Dietary Intake Of Flavonoids And HDL- And LDL-Cholesterol In Two Black Ethnicities With And Without Type 2 Diabetes

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Abstract
Flavonoids are a class of over 6,500 plant metabolites that have been associated with reduced mortality from cardiovascular disease. A cross-sectional analysis of dietary flavonoids and serum cholesterol in 507 Blacks with and without type 2 diabetes (258 Haitian-Americans and 249 African-Americans) showed differences by ethnicity and diabetes status. Haitian-Americans consumed more of most flavonoids as compared to African-Americans. Individuals with type 2 diabetes consumed less of most flavonoids as compared to those without diabetes. Flavonoids were differentially associated with low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL) by diabetes status. Flavanones were associated with lower LDL for participants without diabetes and higher LDL for those with diabetes, independent of ethnicity and adjusted for age, gender, cholesterol medications, daily energy, dietary fat, body mass index (BMI), and smoking. Flavan-3-ols were positively related to LDL while polyflavonoids (theaflavin and polymers, proanthocyanidins) were inversely related to LDL for the group without diabetes only. Higher anthocyanidins and flavan-3-ols and lower polyflavonoids were associated with higher HDL (same adjustments) for those without diabetes, whereas no flavonoids were associated with HDL for individuals with type 2 diabetes.

INTRODUCTION
Flavonoids (bioflavonoids) are a diverse group of more than 6500 polyphenols (phenyl benzopyrans) which function as phytochemicals. Flavonoids are secondary plant metabolites found primarily in fruits, vegetables and seeds and are generally classified into categories based on their chemical structure. There is a lack of consensus for some of the flavonoid-subgroups. This may be due to the fact that subclasses of flavonoids are differentiated on the basis of the number and nature of substituent groups attached to the rings. Isoflavones have been considered a separate category and the distinction of polyflavonoid is not always made. Classification by the degree of unsaturation and degree of oxidation of the three carbon segment results in the following major classes: flavones, isoflavones, flavonols, anthocyanidins (or anthocyanins), flavanones, flavan-3-ols, chalcones and aurones.

It has been demonstrated throughout the literature that diets high in plant-based foods are protective against the development of cardiovascular disease, diabetes and cancer and that flavonoids have been thought to contribute to the protective role. Flavonoids incorporated in the diet exhibit several biological effects, such as anti-oxidant and anti-inflammatory properties. Flavan-3-ols and proanthocyanidins have been associated with reduction of risk for cardiovascular disease by increasing the release of endothelial nitric oxide and inducing vasodilatation. The antioxidant property of dietary flavonoids may be of particular benefit to persons with diabetes since their hyperglycemic condition depletes natural anti-oxidants and may result in oxidative stress.

Dietary flavonoids (flavonols and flavones) were inversely related to mortality from coronary heart disease for a five-year cohort of N=805 men aged 65-84 years. Dietary anthocyanidins and flavanones decreased coronary heart disease deaths, but not stroke, in post-menopausal women (N=34,492). Reduction of cardiovascular risk and mortality by dietary flavonoids may be attributed, in part, to the oxidation and serum levels of LDL. Although there is evidence that certain flavonoids improve cholesterol in numerous animal models, the pathways by which the categories of flavonoids may help prevent or improve lipid profile in humans is unclear. Based on animal studies, flavanones may have biochemical effects that result in
hypolipidemic properties such as inhibiting cholesterol acyltransferase activities in the liver and upregulating LDL receptors. A proposed mechanism of dietary flavonoids resulting in lower serum LDL is their antioxidant capacity with respect to copper-triggered LDL. Flavan-3-ol, epigallocatechin gallate (EGCG) and flavanone hesperetin reduced oxidized LDL activity in human endothelial cells by eliminating lipid peroxidase products. Several intervention studies in healthy adults and hypercholesterolemic adults indicated flavanones from citrus, hesperidin and naringin were associated with lowering of LDL. Albeit, a randomized placebo-controlled trial of these flavonoids in high concentrations in hypercholesterolemic adults (N=204) found no significant change of LDL, triglycerides or HDL. Anthocyanin supplementation improved LDL and HDL while decreasing the mass and activity of plasma cholesteryl ester transfer protein (CEPT) in a randomized clinical trial of hypercholesterolemic adults (N=60). To date, human studies of flavonoids and LDL have been controversial and those with HDL are scarce.

Beneficial health effects of flavonoids in foods have the potential to reduce health disparities by use of pharmaceutical products (of extracted flavonoids) or through dietary interventions aimed at increasing fruit and vegetable consumption among minorities. Blacks consumed less total flavonoids, as classified by the USDA flavonoid database, than White non-Hispanics based on a study of U.S. adults. This study aimed to compare flavonoid type with serum LDL and HDL in two ethnicities of Blacks: Haitian- and African-American with and without type 2 diabetes. We expect higher intake of certain flavonoids will collectively be associated with lower LDL and higher HDL independent of ethnicity and diabetes status.

MATERIALS AND METHODS

SUBJECTS

ASSESSMENT OF DIETARY INTAKE AND TOTAL USDA FLAVONIODS

Dietary intake was measured using the Harvard semi-quantitative FFQ developed by Walter C. Willett and has been extensively validated in diverse populations. On this FFQ, participants self-reported average consumption of specified amounts of various foods over the past year, choosing from frequency responses ranging from ‘never’ to ‘six or more servings per day’. Daily servings of food groups were calculated by summing the frequency factors (which corresponded to the reported consumption frequency) for all related food items. Variables were created for the total 2007 USDA classification of flavonols, flavones, flavanones, flavan-3-ols, anthocyanidins, polyflavonoids (total theaflavin and polymers proanthocyanidins), flavonoids with no proanthocyanidins and flavonoids.

STATISTICS

Prior to analysis continuous variables were tested for linearity with Q-Q plots and were transformed if needed. Final variables did not violate the Kolmogorov-Smirnov (KS) test for normality. All flavonoids were natural log transformed to achieve linearity and passed the KS test for normality. Known confounders for HDL and LDL [age, gender, ethnicity, cholesterol medications, dietary fat and body mass index (BMI)] and daily energy were present in all multiple regression models. Pearson’s and Spearman’s correlations were performed for the combined sample and stratified by diabetes status for all potential confounders of flavonoids with HDL and LDL. Student t-tests were used to compare flavonoid intake by ethnicity, diabetes status, ethnicity stratified by diabetes status, and diabetes status stratified by ethnicity. A one-way analysis of variance (ANOVA) was performed to assess mean differences by diabetes status and ethnicity of general characteristics, biomarkers and flavonoid classes. For each group, with and without diabetes, two-block linear regressions were performed: Block 1: Backward method (probability of F-to-remove ≥ 0.100) for flavonoids (anthocyanidins, poly flavonoids, flavan-3-ols, flavones, flavanone, flavonoid-no-proanthocyanidins, flavonols, all natural log transformed) and Block 2: Enter method (forced entry) for ethnicity, gender, current smoker, total daily energy (Kcal/d), BMI, dietary fat, cholesterol medications, and age was conducted to determine associations with the dependent variables HDL and LDL (both natural log transformed). The results were considered statistically significant if the two-tailed p-value was < 0.05. Statistical analysis was performed using SPSS (version 18).

RESULTS

Several differences in flavonoid intake were observed comparing with and without diabetes. For the combined sample, participants with diabetes as compared to those without diabetes consumed less of all flavonoids [flavanones (p < 0.001), flavan-3-ols (p = 0.022), polyflavonoids (p = 0.005), flavones (p = 0.003), flavonoids no proanthocyanidins (p = 0.002) and total flavonoids (p = 0.002)] except flavonol (p = 0.883) and anthocyanidins (p =
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0.148) (data not shown). There were differences by ethnicity for the combined sample with and without diabetes. Haitian-Americans consumed more flavonol (p < 0.001) and flavones (p = 0.029) than AA (data not shown). The general characteristics of the participants compared by diabetes status and stratified by ethnicity are presented in Table 1. There were differences in several flavonoids by diabetes status for HA, whereas there were no differences for AA. One-way Analysis of Covariance (ANCOVA) comparing HA and AA, stratified by diabetes status, showed that HA without diabetes consumed more flavonols (p = 0.015) and flavones (p = 0.028) than AA without diabetes (data not shown).

Figure 1

Table 1. General Characteristics of the participants stratified by diabetes status and ethnicity

| Abbreviations: T2D=type 2 diabetes, HA=Haitian-American; AA=African-American; HS=high school; cholesterol med.= cholesterol medications; KW= Kruskal Wallis test; IQR = interquartile range (75th quartile-25th quartile); w/wo T2D = with and without type 2 diabetes; BMI=body mass index; proantho= proanthocyanidins). 
| Abbreviations: T2D=type 2 diabetes, HA=Haitian-American; AA=African-American; HS=high school; cholesterol med.= cholesterol medications; KW= Kruskal Wallis test; IQR = interquartile range (75th quartile-25th quartile); w/wo T2D = with and without type 2 diabetes; BMI=body mass index; proantho= proanthocyanidins). 

† KS= Kruskal Wallis test given as mean rank comparing ethnicities within diabetes status. KS test is given as medium (IQR).

‡ HDL and LDL cholesterol for HA (n=257).

ű Total flavonoids without proanthocyanidins.

The final models for the association of flavonoids with HDL and LDL are presented in Tables 2 and 3, respectively. The results are stratified by diabetes status and all models were adjusted for ethnicity, age, gender, current smoker (yes/no), total daily energy intake, and cholesterol medication (yes/no). Lower LDL was associated with higher intake of flavanones for participants with diabetes; whereas, higher flavan-3-ols, and flavanones and lower polyflavonoids were related to lower LDL in the group without diabetes. There was a positive association for HDL with anthocyanidins and flavan-3-ols and an inverse relationship with polyflavonoids for the group without diabetes. There was no relationship between HDL and flavonoids for the group with diabetes.

Figure 2

Table 2. Association of flavonoids with HDL-cholesterol stratified by diabetes status

<table>
<thead>
<tr>
<th>Variability†</th>
<th>HDL (dependent) independent</th>
<th>Variability†</th>
<th>HDL (dependent) independent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (standardized coefficient)</td>
<td>95% CI for coefficient</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>lower</td>
<td>upper</td>
<td>P</td>
</tr>
<tr>
<td>Antocyanidins</td>
<td>0.200</td>
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<td>0.038</td>
</tr>
<tr>
<td>Flavan-3-ols</td>
<td>0.080</td>
<td>0.193</td>
<td>0.188</td>
</tr>
<tr>
<td>Polyflavonoids</td>
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<td>-0.256</td>
</tr>
<tr>
<td>Ethnicity (African-American)</td>
<td>0.025</td>
<td>0.028</td>
<td>0.028</td>
</tr>
<tr>
<td>Age</td>
<td>0.080</td>
<td>0.080</td>
<td>0.070</td>
</tr>
<tr>
<td>Cholesterol Medications</td>
<td>-0.146</td>
<td>-0.127</td>
<td>-0.104</td>
</tr>
<tr>
<td>Current Smoker (yes)</td>
<td>-0.066</td>
<td>0.177</td>
<td>0.177</td>
</tr>
<tr>
<td>Daily energy (kcal)</td>
<td>-0.133</td>
<td>-0.206</td>
<td>-0.206</td>
</tr>
<tr>
<td>Dietary fiber (g)</td>
<td>0.556</td>
<td>0.380</td>
<td>0.380</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.207</td>
<td>-0.456</td>
<td>-0.456</td>
</tr>
</tbody>
</table>

Model summary without diabetes

| F (11, 238) = 4.72 | R = 0.493 | ns. R² = 0.146 | P = 0.004 | R² = 0.241 |

PARTICIPANTS WITH DIABETES

<table>
<thead>
<tr>
<th>Variability†</th>
<th>HDL (dependent) independent</th>
<th>Variability†</th>
<th>HDL (dependent) independent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (standardized coefficient)</td>
<td>95% CI for coefficient</td>
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<td></td>
<td>lower</td>
<td>upper</td>
<td>P</td>
</tr>
<tr>
<td>Ethnicity (African-American)</td>
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<tr>
<td>Age</td>
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<td>0.092</td>
<td>0.092</td>
</tr>
<tr>
<td>Cholesterol Medications</td>
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<td>-0.256</td>
<td>-0.256</td>
</tr>
<tr>
<td>Current Smoker (yes)</td>
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<td>-0.339</td>
<td>-0.339</td>
</tr>
<tr>
<td>Daily energy (kcal)</td>
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<td>-0.226</td>
</tr>
<tr>
<td>Dietary fiber (g)</td>
<td>0.255</td>
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<td>0.283</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.471</td>
<td>0.483</td>
<td>0.483</td>
</tr>
</tbody>
</table>

Model summary with diabetes

| F (11, 256) = 7.39 | R = 0.433 | ns. R² = 0.206 | P = 0.001 | R² = 0.162 |
DISCUSSION

Flavonoids are phytochemicals that have gained importance in nutritional science for their effect on human health. These data provide new insight into dietary flavonoids and their association with type 2 diabetes and serum cholesterol. Individuals with type 2 diabetes, in the current study, consumed less flavonoids than those without type 2 diabetes. Participants with diabetes may abstain from fruit and sugar-containing juices as a means of limiting carbohydrate intake in an effort to control their diabetes. Lower fruit intake could, in part, account for lower dietary flavonoids.

Flavanones are abundant in citrus fruit and are present in lesser amounts in parsley, mint and hot pepper. For participants with type 2 diabetes, flavanones were associated with higher LDL for persons with diabetes and lower LDL for persons without diabetes. This data suggest a confounder may be present only for the group with diabetes. Adjustments for fat intake and BMI did not change the direction or significantly affect the magnitude of coefficients for flavanones with respect to LDL or HDL. Similarly, Arai et al found that adjustments for fat and fiber intake did not change the relationships between flavonoids and plasma LDL for a sample of Japanese women.

Several flavonoids either in food sources or supplements have been found to improve LDL and HDL in clinical trials and have been correlated with LDL and HDL in cross-sectional studies. For example, Qui et al reported that an anthocyanidin supplement (160 mg) versus a placebo group administered for 12 weeks improved LDL and HDL levels of 120 subjects with hypercholesterolemia. Our findings of anthocyanidins and HDL for the group without diabetes corroborate with Qui and colleagues’ study. Flavonols and flavones were inversely correlated to LDL in a cross-sectional study of 120 Japanese women aged 29-78 years; however, they did not test any other flavonoids. Naringin (a flavanone found primarily in grapefruit and other citrus) administered in supplement form for an 8-week trial was found to lower LDL concentrations by 17% in the hypercholesterolemic group (n=30) as compared with healthy controls (n=30). Flavanones (hesperidin (found mostly in oranges) and naringin did not improve cholesterol (total, LDL or HDL) in a 4-week intervention of 194 hypercholesterolemic men and women; however, the study was of a short duration. Phenols extracted from the fruit and measured as milligrams of gallic acid per kilogram of fresh weight were found to increase HDL and LDL for non-smokers and decrease LDL for smokers in a 26-week intervention of fruit (one apple, one pear and 200 ml of orange juice each day) in 14 smoking- and 18 non-smoking-normo-lipid adults (aged 23-60) from Northern Mexico. Although LDL was raised for non-smokers, their baseline and final level were within the normal range.

On the other hand, some interventions report no improvement of cholesterol with the administration of select flavonoids. A systematic review indicates there has been mixed reporting of epidemiologic evidence for cardiovascular effects of diets rich in flavonoids. No heterogeneity of flavonoid-type and HDL was found by meta-analysis of 102 trials and minimal effects were found with green tea (catechins, flavan-3-ols) and for soy protein isolate (isoflavones) for the reduction of LDL. The evidence for other flavonoids classifications was that they had no effect on LDL.

Lack of agreement with food flavonoids and cholesterol may be due to varying composition of flavonoid type and amount. For some studies, the bioavailability of the flavonoids was not established since their assessment was...
based on a FFQ and not assessed in blood. Environmental conditions of the food sources as well as human genetic differences could account for differences in availability, absorption and metabolic use of flavonoids. Not only may the composition of flavonoids in a particular food (for example, pomegranate) vary by nature (species, maturation, climate) and environmental factors (cultivation, storage and processing), but there are no uniform analytical methods for extracting and analyzing flavonoids in foods. Although there are standard methods available, the results between methods may vary. Flavan-3-ols, flavonol (quercetin) and anthocyanidins (cyaniding-3-O—galacoside) were the most prevalent flavonoids found in commercially available pomegranate juice by High Pressure Liquid Chromatography with photodiode array mass spectroscopy (HPLC-PDA-MS). Yet Rosenblat and colleagues used a different HPLC method and reported a different polyphenol composition (no flavan-3-ols and higher anthocyanidins).

Another area of difficulty when assessing the effect of flavonoids on cholesterol is the complex nature of the food source. The presence of phytochemicals in foods in addition to flavonoids such as other polyphenols (phenolic acids, phenolic alcohols, stilbenes and lignans), dietary fiber, and other known antioxidants such as vitamin E, beta carotene and lutein may have synergistic effects with particular flavonoids that are responsible for the improvement of serum cholesterol through multiple mechanisms.

The lack of consensus may also be confounded by nutrigenomic interactions. Flavonoid-type may differentially affect the pathways of LDL by gene activation and enzyme regulation. Epigallocatechin gallate (EGCG), a flavan-3-ol, and hesperitin, a flavanone, each have a specific role in inhibiting the intracellular accumulation of oxidized LDL in human cells. Future studies should consider the possibility of ethnically-specific polymorphisms interacting with flavonoids in regulating cholesterol metabolism.

The data presented here should be considered preliminary since the sample size was modest and the study was collected at a single time point. These results suggest randomized control studies aimed at improving cholesterol with foods or supplements containing flavonoids are warranted. In consideration of safety, flavonoids in supplemental form (beyond the normal dietary intake) would require evaluating the kinetics and toxicity with respect to botanical-drug interactions, dosage level and the matrix (additional ingredients in the supplement). The current study had several limitations. First, it was a single time point, and causal relationship between flavonoids and serum cholesterol could not be established. Second, flavonoid types and amounts were based on analysis of a FFQ which was obtained by self-report. Self-reported food data may be under or over-reported. In addition, the intrinsic (bioavailability) and extrinsic (environmental) factors of the flavonoids varied and were not measured.

CONCLUSION

This data showed differences in consumption of several flavonoid types between ethnicity and disease state (with or without type 2 diabetes). Dietary flavonoids were associated differently with HDL and LDL by diabetes status but not ethnicity. To its merit, the present study adds much needed epidemiological data for two Black ethnicities. Haitian- and African- Americans are likely to have different dietary preferences and nutrigenomics. Another novel aspect of this study was the comparison of Blacks with and without type 2 diabetes with respect to flavonoid intake and the association with HDL and LDL.

References
2. Robards K, Antolovich M. Analytical chemistry of fruit bioflavonoids—a review. Analyst 1997; 122: 11R-34R.
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