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Dietary Patterns and Cognitive Decline in Aged Populations

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DIETARY PATTERNS AND COGNITIVE DECLINE

IN AGING POPULATIONS

By

Austin Bowles

A report submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

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ABSTRACT

Dietary Patterns and Cognitive Decline

in Aged Populations

by

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Utah State University, 2011

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In this paper, we discuss distinctive features of longitudinal studies, and illustrate two regression-based methods for the analysis of longitudinal data. A study of dietary patterns and cognitive decline (Cache County Memory Study) is used to motivate our discussion and analysis. Cognitive decline is a risk factor for Alzheimer's disease, the sixth leading cause of all deaths among Americans. The study attempted to identify dietary patterns associated with reduced risk of age-related cognitive decline in elderly populations. Higher levels of adherence to the Dietary Approaches to Stop Hypertension (DASH) and/or Mediterranean diets were found to be associated with increased cognitive function at the beginning of the study. These differences were not strengthened or weakened over time, but were maintained over the 11 year duration of the study. Diets characterized by high intake of whole grains and nuts were also found to be associated with higher baseline cognitive function, but there was no evidence that these diets are associated with increased or decreased rates of decline after baseline.

(52 pages)

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Austin Bowles

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COGNITIVE DECLINE IN AGING POPULATIONS

Our objective in this study was to examine the association between the rate of age-related cognitive decline and dietary patterns. Neurodegeneration is a risk factor for Alzheimer's disease, the sixth leading cause of all deaths in the United State (1). Life style changes that slow or prevent cognitive decline may delay the onset of Alzheimer's disease. It has been projected that delaying the onset of Alzheimer's disease by just five years could reduce its prevalence by 50 percent (2). Hence, dietary patterns with even modest effects could translate into large reductions in the incidence of Alzheimer's disease in the population.

While previous studies have examined the associations between single nutrients and risk of age-related cognitive decline (3), we hypothesized that certain dietary patterns (i.e., emphasizing and discouraging the intake of multiple nutrients) may protect against age-related neurodegeneration. Studies examining the effects of single nutrients have provided mixed results. This may be because single nutrient analysis ignores the complexity of diet (4). Nutrients act synergistically to support or disrupt multiple biological processes. Thus, it is reasonable to study dietary patterns (rather than just single nutrients) and their association with risk of age-related cognitive decline.

Cache County Study on Memory, Health and Aging

The data examined in this study were obtained from the Cache County, Utah, Study on Memory, Health and Aging (hereafter referred to as the Cache County Memory Study, CCMS). The CCMS is a large population-based prospective study of the prevalence and incidence of dementia among elderly residents of Cache County Utah. In

1995 all residents of Cache County over 65 years of age were invited to participate in the study, and 90% (n = 5,092) completed the baseline interview (1995 – 1996). Re-assessments of the same cohort were completed in 3 years, 7 years, and 11 years after the initial interview. The study was approved by the institutional review boards of Utah State University, the Johns Hopkins School of Public Health, and Duke University Medical Center.

The baseline interview collected information on demographic characteristics, health history, family history of dementia, use of medications, alcohol, tobacco, and other life-style factors. Most participants provided a cheek-swab DNA sample that was used for apoliprotein (APOE) genotyping (n = 4,962). The Modified Mini-mental State Examination (3MS) (5) was used to assess cognitive function at baseline and was re-administered at the subsequent assessments. The 3MS is a 100-point, expanded version of the Mini-mental State Examination (6) that has been used in many epidemiological studies and found to be useful as a global measure of cognitive function and decline among non-institutionalized elderly men and women (7). Participants diagnosed with dementia at the baseline interview or at subsequent assessments were not asked to complete additional 3MS examinations.

Average daily dietary intake was assessed using a 142-item food frequency questionnaire (FFQ) patterned after the methods developed for use in the Nurses' Health Study. Similar questionnaires have been shown to provide reasonable estimates of usual dietary intake among populations of elderly women (8; 9). The questionnaire asked participants to report their frequency of consumption of the listed food items or groups.

Nutrient composition of food items was obtained using a time-specific version of the

Food Processor Program (ESHA Research, Portland, Oregon) nutrient composition

database. Daily intakes of nutrients were computed by multiplying the nutrient content of

the food item by the reported frequency of intake and summing over all food items.

Daily intake of nutrients and servings of food groups were adjusted for total energy

intake (8).

Of the 5,092 participants who completed the baseline interview, 355 were

considered cognitively impaired (3MS ≤ 60) and were not asked to complete the FFQ.

Of the 4,737 who were asked to complete the FFQ, 3,829 (81%) completed the

questionnaire. An additional 197 participants were later excluded because of implausible

energy intake (≤ 500 or ≥ 5,000 kcalories per day).

Four additional dietary variables were created from responses to the FFQ. Two of

these derived variables measure each subject's adherence to two well-defined dietary

patterns: the so-called Mediterranean and Dietary Approaches to Stop Hypertension

(DASH) diets. The other two derived variables specify patterns identified empirically,

using multivariate variable reduction methods such as k-means clustering and archetypal

analysis.

Mediterranean and DASH Diets

The traditional Mediterranean diet is characterized by large amounts of whole,

minimally processed plant foods with small amounts of animal foods and regular, modest

intake of alcohol (10; 11). Mediterranean diet patterns have been associated with a

reduced risk of total mortality, cardiovascular diseases, cancer and Alzheimer's disease

(12; 13). The Dietary Approaches to Stop Hypertension (DASH) diet substantially reduced elevated blood pressure in clinical trials (14) and is recommended in the current Dietary Guidelines for Americans (15). The DASH diet is similar to the Mediterranean diet but places greater emphasis on low fat dairy products and carbohydrates and is lower in fat and cholesterol. Due to the association of hypertension with cognitive function, it is plausible that the DASH diet may reduce the risk of cognitive decline.

The Mediterranean and DASH diet adherence scores were constructed by assigning scores to food components emphasized or discouraged in these dietary patterns. The Mediterranean diet adherence score included nine food/nutrient components: high intakes of fruits, vegetables, whole grains, fish, legumes, nuts, and ratio of monounsaturated fatty acids to saturated fatty acids; and low intake of red and processed meats.

The DASH diet adherence score also included nine food/nutrient components. They were high intake of fruits, vegetables, low-fat dairy products, nuts and legumes, whole grains and fish; and low intake of sodium, sweets and sweetened beverages, and red and processed meat.

Individuals were assigned a food component score by using quintile cut-offs of the CCMS cohort's distribution of intake for each food component. Quintile rankings (range: 1-5) were used as component scores for fruits, vegetables, low-fat dairy foods, nuts and legumes, whole grains, and fish. A reverse scoring method was used for those components where a lower intake was desired. To create diet adherence scores,

component scores were summed and participants were then binned into quintiles of the respective total diet adherence scores (range of scores: 9-45).

K-Means Clustering and Archetypes

In addition to measuring subjects' adherence to the well-defined DASH and Mediterranean diets, we hypothesized that subjects could be categorized into several undefined diet groups based on their answers to the FFQ. The multivariate methods of k-means clustering and archetypal analysis were used to accomplish this categorization.

The k-means algorithm (16) selects k widely spaced subjects at random to be seeds. All remaining subjects are then assigned to the seed to which they are most similar. This similarity is determined by the multivariate distance between seed and subject, using numerical variables chosen by the researcher. After each subject has been assigned, the mean for the cluster around each seed is computed. Subjects are then reassigned to the cluster mean to which they are most similar. These last two steps are repeated until convergence (i.e., subjects are repeatedly assigned to the same cluster).

To cluster subjects similar in diet, we used food group/nutrient intakes as the numerical variables used by the k-means algorithm. We selected $k=7$ seeds as it yielded fairly interpretable clusters with approximately the same numbers of subjects. For each of these seven clusters, mean intakes of food groups/nutrients were calculated to characterize the dietary pattern of each cluster. **Table A.1** (see Appendix A) summarizes the clusters created by the k-means algorithm.

Archetypal analysis assumes that each individual can be represented as a mixture of pure types or *archetypes* (17). The algorithm chooses these n archetypes by

minimizing the squared error in representing each subject as a mixture of archetypes. Liberally interpreted, this representation describes how similar each subject is to the different archetypes.

As was done in k-means clustering, food group/nutrient intakes were again used as variables in the archetype algorithm. We selected *n=7* archetypes in order to be consistent with and comparable to the k-means clustering results for *k=7*. In addition to scores describing how each subject is a mixture of each archetype, we assigned each subject to the archetype to which it was most similar. Thus, *pseudoclusters* were created for each archetype. Mean intakes of nutrients/food groups were calculated for each group in order to characterize each archetype. **Table A.2** summarizes the groups created by archetypal analysis.

STATISTICAL METHODS FOR ASSESSING

COGNITIVE DECLINE

Some key characteristics of the longitudinal 3MS measures from the Cache County Study influence the analytic approaches required for this project. First, the follow-up visits were balanced by design, with 3MS scores collected for each subject at baseline (study entry) and then at 3, 7, and 11 years after baseline (also referred to as waves 1 – 4). In addition, typical of many large observational studies of aging populations, there is significant attrition, due almost entirely to death and the clinical diagnosis of individuals with dementia (refer to these subjects as missing with death or dementia, or DD). As results of this study are conditional by design on dementia-free survival, the problem of loss to follow-up is comparatively small: only 14% (n = 520) of the initially non-demented sample eventually dropped out for reasons other than death or dementia onset (refer to these subjects as missing with no death or dementia, or NDD). **Figure B.1** (see Appendix B) shows the number of subjects missing at each wave and the proportions of NDD and DD missing. **Table A.3** contains summary statistics (including number of remaining participants) with respect to the distribution of 3MS scores by follow-up visit.

We will later describe in more detail how repeated measures models help us to assess how patterns of cognitive change depend on fixed factors such as diet. If attrition is present, the validity of these mixed models depends on the mechanism by which subjects leave the study. As results of this study are conditional on dementia-free survival, we restrict our attention to NDD missing subjects.

To account for the NDD missing, we must consider the reasons for dropout, often referred to as the underlying dropout mechanism. Conventionally, the dropout mechanism is classified as *completely at random*, *at random*, or *not at random* (18), depending on how the probability of dropout relates to the outcome of interest. In the context of our study of cognitive decline, missing data would be considered missing completely at random (MCAR) if the probability of a given subject leaving the study at any occasion was independent of all previous 3MS scores and all future 3MS scores, had they been obtained. If the probability of dropout was related to subjects' previous 3MS scores but not their future examinations, we would consider missing data to be missing at random (MAR). Lastly, if the probability of dropout depended on subjects' previous and future 3MS scores, we would consider the data to be not missing at random (NMAR).

As the likelihood-based mixed models used in the current study provide valid inferences about changes in mean 3MS over time when data are MCAR or MAR, the term *ignorable* is used to describe these mechanisms. Alternatively, when data are NMAR, almost all standard methods of analysis are not valid. Hence, the term *nonignorable* is used to describe data that are NMAR.

Referring to Table A.3, there are no major differences between NDD missing subjects and continuing participants with respect to 3MS scores. There is no significant difference between baseline 3MS scores for those that are NDD missing at wave 2 and those that continue on to Wave 2 (p = 0.8232). Similarly, the difference in wave 2 scores between participants that continue to wave 3 and those that are NDD missing is non-significant (p = 0.1135). However, subjects that are NDD missing at wave 4 have

significantly lower wave 3 scores than those that are present at wave 4 (p = 0.0110). This last finding suggests that the dropout mechanism is not MCAR.

The distinction between MAR and NMAR cannot be verified with available data since it requires knowledge of the unobserved 3MS scores. However, further analysis reveals that NDD missing and continuing participants do not differ with respect to demographic characteristics and health status. Furthermore, we have no reason to believe the probability of NDD dropout is related to subjects unobserved 3MS scores. Thus, while we cannot prove that the dropout mechanism is MCAR, it is reasonable to assume that the mechanism is ignorable.

Modeling the Mean and Covariance Structure

While our methods of analysis appropriately handle the dropout inherent in the study, they are still invalidated if correlation is not accounted for. As a consequence of repeated measures taken on the same subjects, a distinctive feature of longitudinal data is that the repeated measurements obtained from a single subject are correlated. Because these measurements were made on the same subjects, generally they are positively correlated. For example, an individual that scores relatively high on the 3MS at baseline is likely to score relatively high when measured three years later. This covariance or time dependence invalidates the critical assumption of independence that is fundamental in many standard statistical techniques. But if accounted for and modeled correctly, the covariance increases the efficiency or the precision with which regression parameters are estimated.

The models used for the analysis of the CCMS data can be expressed in terms of the general linear regression model

$$E(3MS_i) = X_i\beta, \tag{1}$$

where $E(3MS_i)$ is the mean vector of 3MS scores at baseline and follow-up for the ith subject, X_i is the subject's matrix of covariates (design matrix), and β is the vector of fixed effects (e.g., the effect of diet on 3MS). The response vector, $3MS_i$, is assumed to arise from a multivariate normal distribution with variance-covariance matrix $\text{Cov}(3MS_i)$. In order to estimate β we must model and estimate the variance-covariance matrix. As the structure of the covariance matrix is conventionally determined before examination of fixed effects, we first consider how alternative covariance models are compared.

Our dataset features four repeated measures of 3MS for most individuals. Thus the variance-covariance matrix for individual i is:

$$\text{Cov}(3MS_i) = \begin{bmatrix} \sigma_1^2 & \sigma_{1,2} & \sigma_{1,3} & \sigma_{1,4} \\ & \sigma_2^2 & \sigma_{2,3} & \sigma_{2,4} \\ & & \sigma_3^2 & \sigma_{3,4} \\ & & & \sigma_4^2 \end{bmatrix}. \tag{2}$$

In this representation, σ_1^2 is the variance of 3MS scores at baseline, σ_2^2 is the variance at the second examination (three years after baseline) and so on. The parameter $\sigma_{1,2}$ is the covariance between baseline 3MS and follow-up 3MS at the second examination, and the other values are interpreted similarly. Since the matrix is symmetric, redundant parameters are not included in the representation above. We also assume homogeneity across individuals (i.e., $\text{Cov}(3MS_i) = \text{Cov}(3MS) \ \forall \ i$).

In order to appropriately estimate the regression parameters, each parameter in the variance-covariance matrix above needs to be estimated. When the number of observations across time is small (four in the current study) and the data set is balanced or incomplete, it may be reasonable to estimate each of the variance-covariance parameters with no constraints on these parameters. This is referred to as an "unstructured" covariance structure. With four repeated measures, there are ten parameters to estimate (four variances and six pairwise covariances). When the number of covariance parameters is large relative to sample size, estimation using an unstructured covariance structure is likely to be very unstable. However, an unstructured covariance structure is very appealing when the number of parameters is small relative to sample size (e.g., 10 vs. 9,365 in the current study).

The defining feature of an unstructured covariance structure is that no assumptions are made about the variances or covariances. This is especially critical since practical experience suggests that variances are rarely constant over time (19). Additionally, covariance typically decreases as measurements become further separated by time. For example, **Figure B.2** shows how the correlation between baseline 3MS scores and follow-up scores decreases with increased separation in time.

Assuming an unstructured covariance matrix provides a general approach, but in appropriate settings it is also possible to impose some specific structure on the covariance matrix. These alternative matrix structures are referred to collectively as covariance pattern models. The advantage of using a more restrictive covariance model (when

warranted by the data) is greater efficiency, as such models require fewer covariance parameters.

In the simplest so-called compound symmetry model, the variance is assumed to be constant across all occasions, and the covariance (or correlation) is also assumed to be constant between any pair of correlated observations. If we were to assume compound symmetry for within-subject observations in the Cache Study, the variance-covariance matrix would be expressed as

$$\text{Cov}(3MS_i) = \begin{bmatrix} \sigma^2 & \rho\sigma^2 & \rho\sigma^2 & \rho\sigma^2 \\ & \sigma^2 & \rho\sigma^2 & \rho\sigma^2 \\ & & \sigma^2 & \rho\sigma^2 \\ & & & \sigma^2 \end{bmatrix}. \tag{3}$$

The chief advantage of this covariance pattern is that it requires estimation of only two parameters, the common variance σ^2 and pairwise correlation ρ. Given the large sample size of the CCMS, estimating two covariance parameters rather than ten for our analyses may not make much of a difference, but the number of parameters that must be estimated in an unstructured covariance structure increases dramatically with increasing numbers of repeated measures. For example, with eight repeated measures, compound symmetry still requires only two parameters rather than the 36 that would be required for the unstructured covariance structure. For smaller sample sizes, model parsimony is much more critical.

While reducing the number of model parameters can be advantageous, alternative covariance structures must be supported by the data. For example, compound symmetry is seldom used with longitudinal measures, for which empirical observation consistently suggests that the pairwise correlations decrease with increased separation in time. This

so-called autoregressive phenomenon is illustrated for the Cache County data in Figure

B.2. Two common types of autoregressive covariance structures – refer to these here as

AR(1) and AR(2) – alternatively assume constant or non-constant variances down the

diagonal of the covariance matrix, but in both cases there is only a single correlation

parameter . The pairwise correlation between two repeated measures can be expressed as

ρ^t, where t represents the elapsed time between the two measures. Hence, for the Cache

County data AR(1) comprises two covariance parameters (a common variance and the

correlation parameter ρ), while AR(2) requires five parameters (four distinct variances

along with ρ).

Maximum Likelihood vs. Restricted Maximum Likelihood

Models for both the mean and covariance are fitted and compared using a

likelihood based approach. Maximum likelihood (ML) is a very general approach to

estimation. In large samples, under appropriate distributional assumptions, the ML

estimates (or MLEs) of the model parameters (such as the regression coefficients

representing fixed effects) have appealing properties. First, the MLE $\hat{\beta}$ is a consistent,

unbiased estimator of the corresponding fixed effect β. Second, the sampling distribution

of $\hat{\beta}$, when the covariance is estimated from the data, is approximately multivariate

normal with mean β and known covariance. Lastly, ML estimation allows for the

construction of likelihood ratio tests, making it useful for comparing nested models for

the mean.

Although the ML estimates of the fixed effects and the covariance have desirable

large sample properties, its estimate of the covariance can be shown to have significant

bias in small samples. Restricted maximum likelihood (REML) estimation corrects for this bias, and nested models for the covariance structure are conventionally compared using REML-based loglikelihoods.

Analyzing Response Profiles

When repeated measures are obtained at the same sequence of occasions, or subsets of that sequence (as with incompleteness), the data can be summarized by the mean response at each occasion. In the current study, the data can be summarized by the mean 3MS score at baseline and at subsequent follow-up examinations, stratified by diet pattern. For a given group, the sequence of means is known as the mean *response profile*. Analyses using response profiles impose minimal structure on the mean response over time. The main goal of this type of analysis is to characterize the patterns of change in the mean response over time in the different groups and to determine whether these response profiles differ among groups. It thus allows us to study cognitive decline by characterizing mean 3MS scores over time and determining whether 3MS profiles differ among diet patterns.

For ease of exposition, assume we are interested in two diets, Diet 1 and Diet 2. Additionally, assume that 3MS scores are taken at baseline and once at follow-up five years later. The expected 3MS score for individual i at the jth occasion can then be modeled with the following linear equation:

$$E\big(3MS_{ij}\big) = \beta_1 + \beta_2(time_2) + \beta_3(Diet1_i) + \beta_4(Diet1_i)(time_2), \qquad (4)$$

where *Diet1*$_i$ equals 1 if the subject adheres to Diet 1 and zero otherwise and *time*$_2$ equals one at follow-up and zero at baseline.

Three main hypotheses can be posed for the analysis of 3MS profiles (and similarly for other longitudinal studies). They are:

1. Are the mean 3MS profiles similar in the different diets, or do they differ? In many longitudinal studies, this question is of main scientific interest. It asks whether or not subjects' pattern of cognitive decline is associated with their diet. This question concerns the *diet×time* interaction effect, β_4. The null hypothesis is that the profiles do not differ among groups. That is, the group profiles are parallel ($\beta_4 = 0$).

2. Assuming that there is no diet×time effect, are different diets' 3MS response profiles the same, or are they parallel but at different levels? This question concerns the *diet* effect, β_3. It is also of interest in the current study. If the different diets are not associated with different patterns of cognitive decline, we would like to examine if there is at least a difference in baseline 3MS scores ($\beta_3 \neq 0$). Since response profiles are parallel, this would indicate that the difference is maintained and still observed five years later.

3. Assuming that there is no diet×time effect, are the mean 3MS scores constant over time, or do they change with time? This question concerns the *time* effect, β_2. This question is not of particular interest in the current study. It is well-known that 3MS scores in aged populations decline with time. Thus, we expect a time effect. Specifically, we expect the time effect to be negative ($\beta_2 < 0$). That is, mean 3MS is lower five years later than it was at baseline.

To study the association of cognitive decline and DASH adherence by analyzing

response profiles, these hypotheses can be tested in SAS using the following PROC

MIXED syntax:

```
PROC MIXED method=ML;
CLASS time id DASH_5;
MODEL mms = time DASH_5 time*DASH_5 / S;
REPEATED time / TYPE=un SUBJECT=id R;
RUN;
```

where *DASH_5* is the variable indicating which quintile the subjects' DASH adherence

score falls into (Q1 being least adherent and Q5 being the most) and *mms* is 3MS score.

Notice the use of TYPE=UN to specify unstructured covariance and METHOD=ML to

specify maximum likelihood. It is also important to note that *time* is placed in the

CLASS and REPEATED statements. Placing it in the CLASS statement makes it a

categorical variable with four levels: 0 years, 3 years, 7 years, and 11 years after baseline.

By analyzing response profiles, both time and DASH effects were found to be

significant (p-value for both < 0.0001). However, the time×DASH effect was highly

non-significant (p = 0.9627) and dropped from the model. Thus, there is evidence that

3MS scores change with time and that different levels of adherence to the DASH diet are

associated with different baseline 3MS scores. However, there is no evidence that the

pattern of decline is different among different levels of DASH adherence. The estimates

of the fixed effects are summarized in **Table A.4**.

According to our estimates, subjects in the fourth and fifth quintiles of DASH

adherence score about 1.65 points higher on the 3MS than those subjects in the lowest

quintile. Our parameter estimates also suggest that subjects in all quintiles score about 5 points lower on the 3MS at the end of the study than at the beginning. **Figure B.3** represents these findings graphically by plotting mean 3MS by time, stratified by DASH adherence quintile.

While the analysis of response profiles is a fairly straightforward way of analyzing longitudinal data, it is not the most appropriate method for the current study. This is because the analysis of response profiles allows for arbitrary patterns in the mean response over time. It does not impose any time *trend*; therefore it ignores the time ordering of the repeated measures. This results in a rather broad statement about group differences in patterns of change over time. The null hypothesis of no diet×time interaction is a global test that provides only a broad assessment of whether mean 3MS profiles are the same for different diets. In the case where the null is rejected, it does not indicate the specific ways in which the profiles differ.

In the Cache study, it is reasonable to assume *a priori* that 3MS has a linear or curvilinear time trend. Treating time as a continuous variable rather than as a factor will have higher power to detect group differences in mean 3MS scores over time.

Parametric Model for Cognitive Decline

Whereas profile analysis allows for arbitrary patterns in 3MS scores over time, we expect, *a priori,* that cognitive function will decline with time in aged populations. It is reasonable to assume that 3MS will decline linearly or curvilinearly over time. Although profile analysis produces a "perfect" fit to the observed mean 3MS profile, it fails to describe the changes in mean 3MS in terms of some pattern that can be given a

substantive interpretation. Since we can reasonably assume that true cognitive function is monotonically decreasing for the duration of the study, a simple parametric curve can be used to describe how 3MS scores decline over time.

The following equation models mean 3MS for subject i at time j with a linear trend, with subjects adhering to one of two different diets:

$$E(3MS_{ij}) = \beta_1 + \beta_2(time_{ij}) + \beta_3(Diet1_i) + \beta_4(Diet1_i)(time_{ij}) \qquad (4)$$

where $time_{ij}$ is a continuous variable rather than categorical as it was in the analysis of profiles model. Notice that this model easily accommodates multiple measurement occasions, whereas the previous model would have required two additional dummy variables for each additional occasion. If we instead assume that the changes in the mean response follow a quadratic trend, we could use the following model:

$$E(3MS_{ij}) = \beta_1 + \beta_2(time_{ij}) + \beta_3(time_{ij}^2) + \beta_4(Diet1_i) +$$
$$\beta_5(Diet1_i)(time_{ij}) + \beta_6(time_{ij}^2)(Diet1_i). \qquad (5)$$

The same three hypotheses that were tested with analysis of profiles can be tested with these parametric models. To test whether the two diet groups change differently over time, we test the null hypothesis $H_0: \beta_4 = 0$ in the linear model or $H_0: \beta_5 = \beta_6 = 0$ in the quadratic. Assuming that the two diets have the same rate of change (i.e., we fail to reject the previous hypotheses), we can test whether mean 3MS changes over time by testing the null hypothesis $H_0: \beta_2 = 0$ in the linear model or $H_0: \beta_2 = \beta_3 = 0$ in the quadratic. Finally, assuming the two diets have the same trend, we can test whether the two groups differ at baseline by testing $H_0: \beta_3 = 0$ in the linear model and $H_0: \beta_4 = 0$ in the quadratic.

To test for quadratic versus linear trend (i.e., which model to use), we can use the model,

$$E(3MS_{ij}) = \beta_1 + \beta_2(time_{ij}) + \beta_3(time_{ij}^2), \qquad (6)$$

and test $H_0: \beta_3 = 0$. If we fail to reject this null hypothesis, a linear trend is sufficient to describe mean 3MS change over time. If we reject the null, the data suggest that mean 3MS should be described with a quadratic curve.

The parametric models used for the hypothetical two-diet study are easily extended to accommodate multiple diets by including additional dummy variables and diet×time interaction terms. To study the association of DASH adherence quintile and cognitive decline by fitting quadratic curves, the following SAS code can be used:

```
PROC MIXED method=ML;
CLASS t id DASH_5;
MODEL mms = time time*time DASH_5 time*DASH_5
time*time*DASH_5 / S;
REPEATED t / TYPE=un SUBJECT=id R;
RUN;
```

where *t* is a copy of the *time* variable. Including *t* in the CLASS and REPEATED statement prompts SAS to appropriately model the covariance while using the continuous *time* variable in the MODEL statement fits a parametric curve. After fitting this model, it was determined that the time×DASH and time×time×DASH interactions were highly non-significant (likelihood ratio test yielded p-value = 0.8831) and were dropped from the model. As we saw with the analysis of profiles, there is still no evidence that

different levels of adherence to the DASH diet pattern are associated with different rates

of cognitive decline. However, DASH, time, and time×time effects were significant (p <

.0001). Their effects are summarized in **Table A.5**.

The estimates of the baseline differences between the quintiles of DASH

adherence are approximately the same as they were in the response profile analysis. The

difference now is that we have one estimate for the time effect, rather than an estimate for

each occasion. The model estimates that mean 3MS scores decline at a rate described by

the function:

$$\Delta E\big(3MS_{ij}\big) = -0.17\big(time_{ij}\big) - 0.03\big(time_{ij}^2\big), \tag{7}$$

where $\Delta E\big(3MS_{ij}\big)$ is the change in mean 3MS since baseline. **Figure B.4** represents

these finding graphically, stratified by DASH adherence quintile.

While the conclusions of this analysis are similar to those made by the analysis of

response profiles, analysis using parametric curves has more power to estimate fixed

effects and describes the decline over time in a more substantive way. For these reasons,

parametric curves were used to analyze the association between cognitive decline and

dietary patterns while controlling for potential confounding variables.

COMPUTATIONAL METHODS

As indicated in the discussion of response profiles and parametric curves, PROC MIXED in the SAS statistical package was used to fit mixed effect linear regression models to examine associations across increasing quintiles of DASH/Mediterranean adherence scores and average 3MS scores at the four periods of assessment. The same was applied to examine associations between different clusters/archetypes and 3MS scores over the duration of the study. Finally, models were also fit to examine the associations between single nutrients/food groups and 3MS trajectories.

As explained previously, both linear and quadratic terms for time were included in the mixed models to account for the nonlinear trajectories of 3MS performance over time. Variables associated with diet patterns and 3MS scores, as well as other potential confounders as identified in other studies, were included in the models. These included education (no more than a high school education or more than a high school education), age at baseline (years), gender, APOE genotype (0, 1, or 2 copies of the e4 allele), physical activity (frequency of moderate physical activity per week), total energy intake, BMI (weight in kg/height in m^2), history of alcohol intake (never, former, or current), smoking (never, former, or current), and history of vascular disease (yes or no). Reported p-values are two-sided and type I error rate for significance was 0.05.

To complement the single food group analyses, the Least Absolute Shrinkage and Selection Operator (LASSO) was used (19). All food groups and other variables were used in the regression model with baseline 3MS as the response. LASSO selection was used to estimate the effects of these variables, "shrinking" some non-significant effects to

zero, and addressing the problem of multicollinearity. LASSO is a constrained form of ordinary least squares regression implemented by PROC GLMSELECT in SAS. The algorithm allows the user to specify a stopping criterion. For our analyses, cross-validation (STOP=CV) was used. The selection was performed using the following SAS syntax:

```
PROC GLMSELECT data=work.fgq plots=all;
CLASS apoe_3 smoker alcohol_;
MODEL v1mssadj = gender educgr_rc apoe_3 smoker
alcohol_ vasc bmi pct_fat kcal_tot q_ffdairy
q_wholegrains q_LFDairy q_sweets_bevs q_nuts q_legumes
q_red_proc_meat q_fish q_fruit q_vegnp q_mono_q_satfat
q_poultry / SELECTION=LASSO STOP=CV;
RUN;
```

where *v1mssadj* represents baseline 3MS score, *q_ffdairy* and similarly named variables represent the quintiles of consumption of the different food groups, and other variables represent potential confounders. Note the specification of SELECTION=LASSO and STOP=CV.

RESULTS

DASH and Mediterranean

Using mixed effects linear regression models that control for the covariates mentioned previously, higher quintiles of DASH adherence were associated with higher 3MS scores at baseline. Interestingly, the second highest quintile of DASH adherence (Q4) was associated with the highest 3MS score at baseline, followed by Q5, Q3, Q2, and Q1. Those subjects in the fourth quintile had scores 0.97 points higher at baseline than those in the lowest quintile (p-value = 0.0003). Those in the highest quintile (Q5) had scores 0.59 points higher at baseline that those in the lowest quintile (p-value = 0.0488). For reference, a three year increase in baseline age was associated with a 1.11 point decrease in 3MS score. Thus higher quintiles of DASH adherence (Q4 and Q5) had roughly the same effect on 3MS score as did a 2-3 year decrease in age.

All interactions between DASH quintile and time were highly non-significant and removed from the model (likelihood ratio test yielded p-value = 0.8992). Thus, there was no evidence that the association between DASH adherence and 3MS scores at baseline was strengthened or weakened over time. In other words, the baseline differences in mean 3MS score were maintained throughout the duration of the study.

The DASH and Mediterranean diet scores were positively correlated (r = 0.8034). As was found with DASH scores, higher quintiles of adherence to the Mediterranean diet were also associated with higher baseline 3MS scores. Subjects in the highest quintile scored on average 1.08 points higher than those in the lowest quintile (p-value = 0.0001). This is approximately the same as the effect associated with a 3 year decrease in age.

Although adherence to the Mediterranean diet was associated with higher 3MS scores at the baseline interview, these associations were not strengthened or weakened over time (interactions between Mediterranean adherence and time were all highly non-significant). The differences in mean 3MS score were maintained for the duration of the study.

To assess whether the DASH or Mediterranean dietary patterns were associated with higher 3MS performance at baseline or if these associations were simply being driven by one or two of the food groups contributing to these dietary patterns, mixed effects linear regression models were fit using individual food groups rather than adherence scores as predictor variables. Subjects were separated into food group quintiles based on their intake of each food group. Food groups associated with mean 3MS score at baseline were whole grains, nuts and legumes, and fish. Whole grains, nuts and legumes, with the addition of low-fat dairy products and poultry, were also found to be significant in regression analyses using LASSO selection.

The results for the DASH, Mediterranean, and individual food group analyses are summarized in **Table A.6**. Using the lowest quintiles as references, the table reports the increase or decrease in mean 3MS associated with being in the higher quintiles of diet adherence or food group intake.

As reported in Table A.6, the effects of being in the highest quintiles of whole grains and nuts/legumes rather than the lowest (1.21 and 0.94 3MS points respectively) are comparable to the effects of being in the highest DASH and Mediterranean adherence

quintiles. These two food groups are components of both dietary patterns. Thus, these two food groups alone might account for the observed associations between diet adherence and mean 3MS at baseline.

K-Means Clusters and Archetypes

Using mixed effects linear regression models similar to those used in the DASH and Mediterranean analyses, there was no evidence of an association between mean 3MS and dietary patterns defined by k-means clustering. There were no time specific differences in 3MS among the different clusters (i.e., time×cluster interactions were non-significant; p-value = 0.1822). Furthermore, no association between k-means cluster and mean 3MS at baseline was observed (p-value =0.9110).

Unlike the k-means results, the groups formed by archetypal analysis did yield significant results. There was a significant association between archetype group and mean 3MS at baseline interview (p-value=0.0331). These differences in 3MS at baseline were neither strengthened nor weakened over time, but were maintained throughout the duration of the study (time×archetype interactions were non-significant; p-value = 0.2254). The effects associated with the different archetype groups are summarized in **Table A.7**.

Consistent with our results in the DASH and Mediterranean analyses, the archetype group characterized by high consumption of whole grains and nuts (see Table A.2) had a mean 3MS score at baseline that was 1.20 higher than the lowest scoring group, which was defined by high consumption of low-fat dairy and cereal. The whole grains and nuts group also scored 0.74 points higher than the group characterized by high

consumption of refined grains. The estimated 3MS trajectories for each of the seven

archetypes are summarized graphically in **Figure B.5**.

DISCUSSION

Higher adherence to both the DASH and Mediterranean diets was associated with higher cognitive function at baseline. Individuals in the higher quintiles of DASH and Mediterranean adherence had higher mean 3MS scores at baseline than individuals in the lower quintiles. These differences in cognitive function were maintained for the duration of the study. Higher intakes of whole grains, nuts, and legumes were also associated with higher cognitive function at baseline. These food groups were each components of the DASH and Mediterranean adherence scores. This suggests that these food groups may be primarily responsible for the higher cognitive function associated with adherence to the two diets.

Furthermore, archetypal analysis provided similar evidence in support of the benefits to cognitive function associated with whole grains and nuts. These findings have prompted further research examining the association between these foods and risk for dementia as well as their association with longevity. It is also interesting that the group characterized by high consumption of whole grains scored significantly higher than the group characterized by high consumption of refined grains.

Although the dietary patterns of interest in this study were not associated with different rates of decline, it is worth noting that decline may have begun before the baseline interview and that different rates of decline would have been responsible for the differences we observe at baseline. Furthermore, we can only make conclusions for what we have observed in the 11 year frame of the study. The 3MS trajectories may take longer to diverge or converge, or for other differences to be observed.

Additionally, a potential weakness of an observational study like the Cache County Memory Study is that the observed effects of dietary factors may be confounded by associated lifestyle factors. We attempted to control for the usual potential confounding factors of age, sex, education, physical activity, body mass index, history of medical co-morbidities, history of smoking and alcohol intake, but the potential of residual confounding remains.

The CCMS study of cognitive decline was also illustrative as a case study. Using it as an example, missing data mechanisms, covariance structures, and model fitting algorithms were all discussed. We were also able to illustrate the differences between analyses using response profiles and those using parametric curves. Finally, we demonstrated how to fit simple models using the SAS statistical package.

In conclusion, higher levels of adherence to the DASH and Mediterranean dietary patterns were associated with higher baseline 3MS scores. These differences were maintained throughout the 11 years of observation in the Cache County Memory Study. Dietary patterns defined by consumption of whole grains, nuts, and other foods were also associated with higher baseline 3MS scores, with no evidence of decreased or increased rates of decline following baseline. Single food analyses found that foods common to both the DASH and Mediterranean diets (namely whole grains, nuts and legumes, and fish) might be responsible for benefits associated with the dietary patterns. Promoting adherence to diets similar to the DASH and Mediterranean diets or promoting the consumption of food groups common to both diets may provide a means for dietary strategies aimed at cognitive benefits.

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APPENDICES

Appendix A. Tables

Table A.1: Summary of Clusters Obtained from K-Means Algorithm

Cluster	Foods for which average intake is higher than for all other clusters
Desserts and Snacks (n = 249)	Desserts, Snacks
Alcohol (n = 321)	Coffee, Liquor, Wine, Beer, Processed Meat, Full-fat Dairy Products, Eggs, Tea, Garlic, Regular Salad Dressing
Fruit and Vegetables (n = 353)	Fruit, Vegetables, Legumes, Fruit Juice, Tomatoes, Olive Oil, Poultry
Refined Grains and Margarine/Butter (n = 499)	Refined Grains, Margarin, Butter, Low Calorie Drinks
Whole Grains (n = 582)	Whole Grains, Nuts, Potatoes
Low Fat Dairy and Cereal (n = 582)	Low-fat Dairy Products, Cereal, Instant Breakfast
Red Meat, French Fries, and Pizza (n = 1,068)	Red Meat, High Calorie Drinks, French Fries, Fish, Pizza, Organ Meat, Mexican Food, Cream-based Soups

Table A.2: Summary of Pseudoclusters Obtained from Archetypal Analysis	
Psuedocluster	Foods for which average intake is higher than for all other clusters
Low-fat Dairy and Cereal (n = 213)	Low-fat Dairy Products, Cereal, Instant Breakfast
Refined Grains (n = 388)	Refined Grains, Butter, Low Calorie Drinks
Red Meat and Alcohol (n = 1,374)	Red Meat, Coffee, High Calorie Drinks, Fish, Processed Meat, Full-fat Dairy Products, Liquor, Wine, Mexican Food, Poultry, Beer, Organ Meat, Garlic, Eggs, French Fries, Cream-based Soups, Pizza
Fruit and Vegetables (n = 413)	Fruit, Vegetables, Legumes, Fruit Juice, Tomatoes, Olive Oil
Desserts and Snacks (n = 373)	Desserts, Tea, Snacks
Margarine, Salad, and Cereal (n = 896)	Margarine, Regular Salad Dressing
Whole Grains (n = 308)	Whole Grains, Nuts, Potatoes

Table A.3: Distribution of 3MS Scores by Follow-up Visit					
Dropout Status	n	Wave 1 3MS Scores mean(s.d)	Wave 2 3MS Scores mean(s.d)	Wave 3 3MS Scores mean(s.d)	Wave 4 3MS Scores mean(s.d)
Present at Wave 2	2,737	92.20(5.44)	n/a	n/a	n/a
NDD Missing at Wave 2	197	92.11(5.81)	n/a	n/a	n/a
DD Missing at Wave 2	670	86.91(8.44)	n/a	n/a	n/a
Present at Wave 3	1,832	93.17(4.59)	93.83(4.91)	n/a	n/a
NDD Missing at Wave 3	189	92.16(5.57)	93.23(5.42)	n/a	n/a
DD Missing at Wave 3	716	89.73(6.52)	86.93(11.49)	n/a	n/a
Present at Wave 4	1,192	93.83(4.13)	94.92(3.94)	93.43(5.03)	91.86(7.27)
NDD Missing at Wave 4	134	93.43(4.26)	94.00(4.92)	92.24(5.85)	n/a
DD Missing at Wave 4	506	91.55(5.27)	91.21(5.88)	83.65(10.65)	n/a

Table A.4: Estimated Fixed Effects for the Analysis of Response Profiles				
Effect		Estimate	Standard Error	p-value
Intercept		90.2785	0.2357	<.0001
Time	baseline	0(reference)	.	.
	3 yrs	-0.2	0.1141	0.0797
	7 yrs	-3.7274	0.178	<.0001
	11 yrs	-5.1301	0.221	<.0001
DASH_5	Q1	0(reference)	.	.
	Q2	0.3561	0.3316	0.2829
	Q3	0.93	0.3301	0.0049
	Q4	1.6681	0.3239	<.0001
	Q5	1.6461	0.3534	<.0001

Table A.5: Estimated Fixed Effects for the Parametric Model				
Effect		Estimate	Standard Error	p-value
Intercept		90.2741	0.2355	<.0001
Time		-0.1687	0.03968	<.0001
Time*Time		-0.03235	0.003482	<.0001
DASH_5	Q1	0(reference)	.	.
	Q2	0.3688	0.3313	0.2657
	Q3	0.9281	0.3297	0.0049
	Q4	1.6740	0.3235	<.0001
	Q5	1.6492	0.3528	<.0001

Table A.6: Mean Difference in 3MS Scores at the Baseline Interview[*]

Diet/Food Group	Quintile of Diet Adherence Score or Energy Adusted Servings Per Day					
	Q1	*Q2*	*Q3*	*Q4*	*Q5*	*P-value*
DASH	0 (reference)	0.12 ± 0.27	0.21 ± 0.27	0.97 ± 0.27	0.59 ± 0.30	0.0018
Mediterranean	0	0.29 ± 0.28	0.88 ± 0.27	0.86 ± 0.29	1.08 ± 0.28	0.0004
Whole Grains	0	1.28 ± 0.28	1.06 ± 0.28	0.84 ± 0.28	1.21 ± 0.28	<0.0001
Nuts and Legumes	0	0.50 ± 0.28	0.76 ± 0.28	0.97 ± 0.28	0.94 ± 0.28	0.0024
Fish	0	0.09 ± 0.28	0.62 ± 0.28	-0.24 ± 0.28	-0.13 ± 0.28	0.0193
Vegetables	0	0.40 ± 0.28	0.81 ± 0.28	0.44 ± 0.28	0.38 ± 0.28	0.0756
Low-fat Dairy Products	0	-0.18 ± 0.28	0.55 ± 0.28	0.21 ± 0.28	0.28 ± 0.28	0.0861
MUFA:SFA[†]	0	0.47 ± 0.28	0.53 ± 0.28	0.75 ± 0.28	0.36 ± 0.28	0.0914
Fruits	0	0.55 ± 0.28	0.61 ± 0.28	0.63 ± 0.28	0.28 ± 0.28	0.1098
Red and Processed Meats	0	0.15 ± 0.28	0.15 ± 0.28	-0.36 ± 0.28	-0.38 ± 0.28	0.1262
Sweetened Beverages	0	0.20 ± 0.28	-0.11 ± 0.28	0.33 ± 0.28	-0.19 ± 0.28	0.2954
Poultry	0	0.27 ± 0.28	-0.08 ± 0.28	0.29 ± 0.28	0.14 ± 0.28	0.5834
Full-fat Dairy Products	0	0.21 ± 0.28	0.36 ± 0.28	0.26 ± 0.28	0.20 ± 0.28	0.7634

[*] The table reports the mean difference from the reference group plus or minus the standard error (coefficient ± S.E.).
[†] Ratio of monounsaturated fatty acids to saturated fatty acids.

Table A.7: Archetypal Analysis Results		
Archetype	Mean 3MS at baseline (coefficient ± SE)	p-value
Low-fat Dairy and Cereal	0 (reference)	.
Refined Grains	0.46 ± 0.45	0.3027
Red Meat and Alcohol	0.51 ± 0.39	0.1879
Fruit and Vegetables	0.56 ± 0.44	0.2092
Desserts and Snacks	1.00 ± 0.45	0.0287
Margarine, Salad, and Cereal	1.10 ± 0.41	0.0081
Whole Grains	1.20 ± 0.49	0.0141

Appendix B. Figures

Figure B.1: Dropout by Wave

Figure B.2: Correlation Between 3MS Scores with Increased Separation in Time

Figure B.3: 3MS Profiles, Stratified by DASH Adherence Quintile

Figure B.4: 3MS Trajectories, Stratified by DASH Adherence Quintile

Figure B.5: 3MS Trajectories, Stratified by Archetype