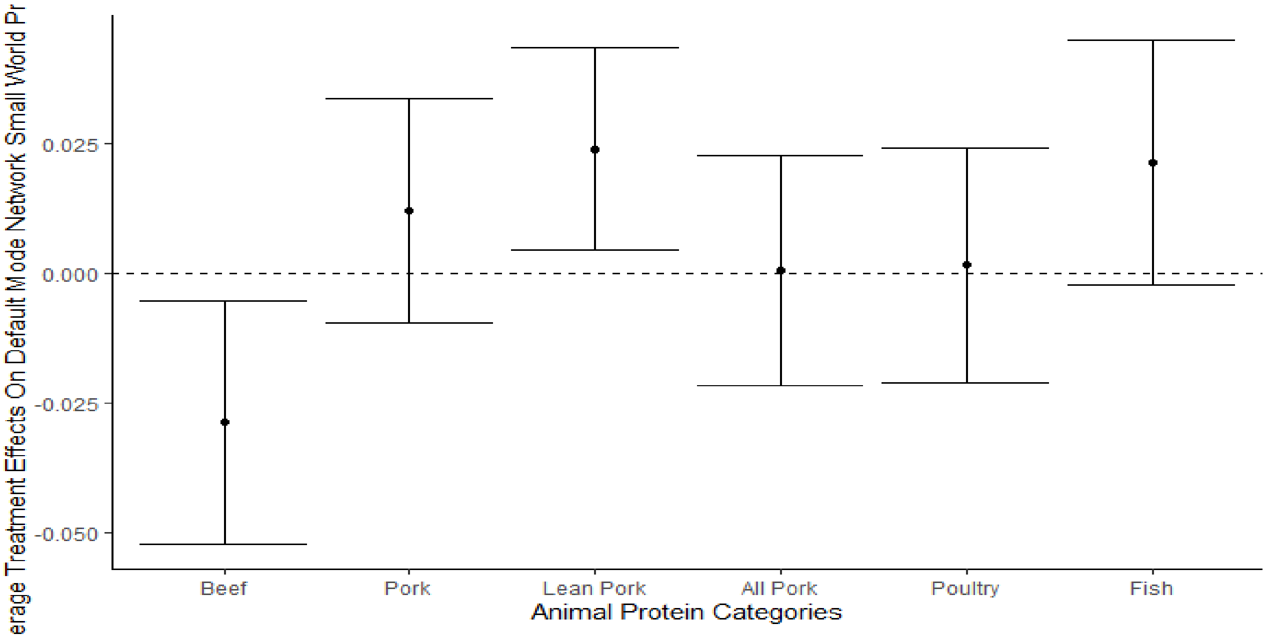


Fig. 5. The vertical bars in the figure illustrate the relationship between the Default Mode brain network and pork- and non-pork protein sources. If the vertical line crosses the dashed horizontal line (0), then the protein category is not significantly related to Brain Age.



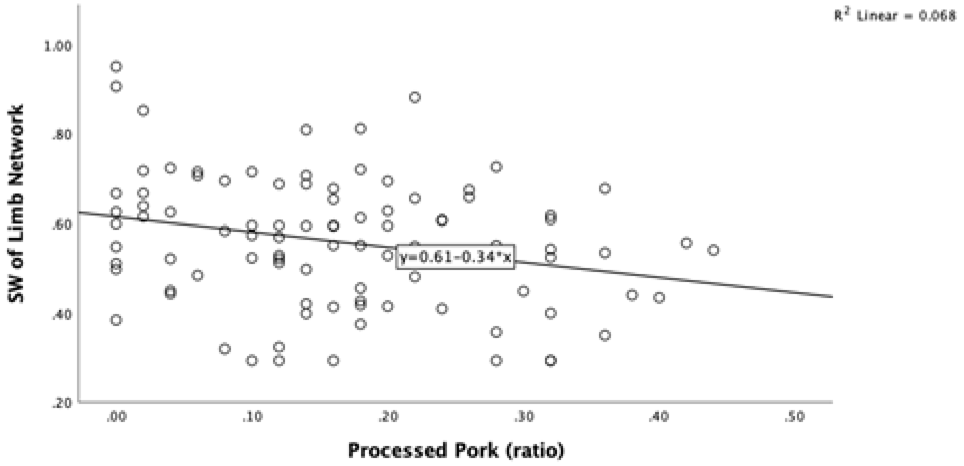
Objective 4: Differentiate Between Different Types of Pork. In this analysis we examined whether the consumption for different types of pork was associated with MRI measures of Brain Age and function (small-world propensity). The full set of results are presented in Table 5.

Brain Age was not associated with any of the pork protein phenotypes. With respect to brain function, the Lean Pork phenotype was positively associated with two brain networks, the visual and ventral attention network. The ventral attention network had a positive association with three of the four pork protein phenotypes: General Pork, Unprocessed Pork, and Lean Pork. Processed Pork had a negative association with the Limbic network (see Fig. 6). We observed several networks, such as the default mode, fronto-parietal and limbic, that were not associated with consumption of any pork phenotype.

	General Pork	Unprocessed Pork	Processed Pork	Lean Pork
Brain Age	-0.07	-0.05	-0.07	-0.02
Visual	0.10	0.05	0.11	**0.24**
Motor	-0.06	-0.07	-0.04	0.15
Dorsal Attention	-0.17	-0.16	-0.16	-0.08
Ventral Attention	**0.19**	**0.22**	0.15	**0.28**
Limbic	**-0.23**	-0.11	**-0.26**	-0.02
Fronto Parietal	0.02	-0.02	0.05	0.02
Default	0.06	-0.01	0.09	0.14

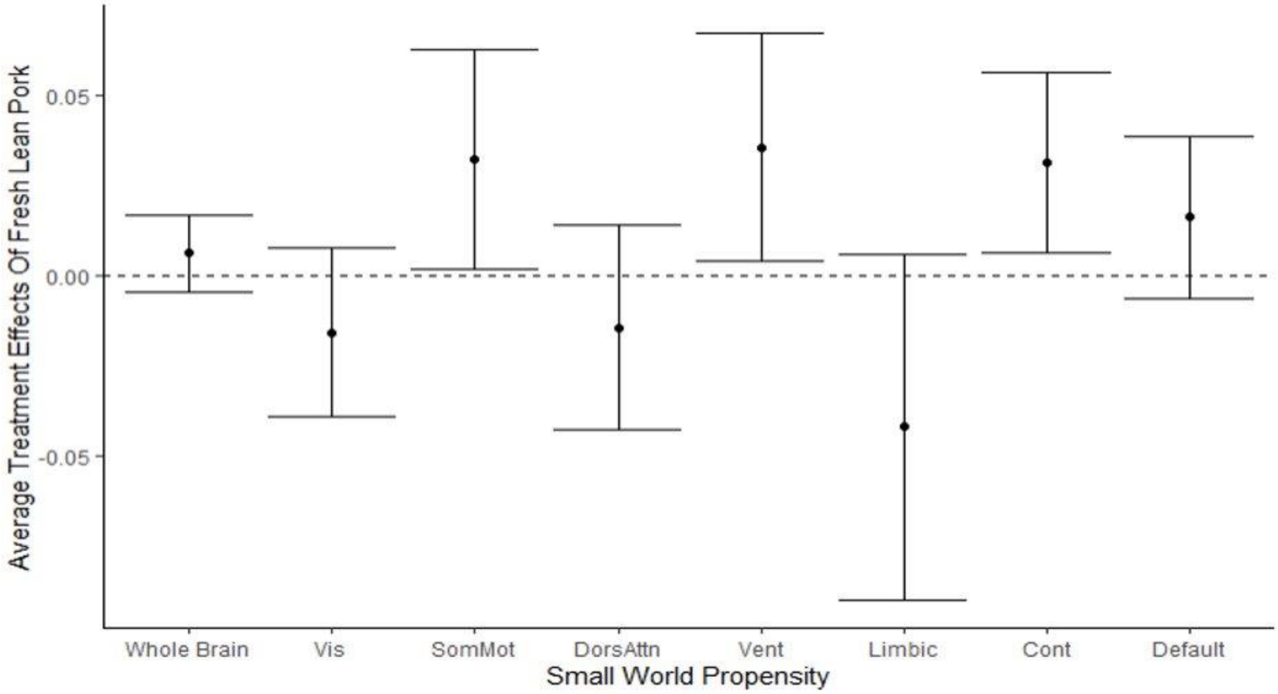
Table 5. Correlation coefficients for Brain Age (row 1) and brain network efficiency (rows 2-8) for each pork protein phenotype. Bolded values represent correlations with a p-value < 0.05.

Fig. 6. Correlation between the Lean Pork phenotype (x-axis) and the limbic network's small world propensity measure.



The results in Fig. 7 replicated and extended the results of Table 5 using a more sensitive machine learning approach to assess the relationship between small-world propensity of brain networks and the Lean Pork phenotype. Our findings indicate that Lean Pork consumption is associated with the efficiency of the somato-motor, ventral attention and fronto-parietal networks, while simultaneously controlling for all other sources of protein in the diet and other foods, such as fruits, vegetables, and grains.

Fig 7. Small-world propensity measure of brain networks and their association with consumption of fresh Lean Pork. If the vertical line crosses the dashed horizontal line (0), then the brain networks is not significantly related to consumption of Lean Pork.



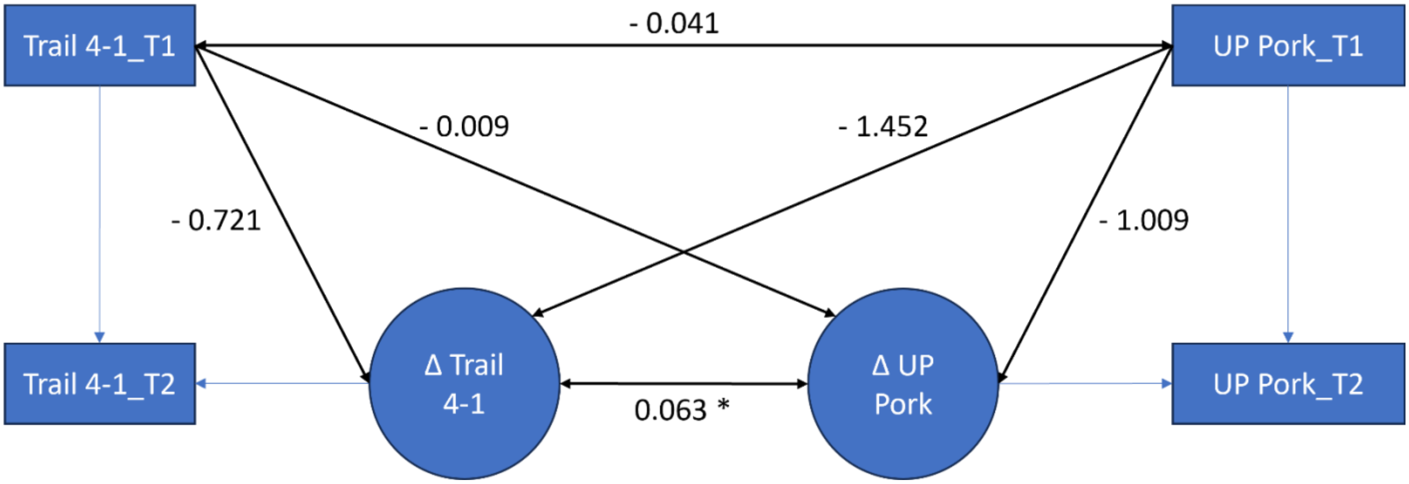
Objective 5: Examine the Longitudinal Effects of Pork Consumption. Participants in the longitudinal follow-up were, on average, two years older at the second timepoint than when they enrolled in the study. Our analysis included examination of descriptive statistics and latent variable modeling.

First, we examined longitudinal changes in cognitive performance and pork consumption across the first and second timepoints. We found no reliable changes over time, suggesting that the participants cognitive performance and dietary patterns remained stable. Table 6 provides descriptive statistics for these findings. While the values in Table 6 vary from Time 1 to Time 2, the differences are not statistically significant according to a t-test comparing the means. In addition, we applied structural equation modeling to jointly assess change over time between pork protein consumption and cognitive performance, and did not observe longitudinal changes (Fig. 8).

	Time 1 Value	Time 2 Value
Overall Pork Consumption	0.17	0.16
Unprocessed Pork	0.16	0.16
Processed Pork	0.18	0.16
Lean Pork	0.06	0.09
Verbal Intelligence	119	121
Fluid Intelligence	115	117
Full Scale IQ	119	121

Table 6. Longitudinal values for Pork protein categories and tests of intelligence. Pork protein consumption values are scaled between 0 (none) and 1 (max).

Fig. 8. Structural Equation Modeling results examining the association between changes in pork consumption and changes in cognitive performance.



Discussion

Our research program applied interdisciplinary methods in nutritional cognitive neuroscience to investigate the role of pork consumption on cognitive performance and brain health. We conducted nutrient biomarker analysis to estimate the concentration of nutrients in each participant's blood. We then applied principal components analysis to derive phenotypes that capture dietary patterns of protein consumption. This approach allowed us to perform a fine-grained analysis of protein consumption that distinguished between different protein categories (pork, beef, poultry, and fish) and preparations of protein (processed versus unprocessed). We examined the association between the observed protein phenotypes and state-of-the-art measures of cognitive performance and brain health (as assessed by structural and functional MRI).

Our research was guided by 5 primary objectives: (1) *Discover Nutrient Biomarkers of Pork Consumption* (i.e., to investigate the key nutrients associated with eating different types of pork and non-pork proteins); (2) *Differentiate Pork Consumers from Non-Pork*

Consumers (i.e., to compare cognitive performance and brain function in individuals who typically consume pork compared to those who typically consume non-pork protein); (3) *Differentiate Pork Consumers from Consumers of Other Types of Protein* (i.e., to investigate how consumption of pork protein compares to non-pork protein with respect to cognitive performance and brain function); (4) Differentiate Between Different Types of Pork (i.e., to examine how consumption of different types of pork protein relate to cognitive performance and brain function); (5) *Examine the Longitudinal Effects of Pork Consumption* (i.e., to assess whether dietary changes in protein consumption were associated with corresponding changes in cognitive performance and brain function).

The results of our study provide evidence for four primary conclusions. First, we observed that several B vitamins, minerals (selenium and zinc), and omega-6 PUFAs were associated with the consumption of pork (as measured by dietary questionnaires and blood-based biomarkers of nutrition). Notably, prior research suggests that these nutrients play an important role in brain health and are associated with cognitive performance on tests of attention, learning, and memory. Second, our study provides novel evidence that these nutrients may be linked to the function of specific brain networks. Our analysis revealed that consumption of different types of pork, but especially lean pork, is associated with the efficiency of information processing within brain networks for visual and attentional processing (as measured by their small-world propensity). Third, to further investigate the role of pork protein in well-established dietary patterns, we conducted an analysis of the contribution of pork and other food categories in the Mediterranean Diet with respect to measures of brain health. Our analysis demonstrated that the consumption of lean pork was associated with favorable Brain Age, a measure of one's chronological age relative to their estimated brain age, providing an index of slow versus accelerated brain aging. Fourth, we investigated whether changes in pork consumption over time accounted for trajectories of cognitive performance and brain function in late life. We found that pork consumption in our longitudinal cohort did not change significantly over time and therefore was not associated with changes in cognition and brain health.

Although we believe the results of the present study are promising, it is important to note the present study is observational and demonstrates several limitations. First, additional research to examine the generalizability of our pork phenotypes is needed, applying similar methods in the context of more comprehensive nutrient biomarker assays and in more diverse populations. Second, the nutrient biomarkers in this study were selected from previous research providing evidence of their favorable effects on brain and cognition. However, the selection of nutrients is not comprehensive and, therefore, may omit important elements that contribute to pork consumption and that are associated with healthy brain aging. Third, these findings do not permit inferences about the causal role of pork consumption on cognitive performance and brain health. Future studies should investigate intervention effects on the pork phenotypes from this study for the promotion of cognitive performance and brain health. Fourth, our sample population represents relatively high performing, well-educated, neurologically healthy older adults. Therefore, these characteristics may limit the generalizability of findings to different, more diverse study populations. Thus, it is important for future research to further test and validate the novel methods used in this study, applying a broader range of nutrient biomarkers, cognitive measures, and neuroscience methods within more diverse populations. Finally, some of the nutrient biomarkers measured showed lower associations with brain network efficiency. Future research will need to determine the significance of these biomarkers for brain and cognitive functioning.

In closing, we emphasize that our findings do not support conclusions about the causal role of pork consumption on cognitive performance and brain health. However, we believe that the results of our study provide promising evidence to motivate the design of future intervention trials to investigate the potential benefits of lean pork protein on Brain Age and targeted measures of brain network function.