Association of Whole Grain Intake with Diabetes and Subclinical CVD in the Multiethnic Study of Atherosclerosis: A Sensitivity Analysis

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Abstract

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Background: A dietary pattern that includes whole grains is widely believed to provide numerous health benefits, such as a reduction in chronic disease risk factors like inflammation, insulin resistance, and subclinical markers of cardiovascular disease. A paper in 2007 by Lutsey et al. looked at the relationship between whole grains and CVD biomarkers. The results of this study found an inverse association between whole grain intake and diabetes risk but were unable to find significant results with CVD biomarkers. In the time since the publication of this paper, MESA corrected an error in how dark bread was counted in total whole grain intake. The purpose of this study was to replicate the analysis in Lutsey et al. using the corrected whole grain intake.

Methods: Whole grain intake was assessed using a 127-item food frequency questionnaire collected between 2000 and 2002 during MESA exam 1. We applied the same exclusion criteria as Lutsey, et al (n=5493) and compared them against the five original outcomes; obesity, insulin resistance, inflammation, newly diagnosed diabetes, and subclinical CVD.

Results: In our analysis, the whole grain data modification greatly altered mean intake and shifted subjects around within the five study quintiles (mean intake of Q1=0.04g; Q2=0.32g; Q3=0.65g;

Q4=1.03g; Q5=1.79g). However, our replication of the paper did not change key outcomes of Lutsey et al.

Conclusion: Our re-analysis supports the association between whole grain intake and diabetes and no relationship was found with subclinical CVD in the MESA cohort. Additionally, this sensitivity analysis demonstrates the limitations of using FFQs and self-reported dietary records to inform national and international guidelines.

INTRODUCTION

In 2007, Pamela Lutsey and a team of researchers used baseline data from the Multi-Ethnic Study of Atherosclerosis (MESA) to study the cross-sectional association between whole grain intake and obesity, inflammation, insulin resistance, diabetes, and subclinical CVD [1]. At the time of this paper's publication, a large body of data existed to support the relationship between whole grains and improved insulin sensitivity, low diabetes incidence, and decreased weight gain [2-4]. Although a diet higher in whole grains was also associated with lower coronary artery disease morbidity and mortality [5-7], no study to date examined the association of whole grain food consumption with subclinical atherosclerosis. Lutsey, *et al*. hypothesized that whole grains would be inversely associated with biomarkers of inflammation, obesity, insulin sensitivity, newly diagnosed diabetes, and finally, subclinical atherosclerosis. The study was conducted using the Multi Ethnic Study of Atherosclerosis (MESA) cohort. This cohort was designed to study the predictors of moving from subclinical to clinical CVD and so all 6814 participants were free of symptomatic CVD at baseline.

The main findings of this paper were that while the largest consumers of whole grain foods had a statistically significant decrease in newly diagnosed cases of diabetes, no relationship seems to exist between whole grain intake and subclinical CVD. By revisiting this data, we aim to determine if statistical methods were robust, if corrections in the data were meaningful, and if the results are still consistent with the current compendium of whole grain research. This work was conducted in the context of a scantron error, in which some of the foods consumed were set to missing in the presence of a modifier (*e.g.,* "do you eat the skin of an apple?"). The correction resulted in more whole grain intake being captured and a subsequent increase to the mean whole grain intake of the study population. Since participants could move from no intake to large amounts of intake, this resulted in dramatic movement of individuals within quintiles of intake. Despite these corrections, our results mirror those of Lutsey and her team. We were unable to find a cross-sectional relationship between whole grain intake and subclinical CVD.

METHODS

Multi Ethnic Study of Atherosclerosis

MESA is a prospective cohort study of 6814 American individuals that began in 2000 and continues today. The subjects were recruited from six different field centers in the United States. At baseline, all were between the ages of 45 and 84 and free from clinically overt CVD. Approximately 38% of recruited participants are white, 28% African American, 22% Hispanic, and 12% are Asian, primarily of Chinese descent. As of 2017, five exams have been completed. The first exam took place between July 2000 and August 2002 and the fifth from April 2010 to December 2011. Personal and medical history, along with anthropometric data and blood samples were taken at each exam, as was a computed tomography (CT) scan of the carotid artery. Food Frequency Questionnaires (FFQ) were collected at exams one and five. The primary aim of the MESA study is to reveal possible risk factors for the development of subclinical CVD [8].

Food Frequency Questionnaire

We reexamined the exam one data in the fall of 2016. Beginning by applying the exclusion criteria from the original paper, we removed from the data set any subjects without FFQs (n=577), individuals with calculated energy intakes of ≥6000 kcal/day (n=16) or ≤600 kcal/day (n=141), and those with diabetes mellitus at baseline (n=610). The final study population was 5493, compared to the original study population of 5496. Using the categories available in the MESA FFQ, Lutsey, *et al* identified five that indicated whole grain intake: whole grain cold cereal, oatmeal, dark bread, bran muffins, and brown or wild rice (table 1.a.). Our recreation (table 1.b.) displays similar results in most categories with one notable difference; the mean intake of dark bread climbed from 0.13 servings per day in 2007 to 0.36 servings in 2016. The reason for this is a data modification that occurred between the publication of the original paper and our recreation. In the original analysis of the MESA FFQ, if a subject noted that they ate whole grain bread, it was removed from the total servings of bread that subject consumed. By 2016, this error was corrected, and dark bread reflected intake of whole grain breads, thus explaining the jump in servings per day and an overall increase in mean whole grain intake among the entire study population

from 0.54 servings per day to 0.80 servings. Whole grain intake now correctly defined, we sorted the study subjects into quintiles of whole grain intake.

Data Collection

Obesity was measured using BMI (kg/m^2), with any BMI ≥ 30 kg/m^2 considered obese. Serum insulin and glucose were taken after an eight hour fast and used to determine insulin resistance (BG=100-125 mg/dL) or newly diagnosed diabetes (BG>125 without a self-report of diabetes). The homeostasis model assessment (HOMA) is expressed as insulin*glucose/22.5. Inflammation was measured through soluble blood measures of of C-reactive protein (CRP), interleukin-6 (IL-6), and homocysteine. Urine albumin excretion was assessed through urine samples, as was albumin creatinine ratio (A/kC). Finally, the main outcome of interest – subclinical CVD – was measured using two outcome variables: carotid artery intima media thickness (measured through B-mode ultrasonography) and presence of calcified plaques (measured by computed tomography scan) reported using an Agatston score.

Analysis

Lutsey, *et al* chose to express results as the mean of each quintile. In our replication of their results, we chose to use odds ratios. While both sets of results were valid measure of association, we find odds ratios (OR) to be more clinically interpretable than adjusted means. Using means, one can show values that are higher or lower, but cannot make a direct comparison between the results, nor estimate the potential difference in outcome that a difference in intake might induce. To determine odds, we converted each linear variable into a dichotomous one by finding the median between low and high values. From there, we ran a logistic regression, using the lowest quintile of intake as a reference category. The direct comparison between the first quintile and subsequent quintiles allows for comparisons. For example, subjects in the highest quintile of intake are 30% less likely to have insulin resistance or newly diagnosed diabetes than subjects in the lowest quintile of intake, provided that the rare disease assumption is met. The results are easy to interpret for researchers and lay audiences alike. Means are less simple to interpret.

Statistical Methods

Lutsey, *et al* ran three different models (table 3a.) to rule out the possibility of confounding and presented their findings in means and percentages per quintile. Here, the p-trend is statistically significant (<0.05) in model 3 for an inverse association between whole grains and: BMI, insulin, HOMA, and homocysteine. While there are other statistically significant p-trends in some of the other disease outcomes, all are attenuated by running the mechanistic model (model 3).

The revised results table (table 3b.) is no longer a comparison of means and percentages between quintiles. To make a more direct comparison, the results are expressed in odds ratios using quintile 1 as a reference category. The dichotomous variables for our logistic regression are either provided by the original paper (impaired fasting glucose, new diabetes diagnosis, A/kC, plaque, and Agatston score) or universally recognized standards (obesity, high blood glucose). For the remainder of outcomes, the median was used to determine high versus low. Instead of using p-trends, table 3b. displays p-values for each outcome variable by quintile. All data was analyzed used Stata v.13.

RESULTS

Whole Grain Consumption

The mean consumption of whole grains once the MESA data correction is applied is 0.79 servings/day. In the initial paper, the mean consumption was 0.54 servings/day, indicating that the data correction impacted the overall consumption of whole grains among the study population. The mean intake in the first and fifth quintile went from 0.02-1.39 in the Lutsey, *et al* paper to 0.04-1.79 post-correction. Furthermore, the addition of whole grain bread moved a subset of subjects from the first quintile of intake into the fifth, thus the distribution of individuals within the quintiles is likely quite different between the Lutsey, *et al* paper and our analysis.

Subclinical Atherosclerosis

No reduction of risk for cardiovascular disease markers was observed between quintiles when controlled for potential confounding. Whole grain intake was not associated with any reduction in either common carotid (p=0.679) or internal carotid intima media thickness (p=0.207), nor was it associated with lower calcified arterial plaque (p=0.236).

Insulin Resistance and Newly Diagnosed Diabetes

Subjects in the highest quintile of intake are 30% less likely to have insulin resistance or newly diagnosed diabetes than subjects in the lowest quintile of intake (OR 0.70; $p<0.05$). Lutsey, *et al* using means found that 38.4% of subjects in the first quintile had insulin resistance or newly diagnosed type 2 diabetes compared to 32.1% of subjects in the highest quintile.

Other Outcomes

Using the behavioral model from Lutsey, *et al*, quintile 5 experienced a statistically significant reduction of risk of high HOMA compared to quintile 1 (OR 0.72, $p<0.05$) and quintiles 4 and 5 showed a statistically significant reduction of risk compared to quintile 1 for high homocysteine (OR 0.7, $p<0.05$ and 0.61, $p<0.05$ respectively). There was also statistically significant reduction in impaired fasting glucose in quintile 4 (OR 0.62, $p<0.05$) and quintile 5 (OR 0.70, $p<0.05$), when compared to the first quintile. Though not statistically significant, the higher quintiles experienced lower risk of obesity and insulin resistance.

DISCUSSION 1: DISCUSSION OF RESULTS

Much like Lutsey, *et al.,* we found inverse associations between whole grain intake and insulin resistance, newly diagnosed T2DM, and levels of homocysteine. Those in the highest quintile of whole grain intake had lower HOMA levels ($p=0.003$), fewer incidences of impaired fasting glucose (IFG) ($p=0.008$), and improved homocysteine levels ($p<0.000$) when compared to the lowest quintile of intake. The second highest quintile of whole grain intake had statistically significant reductions in homocysteine ($p=0.001$) and IFG ($p=0.000$) as well as diagnoses of T2DM ($p=0.051$). Obesity and inflammation were both negatively associated with increased whole grain intake, but these results were not statistically significant. No relation appears to exist between subclinical markers of CVD and whole grain intake. In this section, we will examine these results within the context of other research in the area of whole grain intake and chronic disease biomarkers to better understand the context for the lack of an observed association between subclinical CVD and whole grain intake in the MESA.

Whole grains, Impaired Fasting Glucose, and newly diagnosed Type 2 Diabetes Mellitus

Subjects in the fourth and fifth highest quintiles of whole grain intake had a statistically significant reduction in impaired fasting glucose when compared to the lowest quintile. The odds of having a fasting glucose over 100mg/dL in the highest quintile are 30% lower than in the lowest quintile of intake and 38% lower in the fourth quintile, after controlling for potential confounders. The fourth quintile also saw a statistically significant reduction in the chances of having newly diagnosed diabetes of 51% compared to the first quintile. It is unclear why the fifth quintile does not have similarly significant results (OR 0.71, p=0.316), given that a majority of available research supports an inverse relationship between whole grain intake and diabetes risk. However, we did not remove outliers from this group and it is possible that some outliers in quintile five have more abnormal dietary patterns and could potentially skew results.

Lutsey, *et al* examined the cross-sectional association between whole grains and IFG/diabetes, so it cannot be assumed from this analysis that whole grains are protective against diabetes. In a controlled trial of 61 individuals with prediabetes and the metabolic syndrome, subjects were randomized to a refined-grain diet (control) or a whole-grain diet for 12 weeks. Participants were instructed to eat all other nutrients as they normally would. By the end of the intervention, the postprandial insulin concentrations dropped by 29% compared to baseline, significantly lower than those in the control group. Despite the drop in insulin, postprandial glucose concentrations were similar among groups. This suggests that whole grains helped improve the postprandial glycemic response [9]. In a 2013 prospective cohort study, Wirstrom, *et al* found a whole grain intake > 59.1g/d is associated with a 34% decreased risk of a deterioration in glucose tolerance compared to individuals who consume <30.6g/d whole grains even after controlling for family history of diabetes [10]. Song, *et al* discovered a similar trend among their cohort of 3871 Korean adults. Individuals in the highest quintile of whole grain and bean consumption had a 20% reduction in insulin resistance (based on HOMA scores) when compared to the lowest quintile of intake. The diet high in whole grains and beans included tomatoes and fruits and was notably rich in phytoestrogens, dietary fiber, vitamin C, and magnesium – all of which are suspected to have a role in mediating insulin resistance [11].r

In an umbrella review of meta-analyses published in 2017, McRae*, et al* found a 20-32% reduction in incidence of diabetes among subjects with high whole grain intake [12]. One of these systematic reviews examined the results of 45 prospective cohort studies and 21 randomized controlled trials to find that consuming 46-80g/d (approximately three to five servings) of whole grains is associated with a 26% reduced risk of diabetes compared to those who ate no whole grains [13]. Chanson-Rolle, et al combined eight prospective cohort studies into a meta-regression analysis and observed that every 20g of whole grains added to the daily diet is associated with a 0.6% absolute reduction in risk of diabetes. This relationship is assumed to be linear over the range of data and equates to a 12% relative risk reduction [14]. While some of the included studies showed statistically significant heterogeneity – thus lessening some of the power and generalizability of the meta-review – the overall observed effect suggests that whole grain intake is associated with a reduced risk of diabetes. Whole grains are low on the glycemic index (GI) and will deliver a slower, more measured release of glucose into the bloodstream compared to high GI foods like white bread and table sugar [15]. This explains some of the protective qualities of whole grains in preventing spikes in blood sugar, though there may be additional benefits found in the phytochemicals of whole grain foods.

The results found by Lutsey, *et al* and replicated through the current research are consistent with the majority of research on whole grains and a reduction in diabetes risk. It is important to note that the elimination of preexisting diabetes cases in the MESA dataset helped to prevent a false association between whole grains and diabetes should any of the subjects with diabetes change their diet post-diagnosis. Nevertheless, whole grain intake is greatly associated with a reduced risk of diabetes. What will come in following sections is an analysis of what this means for nutritional guidelines and clinical applications.

Methodology of Researching Associations between Whole grains and Cardiovascular Disease

The relationship between a dietary pattern that includes whole grains and incidence of CVD is well documented. The Dietary Guidelines Advisory Committee (DGAC) recommends a diet high in fruits, vegetables, legumes, seafood, as well as whole grains. The DGAC includes whole grains due to

their high fiber content. A long-established base of evidence exists to suggest fiber is protective against coronary heart disease [16]. Yet, little research seems to show a clear delineation between whole grain intake alone and a reduction in CVD risk. Like Lutsey, *et al*, we did not find a cross-sectional association between whole grain intake and markers of subclinical CVD. Given that the results of Lutsey, *et al*, and our replication paper both fail to find a relationship between whole grain intake and subclinical CVD, it is possible that no relationship exists. It is also possible that the chosen outcome variables of carotid and internal IMT and arterial plaque calcification do not provide a clear picture of CVD risk or that the mean intake of whole grains was too low to produce clear results.

Research shows that even a slight increase of arterial wall thickness can be a strong predictor of future health events. In a pooled analysis of eight studies, every increase of 0.1mm to the carotid artery wall was accompanied by a 10-15% increase in myocardial infarction risk and 13-18% greater risk of ischemic stroke. The included population-based studies had a total sample size of 37,197 subjects and a mean observation period of 5.5 years [17]. If a measurement as small as a tenth of a millimeter coincides with increased risk, it is extremely important to ensure that screening tools be as precise as possible. The intima-media thickness of the common carotid and internal carotid arteries was assessed using B-mode imaging [8]. This method of assessment is simple, noninvasive, and inexpensive [18]. However, Shah, et al, found a greater number of arterial plaques using contrast-enhanced ultrasound (CEUS) (n=367) than B-mode imaging (n=350) in 175 individuals free from known CVD. Using CEUS, researchers noted improved near-wall IMT visualization of the common carotid artery and improved IMT visualization in both the near- and far-wall of the internal carotid artery compared to B-mode imaging. IMT generally increases with age due to hypertension-induced smooth muscle hypertrophy. Shah, et al suggest arterial plaques are more accurate than IMT in assessing the burden of atherosclerosis [19]. It is likely that B-mode imaging was used by MESA researchers due to the simplicity and cost-saving benefits of this measurement tool and thus, the study's reproducibility. Since precision is vital and the margin for error is extremely narrow, arterial wall thickness can be used as an indicator if it is verified by an additional marker of subclinical CVD.

To add to the IMT data, Lutsey, *et al* evaluated the presence of coronary artery calcification (CAC) using the outcomes of percentage of population with plaque, Agatston score, and percentage of population with an Agatston score greater than zero. A score of zero indicates a complete absence of arterial plaque and is rare because most individuals will have some degree of plaque formation [20]. Arterial plaque was assessed using computed tomography (CT) and Agatston scores were calculated by blinded CT image analysts. Using presence of plaque as a dichotomous variable, subjects were grouped into CAC scores > 0 if any CAC was present or CAC scores <0 if no CAC was present [1]. No reduction in risk of CAC was observed among the higher quintiles of whole grain intake compared to the lowest quintile.

To assess coronary artery calcification, subjects lay on a CT scanner – a painless process that takes approximately ten minutes. From this reading, the density and area of calcified plaques is calculated and represented as an Agatston score. The amount of calcification increases with age and more quickly in men than women. The majority of asymptomatic men over 55 and women over 65 years-old have calcified plaque in their coronary arteries [20]. The Agatston score was designed to detect the presence of calcified plaque only. However, noncalcified plaque is also a risk factor for cardiovascular events. Koulaouzidis, *et al*, retrospectively sampled 447 symptomatic patients with CAC scores of zero and found that approximately 10% of these subjects had noncalcified coronary artery plaques (NCAP). This study differs from the MESA data as the MESA population was asymptomatic for CVD at baseline. However, it demonstrates that the relationship between CAC and coronary artery disease is not absolute; "the presence or absence of calcium does not allow a reliable recognition of the vulnerable coronary artery plaques [21]." Similar results were found by Kelly, *et al* in their study of 729 hospital patients admitted for atypical symptoms, abnormal or indeterminate nuclear stress test findings, or CAD assessment for patients with other risk factors. Forty-five percent had a normal calcium score and of these 325 patients, 167 (51%) had some NCAP present and 17 patients had moderate to severe stenosis. These NCAPs were found using coronary CT angiography (CTA) and not computed tomography, finding that this method produces significantly more diagnostic information than calcium score. "Essentially,

coronary CTA adds certainty to the evaluation of the coronary arteries, whereas the calcium score generates probabilities [22]."

While neither the C-IMT and I-IMT nor the Agatston score offers complete insight alone, combined, these values can show a clearer picture of CVD risk. Yet, no cross-sectional relationship was found between subclinical CVD and whole grain intake by Lutsey, *et al* or in the current replication. It is quite possible that eliminating symptomatic atherosclerosis from the study population limits the degree to which cross-sectional analysis will turn up any significant results. As of 2017, between 15 and 17 years have passed since baseline CVD risk was assessed. Intima-media thickness was assessed again at exams two and three and ancillary studies collected this data during exams four and five. While no cross-sectional relationship was found, perhaps a longitudinal study would produce different results. In 2009, Nettleton, *et al* examined the relationship between dietary patterns to CVD risk in the MESA cohort. After an average of four years of surveillance, participants above the 80th percentile of whole grain and fruit intake had a 46% lower risk of CVD than those below the 20th percentile [23]. While this data does not exclusively examine whole grain intake, it suggests that whole grains are part of a dietary pattern that may have protective effects over time. Now that exam 5 data is available to researchers, a replication of the Nettleton study over a longer time period may show an even more robust association.

In a 2017 meta-analysis exploring etiologic effects of foods and nutrients on cardiovascular disease and diabetes, the Nutrition and Chronic Diseases Expert Group (NutriCoDE) did find a small, but significant association. Pooling the results of seven cohort studies, NutriCoDE observed a 9% reduction in CVD risk with every 50g of whole grains consumed [24]. Fifty grams is equivalent to 1.76 ounces, nearly two servings, of whole grains. Because the effect is small, it is possible that the MESA cohort (which had an average intake of 15g) simply did not consume enough whole grains to reach statistical significance. NutriCoDE concluded that diets low in whole grains are related to greater risk of CVD, coronary heart disease (CHD), and diabetes. The group went on to suggest that two and a half 50g servings (4.5 ounce equivalents) per day is ideal for reaping the protective benefits of whole grains instead of the three ounces recommended by the DGAC [24]. This meta-analysis was published in April

2017, therefore it is newer than the 2015 Dietary Guidelines and reflective of a larger, more updated body of research.

DISCUSSION 2: LESSONS LEARNED

Why replicate papers?

Replication in research is essential to validate results and account for any changes to methods or analysis. In the ten years between Lutsey, *et al* first analyzed the data, a major correction to the MESA data set occurred. This increased the overall mean whole grain intake among the entire cohort, but did not dramatically alter results. However, a data modification of this magnitude could have easily changed outcomes. It is impossible to know what affect it would have had without replicating the initial paper. Furthermore, when new eyes view data, the interpretation is bound to change. Lutsey, *et al* represented results as means of intake among quintiles. This shows a trend, which can demonstrate statistical significance, but falls short of showing clinical significance. By reexamining results with the intent of applying conclusions as a practitioner, odds ratios are better able to convey risk of benefit. Odds ratios allow a comparison of one behavior or characteristic in direct relation to another while means show linear trends. Our results show a 30% decrease in risk of diabetes for those who eat the most whole grains *compared to* those who eat the least. Or, by eating one additional serving of whole grains (28g), an individual could reduce their chances of diabetes by 30%. Neither a linear or logistic approach is more correct than the other, the only difference is in how the data is used. Revisiting papers also provides the opportunity to compare recent literature with the paper's original conclusions. When Lutsey, *et al* conducted their initial research, no one had examined the relationship between whole grains and subclinical CVD [1]. In 2017, this is no longer the case. Other published research into whole grain intake and CVD so far confirms these conclusions. Furthermore, considerable research into the relationship between whole grain intake and development of type 2 diabetes conducted in the time since Lutsey's paper was published confirm the results.

Finally, paper replications allow us to ask if the research question is still valid. Dietary intake trends change constantly and as a result, research on consumption can become less applicable as time

goes on. Firstly, total whole grain consumption has increased since data was gathered. According to NHANES data, in 2001-2002, Americans consumed an average of 0.72 oz. equivalents of whole grains per day. By 2012, this had increased to 0.97 oz. equivalents [25]. And the type of grains have changed. According to the Whole Grains Council, kamut is experiencing a 67% growth on restaurant menus in 2016, farro sales growth is projected to increase 105% over 2015 sales figures, and quinoa is now a restaurant staple – appearing on 9% of all American menus [26].

Evaluating Dietary Intake

Determining dietary patterns among large study cohorts is a daunting task. There are three common methods utilized by researchers to assess what people eat: food diaries, dietary recalls, and food frequency questionnaires. Each have their benefits and drawbacks, but none paint a clear picture of true dietary intake as all rely on self-reporting. Understanding the pros and cons of each method helps determine what is the most practical and useful method for a large cohort study, such as MESA, and what information may still be left out.

Diet Record

The diet record (DR) or food diary method refers to a detailed list of foods consumed with correct amounts and preparation methods taken over a specified period of time. This is the only data collection method that is prospective rather than retrospective [27]. Although recording foods while they are being consumed is beneficial in that it does not rely on memory, there are several drawbacks to this method. Firstly, subjects know they are being observed and are thus likely to alter their eating patterns based on convenience or fear of judgement. An individual may be less likely to make a homemade soup if he or she must then remember all the ingredients and amounts and calculate how much of the recipe was consumed. Or, one might skip a usual donut at the coffee shop in an effort to paint oneself in a positive light. Furthermore, assuming subjects are filling out their diary throughout the day may be incorrect. Some individuals are likely to wait until the end of the day, or end of the trial period to complete their records, making it more like a dietary recall and thus, retrospective [28].

Another major assumption with DRs is incorrect estimates of portion sizes. This error can be minimized by providing detailed instruction prior to the beginning of the study period, or by asking subjects to weigh their food and use common measurement tools like cups and spoons [27]. While the error can be minimized, it cannot be eliminated entirely. Asking if a banana is small, medium, or large places a good deal of judgement upon the person eating that banana.

Dietary Recall

Much like a food record, a dietary recall reflects intake over a short time period, typically 24 hours. Unlike a food record, a recall is reflective – the subject remembers what they consumed the previous day as well as the portion sizes and cooking styles. Typically, recalls are done with the assistance of someone trained to administer them. Using the multi-pass method, a recall administrator can prevent recollection error by going over the meals of each day a few times and capturing forgotten items. Yet, this method of assessing dietary intake carries the same flaws as a prospective food record. Even though the food has already been consumed and intake is untainted by bias, what the subject chooses to disclose introduces several opportunities for manipulation of reported intake. In fact, energy intake is generally underreported in 24-hour recall assessments [28]. And like food records, recalls are limited by a short time frame, usually a single day. It is unlikely that a single day for anyone is reflective of their overall dietary pattern. Thus, either a large samples size to estimate population trends or multiple days during a study period are required for studying dietary patterns.

Food Frequency Questionnaire

Food frequency questionnaires are often used in research due to their relative ease of administration and low cost. FFQs are grouped into two main sections, a list of foods and frequency response questions [27]. From this data, researchers can calculate average servings per day for specific foods and a rough idea of total energy intake. They are also useful in determining longer-term patterns of dietary intake [27]. Unlike diet recalls and food diaries, FFQs capture information on a longer period – usually a year. While there is limited precision in this method (e.g. individuals cannot recall exact foods over the last year) most people are good at identifying foods they eat often. Thus, FFQs should have a

wide enough range of foods that individuals can find items that reflect their dietary pattern. This presents a challenge. In order to keep the burden of completing the survey low, there is a limit to the number of questions that can be on it. Long questionnaires cause fatigue and hamper concentration, producing less accurate results. The theoretical limit to the length of an FFQ is approximately 130 questions [27]. Determining what types of foods should be included and what can be left out is highly dependent on what information is sought. Using MESA as an example, foods that are consumed regularly by Americans of four different ethnic groups needed to be included. How do we represent four potentially distinct diets while simultaneously limiting the question bank? Some believe that there is no use including two highly correlated foods, such as kale and chard. One can be used as a representation for the other, but this approach is simply a theory and has not been demonstrated to be accurate in practice. Another approach might be to highlight foods that are thought to be highly correlated to potential outcomes of study [27]. Going back to MESA, since atherosclerosis is the main outcome of study, many of the questions on the FFQ are related to animal fats, as described below. Since saturated fat and cholesterol are thought to be highly correlated to atherosclerosis and other heart disease outcomes, providing several questions about food sources of these fats will produce the kind of data useful for describing these relationships.

In order to be useful, FFQs must be reproducible, validated, and calibrated. Participants should fill out the questionnaire at two time points that are far enough apart to prevent answer duplication. This helps to adjust for any error in reporting from a single administration. Validation can be done using a more accurate method of intake analysis, such as a diet recall or food record [27]. Doubly labelled water (DLW) is the gold standard validation method, since it cannot be influenced by subjects. This test involves administering labelled hydrogen and oxygen isotopes and measuring turnover rates of body water into carbon dioxide. A DLW study can confirm total energy expenditure, but does not provide any insight into dietary patterns. In fact, there is currently no validation method for habitual intake of foods [29]. The results of the validation study are used to calibrate the responses to the FFQ and providing more accurate nutrient and total energy intakes. Nevertheless, an FFQ cannot be used to accurately assess total energy intake as it is simply an estimation of what an individual consumes, based on his or her own

memory. For the purpose of this study, whole grain intake is an appropriate food to measure through FFQ, it is not a measure of total caloric intake and individuals may be likely to recall their frequency of whole grain foods, insofar as it is typically a deliberate choice based on health.

MESA FFQ: Reproduction, validation, and calibration

MESA researchers developed their FFQ to model that of the Insulin Resistance Atherosclerosis Study, which was previously validated in non-Hispanic white, African American, and Hispanic populations. To better reflect the dietary intake of the Chinese study participants, Chinese foods and culinary techniques were included into the 127-question FFQ. The questionnaire had nine categories and for each item, subjects were asked whether or not they ate that particular food and how often (from never or rarely up to three times a day for food and six times per day for beverages). They were also questioned on how large their portions were and servings per day was calculated from this data. Researchers collected 6,237 staff-assisted self-administered FFQs for Exam I between July 2000 and August 2002 at six field centers across the United States. The IRAS FFQ was measured against eight 24-hour dietary recalls and nutrient intake was compared between the two methods. The correlation coefficient was 0.62 for non-Hispanic whites, 0.5 for African Americans, and 0.41 for Hispanics. The IRAS FFQ was also doubly administered for some participants and the mean correlation coefficient for nutrients between the two surveys was 0.62 and did not differ by ethnic subgroup [1]. Although DLW is the current gold standard validation method, it is not designed for measuring habitual intake. Validation simply requires that a comparison be made with a superior standard [27]. A diet recall is stronger than an FFQ, and far cheaper and less burdensome for subjects. Its use as a validation and calibration tool is appropriate.

Since MESA researchers used a previously developed FFQ, they knew how accurate it would be based on the error checks done by IRAS. However, the FFQ had some surprising omissions. For example, corn was not a separate food item, instead listed alongside hominy. Corn is a whole grain, hominy is not. The decision to consider corn a grain or a vegetable will vary and will affect how data interpretation. In the MESA FFQ, while corn/hominy is listed as a vegetable, corn tortillas are combined into a list item with flour tortillas and found in the “Mexican food” category, while tortilla chips are categorized as

bread. Potatoes also straddle the line between vegetable and starch, but in the MESA FFQ they are included in the "rice and potatoes" category. There is no simple answer of how to characterize starchy vegetables like corn and potatoes. Wheat, rice, and grasses will always be considered grains, no matter their preparation. Yet, corn does not have this same distinction. This creates potential problems when trying to assess dietary patterns. Since corn is such a staple in many diets, its definition matters. When translating research into nutritional guidelines, it is important to know if corn is factored into the diet high in vegetables that is considered protective against chronic disease, or if it is a simple carbohydrate best consumed in moderation. The omission of corn as a separate list item is understandable when considering that FFQs must be short enough to prevent fatigue. Hominy needed to be included due to its prevalence in Latin American cooking and listing them as separate items would have been a wasteful use of questions. Yet, the list of whole grain foods available in the MESA data is incomplete.

What is the definition of a whole grain?

Lutsey, *et al* aggregated all of the whole grain items into five groups: whole grain cereals, dark bread, oatmeal, brown or wild rice, and bran muffins. After reviewing the MESA FFQ, there do not appear to be other options that are specifically whole grain foods. Yet, in order to determine what counts as a "whole grain" and what does not, the term must first be defined. There is no current consensus on how to define whole grains. In 2010, the consortium HEALTHGRAIN EU set about to develop a standard definition of whole grains that would reflect industrial processing practices, be easily translatable to nutrition guidelines and labels, and that could be applied broadly across the European Union. Their definition is as follows, "Whole grains shall consist of the intact, ground, cracked, or flaked kernel after the removal of inedible parts such as the hull and husk. The principal anatomical components – the starchy endosperm, germ, and bran – are present in the same relative proportions as they exist in the intact kernel. Small losses of components – that is, less than 2% of the grain/10% of the bran – that occur through processing methods consistent with safety and quality are allowed [30]." The Whole Grains Council (WGC), an industry group created their own definition in 2004: "Whole grains or foods made from them contain all tahe essential parts and naturally-occurring nutrients of the entire grain seed in their

original proportions. If the grain has been processed (e.g., cracked, crushed, rolled, extruded, and/or cooked), the food product should deliver the same rich balance of nutrients that are found in the original grain seed [26].” This definition is less specific than the HEALTHGRAIN EU definition, only implying that a processed whole grain must deliver the same mixture of nutrients without giving precise parameters for component losses. How do consumers know if the product they are purchasing is whole grain? The WGC places a label on items that contain whole grains with slight differences for 100% whole grain, 50%, and products that simply contain a minimum of 8g (one half serving) whole grains per serving [26].

In addition to the lack of a standard definition, the whole grain market has grown substantially since MESA Exam 1 and continues to grow each year. In 2000, an average of 300 new whole grain products was launched in worldwide markets. By 2010, this number was closer to 3,000 [30]. This includes the now highly popular quinoa, which was not included on the MESA FFQ due to its virtual absence from the American diet in 2000. Immigration into the United States has also brought new grains into American supermarkets such as teff from East Africa, which *The New York Times* touted as the “new super grain” in 2016 [31].

DISCUSSION 3: BROAD APPLICATIONS

Knowledge translation and the Dietary Guidelines for Americans

Research is most useful when it can be translated into practice. Translational research is defined as the process of applying laboratory sciences to human studies and then into evidence-based practice in real-world settings. It can take roughly 17 to 24 years to translate 14% of research into routine practice. From study design to implementation, to publication, to inclusion in a systematic review, it is easy to see how designing and implementing guidelines can be a slow process [32]. Yet, the preference for evidence-based conclusions over expert consensus makes for stronger recommendations.

These recommendations are then translated into guidelines by the DGAC, appointed every five years by the Departments of Agriculture and Health and Human Services. The committee is tasked with updating the dietary guidelines (DGAs), which then guide all nutrition education and policy decisions in the United States. The frequent updates make for a more rapid application of new research, incentivizing

researchers to publish in time for the development of the guidelines. The guidelines are meant to reflect the dietary reference intakes (DRIs) set by the Institute of Medicine's Food and Nutrition Board. These DRIs are not regularly updated due to budgetary constraints within the IOM. These guidelines are not free from economic and political interests, industry buy-in, competing priorities, and other interests that are taken into account along with the scientific evidence [27]. Food production, manufacturing, wholesale, and retail are all private interests and subject to economic growth or failure. Therefore, corporate interests can often win out over scientific evidence. For example, the health effects of trans-fats were well documented and the DGAs in 2005 reflected this, but the inclusion of trans-fat on food labels took a full decade from when the research conclusions were reached [27]. It is important to note that the nutrition labels are not under the purview of the DGAC, rather the Nutrition Facts Panel of the Food and Drug Administration [33].

Once implemented, guidelines need to be periodically evaluated to ensure that they are still evidence-based and effective. A good example of this is the 2015 guidelines loosening of regulations on dietary fat. The 1980 DGAs recommended to limit dietary fat to 30% of total daily calories. This was amended in 2005 to 20-35% of total calories. By the release of the 2015 DGAs, several randomized controlled trials showed the benefit of diets higher in fat on cardiovascular health outcomes over the low-fat-high-carbohydrate diets popular in previous decades. The DGAC no longer listed cholesterol as a nutrient of concern and removed the upper limit on consumption of fat. The Nutrition Facts Panel is slower to move, still listing the 1980 figure of 30% on labels [33]. This example underlies the importance of constant review and application of new research as it becomes available. It also demonstrates the drawbacks of having different aspects of public nutrition information controlled by different entities. The speed of emerging research, slow as it may already be, is then hampered by less nimble government committees.

Dietary Guidelines and whole grains

Specific recommendations for whole grain consumption first appeared in the 2005 DGAs. The DGAC recommended a minimum of three ounce-equivalents per day. The committee chose to express

serving size in ounce equivalents because an average slice of bread, cup of cereal, or bowl of cooked rice/pasta all weigh approximately one ounce [26]. This measurement system is potentially confusing to consumers who are used to seeing servings expressed in cups as opposed to weight. Therefore, another method of monitoring whole grain intake recommended by the DGAC is for whole grains to make up 50% of total grain intake [26]. They arrived at this conclusion based on systematic reviews and randomized controlled trials that showed a reduction in chronic disease risk [34].

We know that the recommendation is to eat three servings of whole grains per day, but how many Americans actually reach this goal? According to NHANES data, the average American adult ate 0.97 ounce equivalents of whole grains in 2012. Although this is less than one third of the recommended intake, it is a slight improvement over the 2002 survey which showed adults eating 0.72 ounce equivalents. This data is not reflective of total grain intake, which 70% of adults met in 2012. Only 1% of adults met the recommended daily intake of whole grains [34]. The information we have on whole grains and their relationship to chronic diseases like diabetes and cardiovascular disease is based on normal consumption patterns and 99% of people are not eating the recommended quantity of whole grains. Can it then be assumed that if the difference between the highest and lowest quintile of intake was two servings instead of one, the results may be different? Perhaps the cardiovascular benefits of whole grains are not observed at 1.39 ounces (39g) of whole grains per day (the median intake of quintile 5), but could be seen at 3 ounces per day – a clear limitation of this study. In a 2013 meta-analysis of 11 prospective cohort studies of whole grains and chronic disease risk, a 30% drop in diabetes incidence was observed with a 30g/day increase in whole grains. A 40g/day increase was associated with a 40% decrease in diabetes incidence [35]. Another meta-analysis posits a 18% decrease in relative risk of type 2 diabetes with an increase of 45g whole grains per day [15]. Consuming 48-80g whole grains (3-5 servings per day) can reduce cardiovascular disease risk by 21% [13]. It was studies such as these that lead to the DGAC recommending three servings per day for adults.

Increasing whole grain intake vs. limiting total grain intake

As shown by studies mentioned in the previous section, whole grain intake is inversely proportional to chronic disease risk. But what about total grains? The 2015 DGAC report claims that 70% of Americans are eating too many refined grain product [33]. The refinement process strips the bran and germ from the grain, leaving the endosperm. The bran and germ contain most of the fiber and other bioactive components of the grain [30]. By comparison, the endosperm has little nutritional value beyond providing energy, but it is easily digestible and palatable. Diets high in refined grains are associated with metabolic dysfunction, obesity, and chronic disease [33]. Yet, systematic reviews of dietary patterns do not show that refined grain consumption alone is related to increased risk of type 2 diabetes or all-cause mortality [36, 37]. Refined grains appear to be a neutral presence; they are not protective against disease in the same way whole grains are, but they do not seem to contribute to disease, either. The implication being that the dietary pattern consistent with refined grain intake is also high in other nutrients of concern like sodium, saturated fat, and refined sugar.

Low carbohydrate diets (LCD) are also popular, thought to control weight gain by limiting the amount of excess glucose an individual consumes. A 2012 systematic review shows LCDs to be associated with significant reductions in body weight, BMI, abdominal circumference, blood pressure, plasma triclycerides, blood sugar, glycated hemoglobin, insulin, CRP, and an increase in HDL-c. The review authors were quick to note that the benefits shown are short-term [38]. It is not possible to elucidate long term effects of such diets based on this data. While a 2015 meta-analysis confirms the data on improved weight loss, plasma triglycerides, and HDL-c after six months to two years, the increase of LDL-c among those on a LCD compared to a low-fat diet was significant [39]. It is not surprising that a diet high in saturated fat would cause LDL-c to increase. Furthermore, the LCD carbohydrate range among studies selected by Mansoor, et al was 20-40g per day; roughly a serving or less. This deviates substantially from the typical American diet, correlating to less than one sandwich-worth of bread per day or a small bowl of cereal. Fruits and vegetables are similarly rich in carbohydrates and would thus need to be limited as well. This diet is not practical long-term, nor reflective of how most individuals choose to eat.

So what about whole grains is protective? Traditionally, it was thought that the benefit of whole grains was simply in the fiber they contain. However, as newer research indicates, whole grains contain phytochemicals that may confer additional health benefits. The intact grain contains zinc, ferulic acid, and magnesium. These nutrients are believed to work in tandem with one another to manage blood sugar and hypertension [35]. Whole grains contain upwards of 180 different bioactive components including various sphingolipids, flavonoids, lignans, fatty acids, and carotenoids. Dietary wheat may have blood pressure, cholesterol, and blood triglyceride lowering properties owing to the combined efforts of the fiber and phytochemicals [40]. Other plant-based foods like fruit, vegetables, legumes, and nuts have their own blend of fiber and phytonutrients. While the inclusion of whole grains in a balanced diet has been traditionally based on the large quantity of fiber found in the bran and germ of the grain, these are not the only benefits. The biomechanical role of fiber in the management of heart disease and diabetes is well documented and we are now seeing the smaller effects of the phytochemicals. No compelling evidence exists to suggest whole grains do not belong in a healthy diet. In fact, we are not getting enough of it.

Increasing whole grain intake on the population and individual levels

Increasing consumption of whole grain foods in place of their refined alternatives is a difficult task. Whole grain items are usually denser, drier, and more bitter. Many grain-based products that people regularly consume like pastries, pasta, cookies, crackers, and bread are processed and refined. These items are plentiful in grocery and convenience stores and often cheaper than their whole grain alternatives. Cost of foods are a major determinant of dietary pattern, especially for lower income individuals and households [41]. The DGAC may recommend that individuals make half of their grains whole, but how realistic is this for the average person to achieve?

Changing dietary patterns involves education, access, and affordability. Education or information about the effects of whole grains on chronic disease risk is useless if the price of white bread is the same as that of a whole wheat variety. It is even less useful if the whole wheat bread is not sold in the same stores as the white bread. Until these three components are addressed, we will not see population-level change. There is also a large degree of confusion around nutrition priorities, uncertainty around methods

of behavior change, and inadequate measurement systems of dietary intake [42]. Undoubtedly, consumer frustration around changing health recommendations make it difficult for someone without a degree in nutritional science to navigate the ever-changing landscape of dietary recommendations. The industry tends to cater to consumer trends, manipulating foods into several different product offerings. To the advantage of whole grains, they can be incorporated into breads, pastas, cookies, and other marketable products so long as the bran, germ, and endosperm are all present in their naturally occurring ratios. Therefore, it is possible that food manufacturers could see an advantage to selling more products made with 100% whole grains. Overall, whole grains should be part of a dietary pattern that is supported by evidence, driven by policy, and supported by the food industry. This can be accomplished by the formation of genuine and transparent partnerships between food manufacturers, advocacy groups, governments, and other stakeholders to promote healthy dietary patterns over less nutritious ones [42].

Changing population-scale nutrition behaviors is a daunting task. Applying evidence-based guidelines in a clinical setting has its own challenges, but involves fewer stakeholders. Trained clinicians will employ behavior-change strategies to encourage more healthful practices [42]. They will also serve as the translator of nutritional science for their patients or clients. In the case of whole grains, research shows that three to five servings per day can profoundly improve health outcomes [13]. Our research showed that the difference between intakes of quintile one and quintile five was little more than one serving, yet was associated with a 30% decrease in diabetes risk. What does this mean to the average person? A single slice of whole grain bread, a bowl of oatmeal, brown rice, fresh corn, quinoa. A sandwich with two slices of whole grain bread? That's two thirds of someone's whole grain needs for a day. Essentially, research is conducted and published, synthesized into systemic reviews and meta-analyses, collected to drive national guidelines, distilled into policy decisions, and eventually handed down to individuals in the form of concrete advice.

CONCLUSION

Nutritional research is essential to the development of policies and guidelines that influence our diet. Therefore, it is critical that this research be generalizable, relevant, and replicable. Paper replications validate study methods and test conclusions against current research, either refuting them or providing the original paper with more statistical power. This is especially critical for research on health and nutrition, as much of the data is observational and data collection methods are often hampered by the limitations of measurement tools and the unavoidable errors of self-reporting. However, through this paper replication and the analysis of research conducted in the 10 years since Lutsey, *et al*, reached their initial conclusions, there is little doubt that whole grains are protective against diabetes and may also guard against subclinical CVD if eaten in greater quantities than the current US mean intake. The recommendation set forth by the DGAC in the 2015 guidelines of three servings of whole grains per day should continue to be the target of public health campaigns and clinical advice with the ultimate goal of increasing intake within an overall diet pattern rich in fruits, vegetables, and plant-based proteins and fats. The simple fact is that with the average person in highest quintile of intake only consuming half of the recommended quantity of whole grains, no clear conclusion about the relationship between subclinical CVD and whole grain intake can be made from this data.

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APPENDIX

Table 1.a. Descriptions of mean intake of whole grain food groups among MESA participants, original

	# of servings/day		# and proportion consuming		# of svgs/day among consumers	
Whole grain item	Mean	SD	n	%	Mean	SD
Total whole grains >= 1 svg/day >= 3 svg/day	0.54	0.54	4973 1074 18	90.5 19.5 0.33	0.59	0.53
Cold cereal (whole grain)	0.15	0.29	2082	37.9	0.40	0.36
Oatmeal	0.17	0.28	3331	60.6	0.28	0.31
Dark bread	0.13	0.25	2761	50.2	0.25	0.30
Bran muffins	0.03	0.10	1390	25.3	0.10	0.19
Brown or wild rice	0.06	0.17	2301	41.9	0.14	0.23

Table 1.b. Descriptions of mean intake of whole grain food groups among MESA participants, revised

	# of servings/day		# and proportion consuming		# of svgs/day among consumers	
Whole grain item	Mean	SD	n	%	Mean	SD
Total whole grains >= 1 svg/day >= 3 svg/day	0.80	0.72	5117 1846 94	93 34 2	0.85	0.72
Cold cereal (whole grain)	0.19	0.32	2582	47	0.41	0.36
Oatmeal	0.17	0.27	3211	58	0.28	0.31
Dark bread	0.36	0.46	4254	77	0.46	0.47
Bran muffins	0.03	0.10	1336	24	0.10	0.19
Brown or wild rice	0.06	0.16	2213	40	0.14	0.23

Table 2.a. Means & percentages* of demographics, behaviors, diet, blood lipids, and blood pressure by category of whole grain intake in 5493 participants, MESA 2000-2002

	Whole grain intake				
	1	**2**	**3**	**4**	**5**
Whole grain intake					
Median	0.02	0.15	0.39	0.72	1.39
Range	0-0.07	0.08-0.26	0.27-0.52	0.53-0.96	0.97-6.14
N	1069	1137	1072	1121	1097
Demographics					
Male %	55.8	48.3	44.8	44.3	43.0
Race (row %)					

White	12.7	20.7	19.8	23.7	23.2
Black	16.4	19.7	19.3	23.3	21.4
Hispanic	23.3	21.8	20.7	16.8	17.4
Chinese	42.3	21.4	17.0	9.3	10.0
Mean age (years)	59.4	60.8	61.5	62.9	65.0
Education (row %)					
< High school	29.9	19.1	19.7	16.1	15.3
High school	20.3	20.8	19.8	19.6	19.5
Some college	18.2	21.0	19.4	21.2	19.5
Bachelors	16.0	20.8	19.6	22.9	20.8
Graduate/professional degree	14.7	21.9	19.0	21.2	23.0
Behaviors					
Smoked within past 30 d (%)	18.1	13.4	13.6	9.5	8.3
Alcohol (g/d)	5.2	4.6	4.5	3.9	3.5
HRT (fem only) (% current use)	30.4	33.4	30.5	35.5	30.5
Leisure PA (MET min/wk)	2285	2510	2340	2703	2610
Sedentariness score (MET min/wk)	1702	1755	1685	1655	1588
Mean dietary intake					
Energy (kJ/d)	6250	6672	7027	7228	8135
Refined grain (svgs/d)	2.03	1.95	1.92	1.83	1.64
Fruit (svgs/d)	1.89	2.00	2.09	2.44	2.69
Vegetables (svgs/d)	2.46	2.60	2.65	2.82	2.79
Dairy (svgs/d)	1.51	1.62	1.66	1.69	1.79
Fish/poultry (svgs/d)	0.98	0.99	1.00	1.02	0.94
Meat (svgs/d)	0.65	0.61	0.57	0.52	0.41
Blood lipids and blood pressure					
HDL-cholesterol (mg/dl)	51.8	51.8	51.4	51.5	51.3
LDL-cholesterol (mg/dl)	118.1	117.5	119.0	118.3	117.0
Systolic BP (mmHg)	126.3	125.6	125.4	125.7	125.0
Diastolic BP (mmHg)	72.2	71.7	72.0	72.1	71.6

* All means and percentages were adjusted for sex, age, race, education, site and energy intake; except for age, sex, education and energy intake, each of which is adjusted for the relevant combination of these variables only; and race, for which crude values are presented.

Table 2.b. Means & percentages* of demographics, behaviors, diet, blood lipids, and blood pressure by category of whole grain intake in 5493 participants, MESA 2000-2002, revised

	Whole grain intake				
	1	**2**	**3**	**4**	**5**
Whole grain intake					
Median	0.04	0.32	0.645	1.03	1.79
Range	0-.145	.15-.5	.505-.83	.83-1.29	1.295-5.435
N	1137	1249	917	1098	1092
Demographics					
Male %	52.42	45.8	44.49	47.54	46.06
Race (row %)					
White	13.3	20.8	17.2	23.8	24.9
Black	14.0	23.1	18.1	21.2	23.7
Hispanic	27.4	25.5	18.0	16.1	13.0
Chinese	47.9	24.0	10.2	11.3	6.7
Mean age (years)	60.5	61.2	61.8	62.4	63.9
Education (row %)					
< High school	33.41	24.24	16.48	14.19	11.68
High school	23.69	26.26	15.04	17.92	17.10
Some college	18.35	21.49	19.27	21.36	19.53
Bachelors	17.07	21.11	15.15	22.32	24.34
Graduate/professional degree	13.82	21.71	15.96	22.73	25.79
Behaviors					

Smoked within past 30 d (%)	17.24	14.33	12.32	9.93	8.52
Alcohol (g/d)	5.18	5.13	5.68	6.06	5.51
HRT (fem only) (% current use)	27	28	35	38	35
Leisure PA (MET min/wk)	2036	2435	2424	2658	2889
Sedentariness score (MET min/wk)	1581	1642	1699	1716	1764
Mean dietary intake					
Energy (kJ/d)	6657	6828	6816	7196	7874
Refined grain (svgs/d)	2.2	2.0	2.0	2.0	2.1
Fruit (svgs/d)	1.4	1.6	2.0	2.2	2.8
Vegetables (svgs/d)	1.8	2.0	2.3	2.6	3.0
Dairy (svgs/d)	1.5	1.7	1.9	2.0	2.1
Fish/poultry (svgs/d)	0.71	0.77	0.87	0.86	0.97
Meat (svgs/d)	0.59	0.57	0.58	0.59	0.53
Blood lipids and blood pressure					
HDL-cholesterol (mg/dl)	50.0	51.0	52.2	52.2	52.3
LDL-cholesterol (mg/dl)	117.6	119.9	118.8	116.4	116.3
Systolic BP (mmHg)	124.9	125.2	126.5	125.3	126.9
Diastolic BP (mmHg)	72.7	72.0	72.0	71.8	71.2

* All means and percentages were adjusted for sex, age, race, education, site and energy intake; except for age, sex, education and energy intake, each of which is adjusted for the relevant combination of these variables only; and race, for which crude values are presented.

Table 3. a. means and percentages of body mass index, insulin resistance, inflammation, diabetes, and atherosclerosis divided into quintiles and compared to the first quintile of whole grain intake in 5493 participants, MESA 2000-2002

	Model	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**P-trend**
Median intake		0.02	0.15	0.39	0.72	1.39	
N		1069	1137	1072	1121	1097	
BMI (kg/m^2)	1	28.2	28.2	28.0	27.7	27.4	<0.0001
	2	28.2	28.2	27.9	27.8	27.6	<0.0001
Insulin † (mU/l)	1	5.44	5.48	5.45	5.15	4.96	<0.0001
	2	5.37	5.42	5.42	5.19	5.16	0.002
HOMA (mU/l*mmol/l)	1	1.70	1.72	1.64	1.54	1.50	<0.0001
	2	1.68	1.70	1.63	1.55	1.53	0.002
CRP † (mg/l)	1	3.56	3.31	3.23	3.18	3.02	<0.0001
	2	3.48	3.26	3.20	3.22	3.12	0.004
	3	3.43	3.22	3.20	3.24	3.17	0.08
IL-6 (pg/ml)	1	1.59	1.50	1.46	1.48	1.45	0.03
	2	1.56	1.49	1.45	1.50	1.48	0.39
	3	1.54	1.47	1.45	1.51	1.51	0.92
Homocysteine (µmol/l)	1	9.68	9.34	9.35	9.01	8.77	<0.0001
	2	9.62	9.32	9.34	9.05	8.80	<0.0001
	3	9.62	9.32	9.34	9.05	8.82	<0.0001
Glucose (mg/dl)	1	99.3	99.0	97.3	98.4	96.9	0.001
	2	99.2	98.9	97.3	98.4	97.2	0.008
	3	99.0	98.7	97.2	98.5	97.6	0.08
IFG or newly diagnosed diabetes	1	38.4	35.4	33.2	34.1	32.1	0.004
	2	38.2	35.1	33.2	34.2	32.5	0.02
	3	37.7	34.4	32.7	34.7	33.8	0.23
Newly diagnosed diabetes	1	4.00	4.70	3.50	3.70	3.60	0.16
	2	3.90	4.70	3.50	3.70	3.80	0.33
	3	3.80	4.50	3.40	3.70	4.10	0.63
Urine albumin excretion † (mg/g)	1	7.30	7.59	6.71	7.00	6.72	0.03
	2	7.22	7.55	6.69	7.04	6.82	0.20
	3	7.19	7.51	6.66	7.07	6.88	0.38
% A/kC >25	1	10.7	12.5	9.20	10.2	8.90	0.05

	2	10.4	12.3	9.10	10.4	9.40	0.28
	3	10.3	12.2	9.00	10.6	9.50	0.39
Common carotid IMT † (mm)	1	0.85	0.86	0.85	0.85	0.85	0.26
	2	0.85	0.86	0.85	0.85	0.85	0.48
	3	0.85	0.86	0.85	0.85	0.85	0.98
Internal carotid IMT † (mm)	1	0.99	0.99	0.98	0.97	0.98	0.49
	2	0.98	0.99	0.98	0.97	1.00	0.59
	3	0.98	0.99	0.98	0.97	1.00	0.42
% with plaque †	1	42.2	40.9	39.2	36.9	39.4	0.21
	2	41.1	40.5	38.9	37.5	40.5	0.97
	3	41.1	40.3	38.8	37.8	40.6	0.84
% Agatston >0 †	1	48.9	48.6	48.6	49.8	46.6	0.05
	2	48.4	48.4	48.6	50.0	47.2	0.15
	3	48.2	48.2	48.5	50.2	47.6	0.33

Model 1 (base model): age, sex, race, education, survey center and energy intake.
Model 2 (behavioral model): model 1 plus current smoking, current alcohol use and dietary intake of fruit, vegetables, refined grains, dairy, fish and poultry, meat, leisure physical activity, and sedentariness score.
Model 3 (mechanistic model): model 2 plus BMI and insulin.
† Geometric mean

Table 3. b. Odds ratios of obesity, insulin resistance, inflammation, diabetes, and atherosclerosis divided into quintiles and compared to the first quintile of whole grain intake in 5493 participants, MESA 2000-2002, revised*

	Q1	**Q2**	P-value	**Q3**	P-value	**Q4**	P-value	**Q5**	P-value
Median intake	0.04	0.32		0.645		1.03		1.79	
Range	0-.145	.15-.5		.505-.83		.83-1.29		1.295-5.435	
N	1137	1249		917		1098		1092	
Obese (BMI >30)	1	0.98 (0.79-1.22)	0.867	0.99 (0.78-1.25)	0.923	0.94 (0.75-1.18)	0.593	0.94 (0.75-1.19)	0.626
Insulin>25 mU/l	1	1.29 (0.75-2.19)	0.355	0.89 (0.48-1.67)	0.723	1.04 (0.58-1.86)	0.895	0.89 (0.47-1.67)	0.707
HOMA	1	0.94 (0.78-1.14)	0.544	0.99 (0.81-1.23)	0.961	0.87 (0.71-1.06)	0.165	0.72 (0.58-0.89)	0.003
CRP	1	0.98 (0.80-1.20)	0.851	0.87 (0.71-1.08)	0.215	0.85 (0.69-1.04)	0.114	0.87 (0.70-1.08)	0.213
IL-6	1	0.88 (0.72-1.07)	0.187	0.95 (0.77-1.18)	0.666	0.84 (0.68-1.03)	0.098	0.96 (0.77-1.19)	0.712
Homocysteine	1	0.83 (0.68-1.01)	0.066	0.81 (0.65-1.01)	0.061	0.70 (0.56-0.86)	0.001	0.61 (0.49-0.76)	0.000
IFG or new DM	1	0.77 (0.61-0.98)	0.034	0.85 (0.66-1.10)	0.211	0.62 (0.48-0.81)	0.000	0.70 (0.53-0.91)	0.008
New DM dx	1	1.12 (0.64-1.95)	0.683	0.90 (0.48-1.68)	0.745	0.49 (0.24-1.00)	0.051	0.71 (0.36-1.39)	0.316
Urine albumin	1	0.89 (0.78-1.09)	0.253	0.99 (0.80-1.22)	0.893	0.87 (0.71-1.07)	0.196	0.82 (0.66-1.01)	0.065
A/kC >25	1	0.91 (0.64-1.30)	0.596	0.90 (0.61-1.32)	0.573	0.75 (0.51-1.10)	0.137	0.74 (0.50-1.10)	0.139
Common carotid IMT	1	0.89 (0.72-1.09)	0.269	1.00 (0.80-1.25)	0.673	0.95 (0.80-1.18)	0.673	1.05 (0.84-1.32)	0.679
Internal carotid IMT	1	0.97 (0.79-1.19)	0.769	1.03 (0.83-1.28)	0.766	1.03 (0.84-1.27)	0.751	1.15 (0.93-1.43)	0.207
% with plaque	1	0.90 (0.73-1.11)	0.322	1.06 (0.85-1.32)	0.617	0.91 (0.74-1.14)	0.418	1.15 (0.92-1.43)	0.236
Agatston >0	1	0.99 (0.80-1.24)	0.963	1.05 (0.83-1.33)	0.659	1.04 (0.83-1.30)	0.757	0.98 (0.77-1.24)	0.853