Apple Juice Consumption Reduces Plasma Low-Density Lipoprotein Oxidation in Healthy Men and Women

DIANNE HYSON, M.S., R.D.,1 DEBORAH STUDEBAKER-HALLMAN, B.S.,1 PAUL A. DAVIS, Ph.D.,1,2 and M. ERIC GERSHWIN, M.D.2

ABSTRACT

Epidemiological studies show that consumption of fruits and vegetables is associated with beneficial effects on human health including reduced risk of coronary artery disease (CAD). Fruits and their juices contain phytochemicals that inhibit in vitro low-density lipoprotein (LDL) oxidation and may account, in part, for their protective effect. However, reports of in vivo antioxidant effects from fruit intake are limited. We conducted a human trial to examine the in vivo effect of consumption of apples (both whole and juice) in an unblinded, randomized, crossover design. Healthy men and women added 375 ml of unsupplemented apple juice or 340 g of cored whole apple to their daily diet for 6 weeks, then crossed over to the alternate product for 6 weeks. Blood samples were obtained at baseline and after each dietary period. Compliance was monitored via biweekly 5-day food records, bodyweight checks, and meetings with study personnel. There were no significant differences between groups in intake of dietary fat, cholesterol, total carbohydrate, sugar, or calories throughout the study. Dietary fiber intake increased by 22% with whole apple consumption. Body weight, fasting serum lipid concentration, and other lipoprotein parameters were unchanged. Apple juice consumption increased ex vivo copper (Cu^(2+))-mediated LDL oxidation lag time by 20% compared with baseline. Apples and apple juice both reduced conjugated diene formation. Moderate apple juice consumption provides in vivo antioxidant activity. In view of the current understanding of CAD, the observed effect on LDL might be associated with reduced CAD risk and supports the inclusion of apple juice in a healthy human diet.

INTRODUCTION

Epidemiological studies have shown that consumption of fruits and vegetables is associated with reduced risk of chronic diseases including coronary artery disease (CAD) (Ness and Powles, 1997; Law and Morris, 1998). Oxidative processes, in particular oxidation of low-density lipoprotein (LDL) cholesterol, are believed to be important etiologic factors in the development of CAD (Steinberg et al., 1989; Berliner et al., 1995; Ness and Powles, 1997). Many fruits and vegetables are rich in phytochemicals that have been shown to have antioxidant properties (Abbey et al., 1993, Frankel et al., 1993, Hertog et al., 1995, Law et al., 1998, Miyagi et al., 1997). The health protective effects of fruit and vegetable intake observed in epidemiological studies may be due, in part, to the presence of antioxidants in these foods. A number of studies have indicated that LDL oxidation is reduced in vitro by a variety of fruits.
or fruit extracts (Frankel et al., 1998, Heinonen et al., 1998, Meyer et al., 1998, Nigdikar et al., 1998, Wilson et al., 1998). We recently reported that the addition of various apple juices to an in vitro LDL oxidation system reduced LDL oxidation by 9–34% (Pearson et al., 1999). In addition, extracts from whole apple, apple peel, or apple flesh reduced in vitro LDL oxidation by 34%, 38%, and 21%, respectively (Pearson et al., 1999). That study demonstrated that apples and apple juice, as noted in earlier studies, contain significant levels of polyphenols, a class of phytochemicals known to have potent antioxidant properties. In the present study, we sought to determine whether long-term consumption of apple juice or whole apples by healthy men and women would have an in vivo effect on the oxidative resistance of LDL.

METHODS

Subjects and study design

The effect of consumption of apple juice and whole apple was examined in an unblinded, randomized, 6-week crossover trial with healthy men and women. Twenty-eight subjects from the university and surrounding community were entered into the study. The subjects were recruited with flyers and newspaper advertisements. Recruitment criteria required all subjects to be of normal body weight, free of active disease and medication usage, non-smokers, normocholesterolemic (<200 mg/dl ± 10%), and free of dietary restrictions or allergies. Subjects consuming nutritional supplements other than multivitamin-mineral preparations were not enrolled. Entry levels of total and high-density lipoprotein (HDL) cholesterol and triglycerides were determined by capillary blood finger stick on a Cholestech LDX lipid analyzer (Cholestech Corporation, Hayward, CA). The study protocol was approved by the University of California Human Subjects Review Committee, and all participants provided informed consent.

Dietary protocol and monitoring

On entry into the study, subjects were required to keep a detailed 7-day food record of their habitual intake. Digital food scales were provided, and a registered dietitian met with each subject to provide guidelines for record keeping and to answer questions. Subjects were then randomly assigned to either the apple juice (AJ) or the whole apple (WA) diet group for a period of 6 weeks. The AJ group was provided with unsweetened, unsupplemented commercial apple juice and instructed to measure and consume 12 fluid ounces (375 ml) daily for the 6-week period. Each subject was provided with a supply of juice and a measuring container. The WA group was provided with a supply of fresh whole apples including the following varieties: Fuji, Golden Delicious, Granny Smith, and Red Delicious. Subjects were instructed to weigh and consume 12 ounces (340 g) of cored whole apples (including peel) daily for 6 weeks. After 6 weeks, subjects crossed over to consuming the alternate product. Subjects were asked to eliminate apples and apple products from their diet other than those provided by the study for the entire 12-week study period. In addition, they were required to maintain their baseline dietary and activity patterns and body weight. Compliance was assessed by several means. Body weight was monitored throughout the study. Each participant was required to keep a detailed 5-day food record every 2 weeks. As a further measure, subjects were required to return biweekly to the facility to pick up their next allotment of apple products and meet with the study coordinator to review their food records and be questioned as to any particular problems they might have encountered regarding compliance. Records of dietary intake were analyzed with the use of Nutritionist Five version 2.0 software (N-Squared Computing, Salem, OR).

Fasting plasma lipid measurements

Twelve-hour fasting blood samples were obtained by venipuncture at baseline and after each 6-week dietary period. Aliquots were taken and stored under nitrogen for LDL isolation as detailed later. Total and HDL cholesterol and triglycerides were analyzed with the use of enzyme-based reagents on a Chiron-Bayer 550 Express Chemistry Analyzer in the University of California, Davis Clinical Nutri-
tion Research Unit, analytical core laboratory, NIH#DK35747. LDL cholesterol concentrations were estimated by the Friedewald equation (Friedewald et al., 1972). Apolipoprotein B and apolipoprotein A-I were measured using an immunoprecipitation reaction on a Beckman-Coulter Array Protein System as described by the manufacturer.

Isolation of low-density lipoprotein

LDL was isolated from frozen ethylenediaminetetraacetic acid (EDTA)-containing plasma by stepwise density gradient centrifugation (total 3.5 hours at 649,826 g in a S120-AT2 rotor) using a Sorvall RC-M120GX Micro-ultracentrifuge (Sorvall Products L.P., Newton, CT). The LDL band was aspirated and dialyzed in the dark overnight at 4°C in 2 L of phosphate-buffered saline (PBS) containing a chelating ion exchange resin (Bio-Rad Laboratories, Hercules, CA). Cholesterol content of LDL was measured with a cholesterol oxidase kit (Boehringer Mannheim, Indianapolis, IN).

Oxidation of low-density lipoprotein

LDL oxidation was measured essentially as described by Esterbauer et al. (1989). Briefly, an aliquot of LDL (normalized to contain 250 mg of cholesterol per liter) was added to PBS in a 1-cm quartz cuvette. The oxidation procedure was run at 37°C and was initiated by addition of CuCl₂ to give a final concentration of 1.66 μmol/L. The kinetics of conjugated diene formation were monitored spectrophotometrically (Shimadzu-UV-160, Tokyo, Japan) by measuring absorbance at 234 nm every 5 minutes until a plateau occurred. Calculations were made to determine lag time, propagation rate, and maximum diene formation during LDL oxidation. The lag time was defined as the interval (in minutes) between the intercept of the tangent of the slope of the propagation phase and the initial absorbance axis. The maximum diene concentration was determined from the difference between the absorbance at the maximum slope of the absorbance curve and the absorbance at time zero using the extinction coefficient for conjugated dienes at 234 nm: ε = 29,500 L/(mol·cm). The maximal rate of oxidation was calculated from the slope of the absorbance curve during the propagation phase and expressed as nanomoles of dienes produced per minute per 75 μg of cholesterol (Esterbauer et al., 1989).

Statistical methods

Repeated measures analysis of variance (ANNOVA) was used to assess dietary effects on plasma lipid parameters, LDL oxidation indices, and dietary intake using Sigmasstat (version 2.0 for Windows 95, Jandel Scientific Software, San Rafael, CA). The plasma lipid values that were examined included total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, apolipoprotein A-I, and apolipoprotein B. Statistical analysis of the dietary intake data included total calories, total carbohydrate, sugar, dietary fiber, total and saturated fat, and cholesterol. The LDL oxidative indices analyzed included lag time, rate of conjugated diene formation, and total production of conjugated dienes. Because the distribution of the oxidation values was not normal, nonparametric analyses were required; the Friedman repeated measures analysis on ranks was used. Tukey’s or Dunnet’s multiple comparison procedures were used for the post hoc analysis. A value of P < .05 was deemed significant. No sex-related effects were noted in any of the variables examined, and therefore the analysis was conducted on the entire subject population.

RESULTS

Subject characteristics

Twenty-five subjects completed the study, including 12 men and 13 women. Two subjects withdrew during the study due to employment demands and one subject was asked to withdraw due to noncompliance. The mean age (±SE) of the group was 39.8 ± 1.9 years. Mean body mass index at study entry was 24.9, and it remained unchanged throughout the study. Eleven subjects consumed a daily multivitamin-mineral supplement at baseline and throughout the study. Five of these subjects consumed daily vitamin C (500–1000 mg), and
four also consumed vitamin E daily (400 IU) throughout the entire study. Although it might have been desirable to minimize or eliminate supplemental intake of antioxidants, it proved very difficult to recruit subjects who were not taking some sort of vitamin preparation. In addition, the presence of supplementation was accommodated at several levels. These include the fact that mandatory maintenance of each subject’s baseline intake throughout the study period was a requirement of the study. Moreover, the study was a crossover design in which each subject served as his or her own control in the repeated measures ANOVA.

**Dietary intake**

Dietary intake data for the subjects at baseline and after 6 weeks of AJ or WA consumption are portrayed in Table 1. There was no significant change in intake of daily energy, carbohydrate, sugar, total or saturated fat, or cholesterol throughout the study. Daily dietary fiber intake increased by 22% during the WA period, which is congruent with the additional fiber provided by the consumption of WA and suggests that the subjects complied with the dietary protocol.

**Fasting lipid measurements**

There was no significant effect of 6 weeks of WA or AJ consumption on total cholesterol, LDL or HDL cholesterol, or apolipoprotein A-I or B levels, as presented in Table 2. There was a small, nonsignificant rise in fasting plasma triglyceride levels.

**Oxidation of low-density lipoprotein**

The indicators of LDL oxidation are presented in Table 3. Consumption of AJ for 6 weeks resulted in a significant increase in lag time, suggesting that AJ increased the resistance of LDL particles to *ex vivo* copper-induced oxidation. The increased lag time noted on WA consumption did not reach statistical significance (*P* = .07). Both WA and AJ consumption resulted in a modest but statistically significant reduction in the total production of

---

**Table 1. Dietary Intake of Subjects Consuming Whole Apple or Apple Juice for 6 Weeks**

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Baseline</th>
<th>WA</th>
<th>AJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy (Kcal)</td>
<td>2422 ± 127</td>
<td>2419 ± 123</td>
<td>2439 ± 164</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>335 ± 19</td>
<td>357 ± 18</td>
<td>339 ± 20</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>132 ± 11</td>
<td>148 ± 8</td>
<td>152 ± 11</td>
</tr>
<tr>
<td>Dietary fiber (g)</td>
<td>23 ± 2</td>
<td>28 ± 1†</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>82 ± 5</td>
<td>77 ± 6</td>
<td>74 ± 6</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>26 ± 2</td>
<td>23 ± 2</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>229 ± 24</td>
<td>216 ± 26</td>
<td>227 ± 23</td>
</tr>
</tbody>
</table>

*All values are daily mean ± SEM.
†*P* < .05, WA vs. baseline, AJ.

**Table 2. Fasting Plasma Lipid Concentrations of Subjects Consuming Whole Apple or Apple Juice for 6 Weeks**

<table>
<thead>
<tr>
<th>Lipid parameter</th>
<th>Baseline</th>
<th>WA</th>
<th>AJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol*</td>
<td>5.57 ± .21</td>
<td>5.47 ± .18</td>
<td>5.52 ± .18</td>
</tr>
<tr>
<td>LDL cholesterol*</td>
<td>3.60 ± .26</td>
<td>3.42 ± .21</td>
<td>3.57 ± .18</td>
</tr>
<tr>
<td>HDL cholesterol*</td>
<td>1.27 ± .08</td>
<td>1.27 ± .05</td>
<td>1.24 ± .05</td>
</tr>
<tr>
<td>Triglyceride*</td>
<td>1.27 ± .2</td>
<td>1.40 ± .23</td>
<td>1.44 ± .18</td>
</tr>
<tr>
<td>Apolipoprotein A-1†</td>
<td>51 ± 2.0</td>
<td>48 ± 2.5</td>
<td>49 ± 2.5</td>
</tr>
<tr>
<td>Apolipoprotein B†</td>
<td>1.9 ± 0.12</td>
<td>1.9 ± 0.10</td>
<td>2.0 ± 0.18</td>
</tr>
</tbody>
</table>

*Values are mean (mmol/L) ± SEM.
†Values are mean (μmol/L) ± SEM.
conjugated dienes, but neither affected the propagation rate of diene formation.

**DISCUSSION**

Epidemiological studies have shown that high intake of fruits and vegetables is associated with reduced risk of cardiovascular disease. Most fruits and vegetables contain significant quantities of polyphenolic compounds in addition to variable amounts of antioxidant vitamins including C, β-carotene, and E. The antioxidant activity of whole fruits and vegetables is of great interest because oxidative processes have been shown to be important in the initiation of atherosclerosis (Steinberg et al., 1989). Oxidation of LDL increases its affinity for scavenger receptors on macrophages, thus facilitating the deposition of lipid in the arterial wall. In addition, oxidized LDL has been shown to induce the production of a number of inflammatory molecules involved in the atherogenic process (Berliner et al., 1995). Dietary antioxidants that protect LDL from oxidation may therefore reduce atherogenesis and the risk of CAD. The antioxidant capacity of many polyphenolic compounds has been well documented in *in vitro* systems and in animal studies (Knekt et al., 1996). Several large epidemiological studies have provided evidence supporting an inverse relationship between relative risk for cardiovascular disease and a high intake of the flavonoid class of polyphenols (Hertog et al., 1993, 1995; Knekt et al., 1996). Therefore, it is presumed that the antioxidants (polyphenols?) present in fruit and vegetables mediate part of the cardioprotective effect of these foods.

Accordingly, there has been an increased research focus on the ability of polyphenolic-rich foods to increase resistance of LDL to oxidation. Several *in vitro* studies have shown that foods and beverages containing phenolic flavonoids reduce LDL oxidation (Miyagi et al., 1997; Leake, 1998; Meyer et al., 1998; Nigdikar et al., 1998; Pearson et al., 1999). When isolated human LDL is incubated with a variety of fruit extracts or juices including blackberries, red raspberries, sweet cherries, blueberries, strawberries (Heinonen et al., 1998) grape juice (Frankel et al., 1998), cranberries (Wilson et al., 1998), and red wine (Frankel et al., 1993), oxidation of LDL is reduced by as much as 70% in some cases. A number of investigators have examined the antioxidant impact of isolated polyphenolic compounds common to these foods (Meyer et al., 1998). They showed that polyphenolic components themselves reduce LDL oxidation, suggesting that the observed antioxidant effects of fruit are probably a result of the variety of flavonoids present in fruit and fruit-based products. Consistent with these findings, our laboratory recently reported that the addition of apple juice and extracts of apple peel, flesh, and whole apple to the LDL copper-mediated oxidation system attenuated *in vitro* LDL oxidation by 9–34% (Meyer et al., 1998).

However, only a few studies have examined the *in vivo* effects of fruit product consumption on LDL oxidation in humans, and these have primarily examined the effects of grape-related products (Nigdikar et al., 1998). One study showed that 2 weeks of daily wine consumption by 17 healthy volunteers reduced copper-induced oxidation of isolated LDL. The authors observed reduced production of conjugated dienes, thiobarbituric acid reactive substances (TBARS), and lipid peroxides (54%, 46%, and

---

**Table 3. Indices of LDL Oxidation in Subjects Consuming Whole Apple or Apple Juice for 6 Weeks**

<table>
<thead>
<tr>
<th>LDL oxidation indices</th>
<th>Baseline</th>
<th>WA</th>
<th>AJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lag time (min)</td>
<td>56 ± 4</td>
<td>61 ± 4</td>
<td>67 ± 4†</td>
</tr>
<tr>
<td>Propagation rate (nmol/min)</td>
<td>1.56 ± 0.13</td>
<td>1.51 ± 0.14</td>
<td>1.38 ± 0.06</td>
</tr>
<tr>
<td>Conjugated diene formation (nmol per 75μg cholesterol)</td>
<td>3.20 ± 1.2</td>
<td>2.97 ± 1.5†</td>
<td>3.04 ± 1.1†</td>
</tr>
</tbody>
</table>

*All values are mean ± SEM.
†P < .05, diet vs. baseline.
72%, respectively) compared with baseline values (Nigdikar et al., 1998). In another study, the in vivo effects of consumption for a 2-week period of either red wine, white wine, or polyphenols isolated from red wine was evaluated in small groups of healthy men (n = 6–9) (Nigdikar et al., 1998). The production of conjugated dienes and TBARS was significantly reduced by all treatments; lipid peroxides were reduced in all but the white wine consumption group. Lag time (the number of minutes before onset of LDL peroxidation) was significantly improved in all groups except the white wine group. It is noteworthy that white wine is relatively lacking in polyphenolic compounds compared to red wine.

In a different study, 15 subjects with coronary artery disease consumed approximately 640 ml of purple grape juice per day for a 2-week period (Stein et al., 1999) and the lag time of copper-induced LDL oxidation was increased by 34%. Very recently, a similar study of grape juice reported in abstract form showed that daily consumption of 1,005 ml of grape juice for 3 weeks increased lag time in four of five subjects, although a measure of statistical significance was not provided (Gross et al., 2000).

Finally, a large intervention study evaluated the effects of feeding diets enriched in fruits and vegetables on several measures of antioxidant capacity and lipid peroxidation (Miller et al., 1998). After 8 weeks of consuming a high intake of fruits and vegetables either with or without reduced fat content, there was a significant increase in the antioxidant capacity of the serum (based on oxygen radical absorbing capacity assays) and a reduction in breath ethane levels compared with results for the control diet. This study provided evidence that antioxidant capacity can be altered by diet without the use of vitamin supplementation.

The observed reduction in LDL oxidation was achieved with only a moderate consumption of daily juice, 375 ml, compared to the amount used in studies with grape juice, approximately two to four times this amount. The amount of juice consumed in this study did not provide a significant source of additional energy (375 ml = 190 kcal/day) and could be taken as a single serving, promoting compliance to the dietary protocol. The ability of moderate amounts of apple juice to prolong LDL oxidation lag time suggests that the use of total oxygen radical absorbance capacity (ORAC) as a measure of antioxidant levels is potentially misleading (Bravo, 1998).

Almost half (44%) of the study subjects were induced LDL oxidation was increased by 20% (P < .05) and production of conjugated dienes was reduced by 7% (P < .05). Consumption of whole apples did not significantly increase lag time but did significantly reduce conjugated diene formation compared to baseline levels (5%, P < .05). Neither apple juice or whole apple intake altered fasting lipid values or the propagation rate of conjugated diene production.

The reasons for the change in LDL oxidative susceptibility with apple juice consumption but not whole apples are unclear. We did not evaluate the mechanisms for the observed effects in this study and therefore cannot attribute the antioxidant effects to any specific component in apple juice. Apples are a more complex food than apple juice and contain relatively high amounts of dietary fiber. It is unknown whether the antioxidant components in apples are less available for absorption than apple juice or whether they are more likely to be subject to bioconversion in the intestinal lumen compared to apple juice. It is not unreasonable to speculate that the availability of nutrition-related components in an intact food are more likely to be influenced by variables such as mastication, reducing absorption of bioactive compounds. Furthermore, it is possible that the processing of apple juice may result in enrichment of some components that have antioxidant potential (Bravo, 1998).
taking a daily multiple vitamin/mineral supplement at baseline and throughout the entire study. Five of these subjects were taking additional vitamin E and vitamin C daily (400 IU and 500–1000 mg, respectively). Studies examining the antioxidant effect of oral vitamin supplementation have provided equivocal results for vitamin C (Abbey et al., 1993) but suggest that vitamin E intake reduces oxidative susceptibility of LDL (Dieber-Rotheneder et al., 1991; Abbey et al., 1993; Jialal et al., 1995). Therefore, some of our subjects may have had higher antioxidant defense at baseline, which would presumably make it more difficult to demonstrate a beneficial effect from apple juice consumption. Despite this potential drawback, we still observed a significant reduction in LDL oxidation in subjects who consumed apple juice for 6 weeks. Confidence in the robustness of these results is further enhanced by the multiple measures that were taken to ensure compliance in this study. Because the subjects were drawn from the University community, the need for strict adherence to the protocols and study requirements to ensure validity of the results was readily apparent to them. In addition, subjects admitted into the study were chosen by a careful process of screening interviews designed to ensure that any subjects selected thoroughly understood the protocol and recognized the need for compliance. All subjects were trained in record keeping and study design and met with the study coordinator or registered dietitian biweekly. Food records were reviewed at each meeting and the subjects were questioned on their weekly patterns and their adherence to the study protocol. The food record analysis showed that mean energy and nutrient intake remained consistent for the subjects throughout the study except for an expected increase in dietary fiber during the whole apple consumption period. There was no significant change in body weight during the study, again suggesting good compliance with maintaining habitual intake.

In summary, the results of this study demonstrate that daily consumption of moderate amounts of unsupplemented apple juice reduces \textit{ex vivo} oxidative susceptibility of LDL cholesterol in healthy, normolipemic men and women. Moreover, they suggest that apple juice constituents by their \textit{in vivo} effect on LDL properties may reduce the risk of CAD. Although further research is needed to gain a greater insight into the determinants of biological activity in the complex mixture of phenolics and other constituents present in apples and apple juice, the findings of this \textit{in vivo} study provide support for the inclusion of apple juice in a balanced, “healthy” human diet.

**ACKNOWLEDGMENTS**

This study was funded in part by grants from the United States Apple Association and the Processed Apple Institute.

**REFERENCES**


Address reprint requests to:

M. Eric Gershwin, M.D.

Division of Rheumatology, Allergy and Clinical Immunology

University of California at Davis

School of Medicine

TB 192

One Shields Avenue

Davis, CA 95616

E-mail: megershwin@ucdavis.edu