Michael Garshick, MD
PGY-1
Columbia University Medical Center

Effects of a dietary intervention to reduce saturated fat on markers of inflammation and cardiovascular disease.

Study Purpose and Rationale:

Current cardiovascular risk factors and primary prevention interventions do not account for nor treat all of the changes seen in atherosclerotic disease. [1-3] Epidemiologic studies have consistently noticed a correlation between the risk of cardiovascular disease and increases in certain biomarkers.[4] Increasingly, markers of inflammation and cardiac biomarkers are being used in models to predict risk of cardiovascular disease. [5, 6] Decreasing these markers of inflammation should lead to a decreased risk of morbidity and mortality.

C-RP is one such well studied inflammatory biomarker.[6] In the Jupiter Trial patients with an elevated C-RP and an LDL < 130 were given statin therapy to lower their C-RP and had a lower morbidity. [7] However, these findings have recently been called into question as the lead author had a significant conflict of interest and another large rigorous study which attempted to tease out if C-RP is causally associated with heart disease showed no causal relationship[1]. In light of this, new markers of cardiac disease are needed to help not only predict but also intervene upon to reduce the risk of coronary artery disease. Currently few if any have been accepted as adequate.[8] Over the past few years, other markers of inflammation and of future cardiac risk have emerged such as lipid particle size, lipid particle number, and Lp-PLA_2 which all have been shown to be positively associated with coronary artery disease. [9-12] .

Lp-PLA_2 is an inflammatory biomarker that binds to apolipoprotein B-100, circulates with LDL cholesterol and is believed to promote atherogenesis by increasing inflammation in the arterial intima.[13] In addition Lp-PLA_2 activity is thought to be up-regulated in atherosclerotic lesions. Experimental models have shown inhibition of Lp-PLA_2 can reduce atherosclerotic burden.[14] In one study analyzing 1000 patients who had CAD and eventually experienced another cardiac event, those with Lp-LPA_2 activity and mass in the middle and upper tertiles were two times more likely to have repeat events when controlled for other variables as compared with those in the lower tertile.[14] With regards to LDL particle size and particle number, it has been noted that these two variables correlate more closely than LDL-C in predicting atherosclerotic burden [15, 16].

Given these new markers of inflammation and their significant strong association with cardiovascular disease burden, interventions that reduce the markers of inflammation should also theoretically reduce the risk of disease burden. There are studies that have noted that a reduction in dietary carbohydrates and fat can result in reduction of lipid particle number, size and Lp-PLA_2. However, the majority of these trials have been done in an extremely controlled environment, are very small and methodically flawed. [13, 17, 18]

In a significant pubmed search, only 2 studies have looked at the dietary changes to affect Lp-PLA_2.[13, 18]. In the most notable review of Lp-PLA_2 and dietary and lifestyle predictors, a large retrospective cohort of patients that eventually went on to have documented CAD from the Nurses' Health Study and HPFS (Health Professionals Follow-Up Study) was looked at for trends in Lp-PLA_2. This study found that weight and smoking were associated with a rise in Lp-PLA_2. It also found that switching protein for carbohydrates led to a decrease in Lp-PLA_2. However, a nutrient density model was done to asses for dietary changes rather than a direct assessment and this was a retrospective case control trial rather than a controlled intervention. In the few trials that have looked at the effects of a dietary intervention on reducing lipoprotein particle size and mass, the overall results have been inconclusive.[17, 19, 20] In the largest study comparing diets, a reduction in LDL particle size and number was noted; however there was no difference when comparing the low carbohydrate and low fat diet.[20] Based on this previous information and a current lack of larger good quality trials, more information and trial data is needed, especially in a "real world setting" on the best way to reduce lipid sub-particles and Lp-PLA_2. The study that is being conducted will attempt to discover if a therapeutic life style change based out of a busy tertiary care center can reduce lipid particle number, lipid particle size and Lp-PLA_2 thereby reducing the risk of cardiovascular disease

The FIT Heart Trial is a randomized controlled trial of a hospital based dietary and lifestyle intervention on relatives or close associates of patients with heart disease.[21] In this trial patients were randomly assigned to either therapeutic lifestyle-change counseling sessions vs. a control intervention. The primary outcome, LDL-C was decreased across both arms but did not differ between the two. Both groups had a significant and equal reduction in saturated fat. Other cardiovascular markers of inflammation were collected but not analyzed during the initial reporting of the study.

Hypothesis:

Our hypothesis is that patients enrolled in both arms of the FIT Heart trial who have had a reduction in saturated fat intake during a 1 year period will have a reduction in Lp-PLA_2, lipid particle size, and lipid particle number.

Methods:

Outcomes:

- % change in dietary components over 1 year (Protein, carbohydrates, mufa, pufa, saturated fat, omega-3s) was measured using the 1998 Gladys Block Food Frequency Questionnaire, a 110-item validated food-frequency questionnaire and validated compliance using the MEDIFI& CTS (Meats, Eggs, Dairy, Fried foods, fat in baked goods, convenience foods, fats added at the table, and snacks) questionnaire

-Anthropometric Measurements (height, weight, waist circumference) all continuous variables.

-Vital signs (blood pressure, pulse) all continuous measurements

-Physical activity level was assessed by METS and smoking status was assessed both using the Behavioral Risk Factor Surveillance system questionnaire was assessed and smoking validated using carbon monoxide monitoring.
-Laboratory Procedures: Venous fasting blood levels measured at baseline and then at 1 year. If the baseline lipids were abnormal, they were measured at 3, 6, and 9 months. Plasma total cholesterol, HDL-C, triglyceride and glucose values analyzed spectrophotometrically on a Hitachi 912 chemical analyzer. Plasma LDL-C values assessed w/ direct homogeneous enzymatic colorimetric assay. LDL-C levels were determined on study subjects with triglycerides < 400mg/dl. Lipid subparticles, Lp-PLA_2 and C-RP were measured using LDX desktop analyzers supplied by Cholestech Corporation (although not analyzed in this study).

Study Design:
The FIT Heart Trial is a randomized placebo controlled trial and has already been reported and methods described in previous publications [21]. In this trial patients were randomly assigned to a therapeutic lifestyle change group or a controlled intervention group and followed for one year.
The TLC group received personalized dietary counseling which included a risk assessment, teaching of lifestyle approaches, dietary education, encouragement and teaching on physical activity and smoking cessation counseling. Health educators contacted and reinforced their interventions at time 0, and then four more times throughout the study. Validated TLC (therapeutic lifestyle change) questionnaires were handed out to assess study compliance at the 6 week and 6 month mark. Lipids were measured at the 3, 6 and 9 month mark and feedback was offered on these lipids. The control group received a one page handout asked to avoid tobacco, choose good nutrition and be more active and filled out dietary and physical activity questionnaires at time 0 and at the 12 month mark. Data collection was done by a trained health educator who administered the validated dietary assessment questionnaires and compliance questionnaires as described above. Labs were stored and analyzed by the Columbia University Clinical and Translational Science Award Biomarker Laboratory with the techniques detailed above. Dietary, physical activity and smoking were assessed as noted above in outcomes section. Vitals and anthropometric data was collected by trained research assistants at the same time labs were drawn. If blood pressure was > 140/90, LDL-C > 190, HDL < 25, Triglycerides > 500 or total Cholesterol > 300 a report was sent to the patient's PCP and a decision was left up to the patient and PCP if they would start a pharmacological therapy.

Statistical Analysis:
The current analysis will be done on an intent-to-treat basis using the last known lab values for patients in the study.
We will use correlation coefficients to assess the percent saturated fat change after 1 year and the percent change in the above stated biomarkers of inflammation and cardiovascular burden.
In the initial analysis, it was found that physical activity was significantly higher in the intervention group than in the control, we will therefore compare between groups the mean percent change in biomarkers after 1 year using a t test.
We will adjust for all covariates in both primary and secondary outcomes using a multiple linear regression model.

All data will be analyzed in STATA.

Sample Size:
The study has previously enrolled 501 participants and 94% of them have completed the study. Using intent to treat analysis, the study will have 80% power to detect a significant ($p < 0.05$) r value of greater than 0.12 correlation. In other words, our study will have 80% power to detect if 1.4% (r^2) or greater of the variation is explained by the measured variables.
Physical activity difference between the control vs. intervention we will have an 80% power to detect a difference of at least 5.5% with regards to Lp-PLA_2, LDL particle number difference of at least 7%, and LDL mean size of at least 3.5%*
**Of note, standard deviations and means for these powers were derived from previous studies of Lp-PLA_2, LDL particle number and LDL mean size. The changes in lab values were divided by their means and multiplied by 100 to get the percent changes.*

Study Questionnaires:
1998 Gladys Block Food Frequency Questionnaire
MEDFICTS (Meats, Eggs, Dairy, Fried foods, fat In baked goods) Questionnaire.
Questions adapted from the Behavioral Risk Factor Surveillance System Questionnaire.

Subject Selection:
Participants were approached if they were a close relation, (defined as spouse, blood relative, individual living with patient for > 5 years) to the patient that had a cardiac presentation secondary to atherosclerotic disease even if they required a cardiac intervention, PCI or CABG. Inclusion criteria included ages 20 to 79 years, male or female of any ethnicity, live less than 3 hours away from medical center. Exclusion criteria was current or planned pregnancy, established CVD, DM, liver disease, CKD, life expectancy < 5 years or recent enrollment, < 3 months, in a study involving novel drugs or TLC groups.

Miscellaneous:

Study Drugs:
Not applicable

Medical Device:
Not applicable

Study Questionnaires:
1998 Gladys Block Food Frequency Questionnaire
MEDFICTS (Meats, Eggs, Dairy, Fried foods, fat In baked goods) Questionnaire.
Questions adapted from the Behavioral Risk Factor Surveillance System Questionnaire.

Recruitment of Subjects:
Subjects will be recruited in the family waiting rooms of patients that have recently undergone a PCI or CABG or had an acute cardiac atherosclerotic event.

Interpreters for Spanish will be on hand to help communicate with potential recruits.

Confidentiality of Study Data:
Recruits involved in this study will be assigned a random ID and their names not included in the datasets. All computers and databases are password protected.

Location of the Study:
Columbia University Medical Center

Potential Benefits:
The Benefits of this randomized controlled trial include increased recruitment knowledge about the benefits of diet and exercise. Recruits may quit smoking, and gain further knowledge about their lipid profiles and blood pressure if they had not seen a doctor previously.

Potential Risks:
The risks from this study are very low and minimal; they do include the risks from increased anxiety about previous poor lifestyle choices, and any undue acute cardiovascular effects from exercise in patients who were not previously physically active.

Ethical Considerations:
Given that patient's PCP will notified of significant abnormal anthropometric or laboratory values, there is little to no unethical considerations that are involved in this trial.
Patients will be compensated 100 dollars at the end of the study for their time and effort.

Alternative Therapies:
Not applicable

Compensation to Recruits:
100 dollars be given at the end of participation

Costs to Subjects:
Misc transportation costs to and from CUMC

Minors as Research Subjects:
Not applicable

Radiation or Radioactive Substances:
Not applicable

References:

1. Elliott, P., et al., *Genetic Loci associated with C-reactive protein levels and risk of coronary heart disease.* JAMA, 2009. **302**(1): p. 37-48.
2. Ridker, P.M., et al., *Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men.* N Engl J Med, 1997. **336**(14): p. 973-9.
3. Peyser, P.A., et al., *Heritability of coronary artery calcium quantity measured by electron beam computed tomography in asymptomatic adults.* Circulation, 2002. **106**(3): p. 304-8.
4. Pearson, T.A., et al., *Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association.* Circulation, 2003. **107**(3): p. 499-511.
5. Danesh, J., et al., *Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses.* BMJ, 2000. **321**(7255): p. 199-204.
6. Koenig, W., et al., *C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992.* Circulation, 1999. **99**(2): p. 237-42.
7. Ridker, P.M., et al., *Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein.* N Engl J Med, 2008. **359**(21): p. 2195-207.
8. Helfand, M., et al., *Emerging risk factors for coronary heart disease: a summary of systematic reviews conducted for the U.S. Preventive Services Task Force.* Ann Intern Med, 2009. **151**(7): p. 496-507.
9. Garza, C.A., et al., *Association between lipoprotein-associated phospholipase A2 and cardiovascular disease: a systematic review.* Mayo Clin Proc, 2007. **82**(2): p. 159-65.
10. Cromwell, W.C. and J.D. Otvos, *Low-density lipoprotein particle number and risk for cardiovascular disease.* Curr Atheroscler Rep, 2004. **6**(5): p. 381-7.
11. Lamarche, B., et al., *Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study.* Circulation, 1997. **95**(1): p. 69-75.
12. Mora, S., et al., *LDL particle subclasses, LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA).* Atherosclerosis, 2007. **192**(1): p. 211-7.
13. Hatoum, I.J., et al., *Dietary, lifestyle, and clinical predictors of lipoprotein-associated phospholipase A2 activity in individuals without coronary artery disease.* Am J Clin Nutr, 2010. **91**(3): p. 786-93.
14. Koenig, W., et al., *Lipoprotein-associated phospholipase A2 predicts future cardiovascular events in patients with coronary heart disease independently of traditional risk factors, markers of inflammation, renal function, and hemodynamic stress.* Arterioscler Thromb Vasc Biol, 2006. **26**(7): p. 1586-93.
15. Rajman, I., et al., *Particle size: the key to the atherogenic lipoprotein?* QJM, 1994. **87**(12): p. 709-20.
16. Rosenson, R.S., J.D. Otvos, and D.S. Freedman, *Relations of lipoprotein subclass levels and low-density lipoprotein size to progression of coronary artery disease in the Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-I) trial.* Am J Cardiol, 2002. **90**(2): p. 89-94.
17. Seshadri, P., et al., *A randomized study comparing the effects of a low-carbohydrate diet and a conventional diet on lipoprotein subfractions and C-reactive protein levels in patients with severe obesity.* Am J Med, 2004. **117**(6): p. 398-405.
18. Tzotzas, T., et al., *Effects of a low-calorie diet associated with weight loss on lipoprotein-associated phospholipase A2 (Lp-PLA2) activity in healthy obese women.* Nutr Metab Cardiovasc Dis, 2008. **18**(7): p. 477-82.

19. LeCheminant, J.D., et al., *Comparison of a reduced carbohydrate and reduced fat diet for LDL, HDL, and VLDL subclasses during 9-months of weight maintenance subsequent to weight loss.* Lipids Health Dis, 2010. **9**: p. 54.
20. Westman, E.C., et al., *Effect of a low-carbohydrate, ketogenic diet program compared to a low-fat diet on fasting lipoprotein subclasses.* Int J Cardiol, 2006. **110**(2): p. 212-6.
21. Mosca, L., et al., *A novel family-based intervention trial to improve heart health: FIT Heart: results of a randomized controlled trial.* Circ Cardiovasc Qual Outcomes, 2008. **1**(2): p. 98-106.