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The Impact of Fiber Supplementation on ADMA Levels

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Abstract

BACKGROUND—Asymmetric dimethyl arginine (ADMA) is an emerging biomarker that has been associated with oxidative metabolism and increased cardiovascular risk. Little information is available regarding the effect of diet on ADMA.

METHODS—We studied 86 adults with overweight/obesity as part of a clinical trial of psyllium supplementation to determine whether 3 months of such supplementation would affect ADMA levels. There were 41 people in the intervention group who received 14 g/day of psyllium in addition to their usual diet compared to 45 controls who followed their usual diet alone. The two groups were comparable at baseline in demographic characteristics and BMI.

RESULTS—Baseline ADMA levels were elevated in this overweight/obese population compared to published reference values in healthy persons (.75umol/L vs. .50umol/L). The change in ADMA levels over 3 months was not different in the psyllium group compared to the control group (−.03umol/L vs. −.01umol/L, $p=.73$).

CONCLUSIONS—These findings do not support a significant effect of psyllium fiber supplementation on ADMA.

Asymmetric dimethylarginine (ADMA) is a naturally occurring oxidative substance of the human plasma. It is a metabolic byproduct of L-arginine metabolism that inhibits the formation of nitric oxide (NO), one of the body's important anti-oxidants. This interference with the formation of NO has significant ramifications, because NO has numerous beneficial effects, including stimulating arterial relaxation and dilation, reducing platelet aggregation and macrophage adherence, maintaining blood vessel elasticity and slowing or reversing atherosclerotic plaque growth. (1) Recent research suggests that obese middle-aged and older individuals have increased levels of ADMA, which contributes to oxidative stress in these individuals (2,3). Elevated ADMA levels increase the risk of developing hypertension and diabetes (2–4). Higher levels of ADMA have also recently been implicated as independent predictors of cardiovascular events (5–9).

Diet plays an important role in oxidative stress (2,10,11,12,13) However, the role of diet in the formation and metabolism of ADMA is less well understood. Ingestion of glucose and fatty acids results in a transient increase in free radicals that acutely triggers oxidative stress, depletion of NO, and endothelial dysfunction, processes that are intimately linked with ADMA metabolism (10,11), while increased dietary intake of fiber is associated with lower oxidative stress (12). Dietary consumption of L-arginine, the metabolic competitor of ADMA, is associated with inflammation (13). Thus, a variety of dietary components appear to influence the oxidative system of which ADMA is a part. Further exploratory analyses are warranted to

investigate key dietary components and determine whether they can play a role in reducing harmful ADMA levels (14–16). Because dietary fiber has a demonstrated impact on oxidative stress, it appears reasonable to investigate the possibility that fiber intake may influence ADMA levels (17).

The purpose of this study was to explore whether daily psyllium fiber supplementation could reduce ADMA levels. This study takes advantage of an opportunity to investigate this question as part of an ongoing trial of psyllium fiber supplementation to reduce markers of inflammation (15,18).

METHODS

This was an exploratory secondary analysis of data from a parent study to evaluate the effect of fiber supplementation on C-reactive protein (18). The study was a randomized controlled trial of fiber supplementation (14g/day of fiber, Metamucil-brand psyllium [Proctor and Gamble, Cincinnati OH]) compared to “no supplement” (usual diet) in 162 overweight/obese adult volunteers age 40–65. The dose of Metamucil was 4 tbsp in water per day; each tbsp contains 3.5 g of fiber, so the total daily supplement dose of 4tbsp contained 14g of fiber. The study was approved by the Institutional Review Board at the Medical University of South Carolina (MUSC) and registered at clinicaltrials.gov (NCT00085800). The study was performed at the General Clinical Research Center (GCRC) at MUSC and approved by the GCRC Advisory Committee. An external data safety monitoring board reviewed progress regularly throughout the trial. The trial was designed to have 80% power to detect standardized mean differences in ADMA between the fiber supplementation and control groups of approximately 0.1mg/l using 43 subjects per group.

Participants were eligible if they were 40–64 years of age, gave informed consent, had a BMI>25kg/m², had stable weight for the last 3 months (no change over $\pm$ 5%), and had no change in medication in the previous 90 days. In addition, participants needed to have a CRP level > 3.0mg/l for women and >2.0mg/l for men, due to the published gender differences in CRP levels (median CRP = 3.3mg/l in women vs. 1.8 mg/l in men; $p < 0.001$) (19,20,21). The primary study focused on people with a BMI>25kg/m² because of the known large impact of BMI on cardiovascular risk, potentially as a result of an inflammatory state (22). Patients were excluded for pregnancy, regular use of a fiber supplement of over 4g per day, a history of known cardiovascular disease (including myocardial infarction, stroke, congestive heart failure, or history of re-vascularization procedure [bypass, angioplasty, or stent placement], uncontrolled hypertension (>170/95), EKG evidence of ischemia or infarction, recent (within 90 days) or planned participation in a formal weight loss program, a history of inflammatory disease including collagen vascular disease, inflammatory arthritis, recent serious infection, or other inflammatory condition. Patients were permitted to continue taking current medications including aspirin, HMG-CoReductase inhibitors, or thiazoladinediones, but were allowed no new medication within 90 days and no change in dosage for the last 30 days.

After a screening visit at the GCRC to determine eligibility, patients were randomized using an online randomization program kept confidential from the primary investigators, and assigned to either the Fiber Group (14g per day), or Control (no psyllium). There were no baseline differences in fiber intake between the two groups. After a baseline laboratory evaluation, history and physical examination, the patients were scheduled to return to the GCRC in 1 month, and again in 3 months for follow up. ADMA levels were obtained at baseline and at the final 3-month follow up visit. Compliance was monitored by self-report and packet counts of unconsumed supplement at each visit. Further details of the methods have been previously published (18)

ADMA determination

ADMA levels were determined on the frozen blood samples in the protocol above, on baseline and 3 month samples in the 14g/day (High Fiber Intervention group) and the 0g/day Control group. Recently a novel, easy to use ADMA enzyme immunoassay has been developed and validated (23). This assay allows any clinical laboratory in which a standard ELISA plate reader is available to measure ADMA in human plasma and serum. With the help of this assay a large series of samples can now be measured in relatively short periods of time.. The ADMA®-ELISA has been validated by comparing it with state-of-the-art liquid-chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) techniques with excellent reproducibility.. Cross-reactivities with other L-arginine analogues present in plasma and serum has been found to be negligible (L-N-mono-methyl arginine [L-NMMA], 1.0%; symmetrical dimethyl arginine [SDMA] 1.2%; L-arginine <0.02%). The ELISA test has a linear range between 0.1 and 3 µmol/l in human serum and plasma and thus covers the whole range of physiological and pathophysiological concentrations. ADMA levels will be determined by ELISA (24,25,26), testing conducted in the MUSC GCRC Core Laboratory.

Data Analysis

The primary endpoint was focused on changes in the ADMA level over 3 months in the high fiber group compared to the changes in the control group. The primary comparison was between the mean changes from baseline to 3 months in the fiber supplementation group compared to the control group at month 3 using a two-sample t-test to allow us to take into account any variation in baseline values that might occur.

There were 43 participants in the Fiber group and 57 in the Control group. Power analysis reveals that we will have >86% power to detect a difference of 15% in the change in ADMA levels from baseline to 3 months, based on a one-sided $\alpha=.05$. These ADMA levels are based on a recent paper in patients with metabolic syndrome compared to healthy controls (26). Compliance was determined by fiber packet counts and supplemented with patient history; all participants in this aspect of the study had consumed greater than 75% of the prescribed fiber supplement and attended the baseline and 3-month follow up visits.

RESULTS

The trial profile and selection of participants are outlined in an earlier paper (18). In brief, there were 420 obese/overweight adults screened for elevated C-reactive protein (CRP), and 171 met entry criteria. Nine participants dropped out after the screening and before the baseline visit, leaving 162 who began the study. There were 132 people who completed the protocol, which was pre-defined as completing the final visit and at least 75% of the intervention supplement. Of these, 46 were in a low fiber supplemented group and did not contribute to the analysis of this current cohort. Forty-one people finished the trial in the high fiber (14g/day) intervention group, and 45 people finished the study in the control group.

The two study groups (fiber supplemented: control) were balanced for demographic and clinical characteristics including age, gender, race, smoking status, and body mass index (BMI) (Table 1). There were also no significant differences in baseline laboratory values except for a small difference in ADMA baseline levels (.70 vs. .80 µmol/L, $p<.05$); fibrinogen, white blood cell count, and CRP were similar in the two treatment groups ($p>0.05$). There were no significant adverse events attributed to treatment in any of the intervention groups.

Baseline ADMA levels were elevated in this overweight/obese population compared to published reference values in healthy persons (.75 µmol/L vs. .50 µmol/L)(20). The change in ADMA levels over 3 months were not different in the psyllium supplementation group

compared to the control group ($-.03\mu\text{mol/L}$ vs. $-.01\mu\text{mol/L}$, $p=.73$). There were no differences in subgroups comparing men with women or white to non-white participants.

DISCUSSION

The results of this study do not suggest a significant impact of fiber supplementation on ADMA levels in middle-aged overweight/obese adults. The impact of psyllium fiber on ADMA did not appear to vary by age, race, or gender in this study.

The results compare to another dietary intervention study that demonstrated no change in ADMA levels after omega-3 fatty acid supplementation (27). In that study, ADMA levels were unchanged after 3 years of supplementation, despite good measured compliance. In the current study, which included only individuals with a pre-defined level of reasonable compliance (consumption of $>75\%$ of the prescribed supplement), the results were similar in causing no change in ADMA levels.

These results are disappointing to those looking for an easy dietary manipulation to reduce ADMA levels, considering the increased cardiovascular risk associated with elevated ADMA levels. In a three-year prospective clinical study ADMA plasma levels in 225 renal patients (28), 120 major cardiovascular events (fatal and non-fatal) and 83 deaths (53 deaths from cardiovascular causes) were detected. In a multivariate Cox regression analysis, only ADMA and age emerged as significant, independent predictors of the incidence of major adverse cardiovascular events and all-cause mortality. Patients whose initial ADMA plasma concentration had been above the 75th percentile had a 3-fold greater risk of experiencing a major adverse cardiovascular event as compared to patients who's ADMA had initially been below the median. More recent research from the CARDIAC study has firmly established ADMA as an independent risk factor for coronary heart disease (5). In this multi-center case-control study of 131 cases and 131 matched controls, the odds ratio for cardiovascular events was 6.04 (2.56–14.25; $P < .001$) for the third tertile (>0.72 micromol/L) versus the first (<0.58 micromol/L), after controlling for other possible confounders.

There are several limitations to this study which need to be mentioned. First, the study was not blinded for the participants, thus some bias may have been introduced due to the lack of double-blinding. Further, only one type of fiber supplement (psyllium) was tested; it was used in this exploratory study because it had already been chosen in the parent study, due to its easy dosing and availability; another type of fiber might yield different results. Next, the length of the study was only three months. It may be that the impact of fiber supplementation requires longer than three months to have a substantial effect on ADMA. Finally, while the study was designed to have over 86% power to detect a 15% change in ADMA, the differences between groups did not meet this criterion, and the study may have been under-powered. However, there was no trend or slope seen in the current study to suggest that increasing sample size would substantially change the outcome. Further, other methods of analysis, such as ANCOVA, accounting for baseline ADMA levels, also could be used, but are unlikely to have altered the negative results of the study.

In conclusion, the current study did not find a significant impact of psyllium fiber supplementation on ADMA levels. This does not negate the epidemiologic evidence that dietary fiber is a factor in reducing inflammation and oxidative stress, but does suggest that ADMA may not be a key modulator in the process. However, further research with other types of fiber or with combinations of nutrients is warranted to investigate other ways in which diet may impact ADMA.

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Table 1

Initial demographic characteristics of the randomized patient population (N=86) by Fiber Group.

	High Fiber	Control	p
N	41	45	
Gender			0.98 [†]
Male	21.9%	22.2%	
Female	78.1%	77.8%	
Race			0.07 [†]
Black	39.0%	46.7%	
White	58.5%	53.3%	
Other	2.4%	0%	
Smoker			0.86 [†]
Never	65.9%	66.7%	
Former	17.1%	20.0%	
Current	17.1%	13.3%	
Diabetic	14.6%	11.1%	0.63 [†]
Hypertensive	29.3%	37.8%	0.40 [†]
Age Mean (SD) (years)	50.9 (6.4)	48.6 (5.8)	0.08 [*]
BMI Mean (SD) (kg/m ²)	33.8 (6.2)	33.9 (6.9)	0.94 [*]
Dietary Fiber Mean (SD) (g/day)	14.4 (4.4)	14.2 (4.7)	0.77 [*]
ADMA (SD) umol/L	.70 (.35)	.80(.35)	0.04 [*]
Fibrinogen Mean (SD) (mg/dl)	370.6 (129.3)	363.7 (80.3)	0.77 [*]
WBC Mean (SD) (K/mm ³)	6.34 (2.1)	6.28 (1.9)	0.89 [*]
CRP Mean (SD) (mg/l)	7.48 (5.5)	8.29 (9.3)	0.62 [*]

[†] χ^2 analysis of the distribution^{*} T-TEST