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# BRACHIAL ARTERY DIAMETER AND VELOCITY OF BLOOD FLOW AFTER HYPEREMIA DURING THE SIX HOURS FOLLOWING CONSUMPTION OF CRANBERRY JUICE

A MANUSCRIPT STYLE THESIS PRESENTED

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## ABSTRACT

WEISE, C.L. Brachial Artery Diameter and Velocity of Blood Flow After Hyperemia During the Six Hours Following Consumption of Cranberry Juice.
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The effects of cranberry juice consumption on brachial artery diameter and velocity of blood flow were investigated. Subjects were aged 40-69 years with documented cardiovascular disease. Subjects refrained from alcohol, fruit juices, or calcium-channel blockers for 24 hours, and supplements 5 days prior to testing. They fasted 9 hours prior to the study. Subjects were randomly assigned to an experimental group ($n = 6$) or a control group ($n = 7$). Baseline measurements were collected, after which participants drank 3 mL/kg of pure cranberry juice, or 3 mL/kg of an isocaloric sugar water mixture (control), and were given half of a plain bagel to eat. Ultrasound and Doppler flow methods were used to measure brachial artery diameter using a longitudinal (M-mode) and cross-sectional technique at pre-cuff and 2 minutes after cuff removal. Velocity of blood flow was measured at pre-cuff, maximum flow, and 1 and 2 minutes after cuff removal. Arterial blood pressure was measured with a manual sphygmomanometer. Measurements were repeated at Hours 2, 4, and 6. There was a significant difference ($p < 0.05$) between the cross-sectional and M-mode method. There were no significant differences in diameter values, velocity of blood flow values, mean arterial pressure, and resistance between the control and CBJ groups at any time point. In general, these measurements significantly decreased ($p < 0.05$) over time.

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# INTRODUCTION

Cardiovascular Disease (CVD) is the leading cause of morbidity and mortality in most industrialized countries (22). There are many risk factors for heart disease including family history, increasing age, smoking, diabetes, hypercholesterolemia, and hypertension. Hypertension, or high blood pressure, puts excess stress on the heart and is associated with damage to the lining, or endothelium, of the blood vessels (5). Endothelial dysfunction is thought to be an early precursor to atherosclerosis (6) and results in a loss of vasodilating ability and increased blood pressure (3).

Vasodilation occurs when vascular smooth muscle in the blood vessels relaxes and vessel diameter is increased. Reactive hyperemia is a type of vasodilation that can be created in the laboratory. When a blood pressure cuff is placed on the arm for 5 minutes, carbon dioxide ($CO_2$) is trapped and cannot be removed. Reactive hyperemia is caused by $CO_2$, as it causes the smooth muscle of these vessels to relax, and as a result, the vessel internal diameters widen (4). When the cuff is removed, $CO_2$ can then escape because blood is once again moving through the arteries and veins of the arm, and the vessels are dilated because of the presence of $CO_2$ during the flow obstruction (12). The blood flow into the arm is enhanced because peripheral resistance is now lower than pre-cuff. As a result, more blood moves into the arm and the velocity of the blood in the brachial artery is increased (24).

Velocity of blood flow increases because blood is no longer shut off from the area with increased blood flow into the peripheral vessels the arm turns red. Increased velocity also causes an increase in the sheer stress on the endothelial lining of the blood vessel because of viscous drag of the blood against the vascular walls (5). This increase in sheer stress on the endothelium increases the activity of the enzyme nitric oxide synthase (NOS) (18). Nitric oxide synthase catalyzes the production of nitric oxide (NO) from arginine. The NO diffuses to the smooth muscle in the local arterial wall causing relaxation and vasodilation (14, 17). Thus increased velocity of blood flow causes this flow-mediated vasodilation (FMD).

Flow-mediated vasodilation thus reduces resistance even further than the initial response to the $CO_2$ and makes it even easier for blood to move into the arm and wash out the $CO_2$ (15). The effect is transient however, because the $CO_2$ eventually gets washed out, the arm goes from hyperemic (red), to pink, and back to normal (4). Blood flow and vessel diameter also return to normal.

Nitric oxide synthase is stimulated by flavonoids (8), which are polyphenol derivatives found in nuts, vegetables, seeds, and fruit (13). Flavonoids in these products may help the vessel make more NO and improve endothelial function when $CO_2$ or FMD occurs (21).

Grapes are used to make wine, which is a very popular beverage in France. The French Paradox suggests that while the French tend to eat high saturated fat diets, have high blood cholesterol, smoke, and have other CVD risk factors, there is a low incidence of CVD in France relative to America (20). By comparison, the diet of Americans

contains slightly less saturated fat (20). Americans are also less likely than their French counterparts to smoke; yet paradoxically Americans have a 2.5-fold greater rate of death due to coronary heart disease than the French (23). It is speculated that this discrepancy is due to the relatively large amount of red wine consumed by the French. Polyphenols are an important part of red wine, have been shown to have antioxidant properties (10), and may be partially responsible for the French Paradox (20).

Like red wine, purple grape juice (PGJ) is also rich in flavonoids and may have cardioprotective qualities. Purple grape juice consumption has been suggested to lead to inhibition of platelet aggregation, (11), reduction in the susceptibility of LDL cholesterol oxidation in people with coronary artery disease (21), and improvement in endothelial function leading to vasodilation in vitro and in vivo (9). However, there are some problems with the consumption of red wine and PGJ. Not everyone can drink alcohol or can drink it in moderation. Also, because many people with cardiovascular disease also have diabetes, they need to watch their intake of sugary foods and beverages. Purple grape juice can be problematic in this regard since an 8-ounce glass of PGJ contains approximately 180 calories from sugar.

Cranberry juice (CBJ) has long been used as a remedy for urinary tract infections (2). Cranberry juice is also rich in flavonoids and may have the ability to prevent platelet aggregation and protect LDL against oxidation (19, 25, 26). Cranberry juice has been shown to vasodilate isolated rat aorta in vitro, and reduce blood pressure in anesthetized rats (16).

Because an 8-ounce glass of pure CBJ has just 60 calories, it may be a viable low-calorie alternative to red wine and PGJ for CVD protection. However, the in vivo effects of cranberry juice on blood flow in people with CVD have not been studied. The purpose of this study was to evaluate the vasodilatory effects of cranberry juice on the brachial artery in people with CVD during the 6 hours following the consumption of a single glass of CBJ.

## METHODS

The University of Wisconsin-La Crosse and Franciscan Skemp Healthcare Institutional Review Boards for the Protection of Human Subjects approved all procedures used in this study prior to contact with subjects. All subjects signed an informed consent form prior to the study and had the opportunity to ask questions (See Appendix A).

### Subjects

Twenty-four non-smoking subjects were recruited from the La Crosse Exercise and Health Program Cardiac Rehabilitation Unit. Subjects were between the ages of 40-69 years. All enrolled participants had documented cardiovascular disease as evidenced by one or more of the following: myocardial infarction, coronary artery bypass graft, angioplasty or stent, or coronary artery stenosis of 50% or more as documented by angiography. None of the participants had Raynaud's disease, diabetes, kidney disease, liver disease, endocrine disorders, uncontrolled hypertension, unstable angina pectoris, or an ejection fraction less than 40% (See Appendix B).

All subjects were instructed to fast overnight for 9 hours before testing. They abstained from calcium channel-blocker and long acting nitrates use for 24 hours prior to testing. They were instructed to abstain from fruits and antioxidant or vitamin supplements for 5 days prior to the testing day, and alcohol or other fruit beverages 24 hours prior to testing.

Blood Flow Protocol

Testing took place at Franciscan Skemp Healthcare, La Crosse, WI. Participants arrived between 6:00-7:00 AM at the Cardiology Department on the second floor of the clinic where they remained until testing was completed for the day. The participants were provided with water and magazines and were asked to bring a non-controversial or non-confrontational book to read quietly during the 6-hour testing period (watching television was not allowed). These measures were performed in an attempt to standardize participant moods during the 6-hour intervention period, so as not to affect blood pressure. On the day of testing, investigators wore standard hospital scrubs to further standardize participant experiences.

The investigators took height and weight measurements upon arrival, and obtained the lipid profiles that the participants brought with them taken in the previous six months. Body mass index (BMI) was then calculated from the height and weight measurements (weight in kg/height in $m^2$). Blood pressure was measured at baseline and before every measurement at 2, 4, and 6 hours by the investigator using a standard blood pressure cuff with attached mercury sphygmomanometer.

The subjects entered an examination room to have baseline velocity blood flow and left arm brachial artery diameter measured with the Doppler/Ultrasound procedure. An Acuson Sequoia Doppler/ultrasound recorder machine with an 8 MHz M-12 Active Matrix Array probe (Mountain View, CA) was used to measure for baseline diameter and velocity of blood flow in the brachial artery. The same technologist collected all ultrasound data in the study. The velocity measurements taken included peak systolic flow velocity, end systolic flow velocity, and end diastolic flow velocity, while the diameter measurements included arterial lumen diameter using the two methods described.

Diameter of the vessel was analyzed using two techniques (See Figure 1). One is called a cross-sectional technique where diameter is measured in cross-section. Diameter was estimated across two angles (x- and y-axis) and these values were averaged to obtain the diameter measurement. This procedure typically took 30 seconds to obtain. The second method is called the M-mode, which is used by researchers at Mayo Clinic. This is a longitudinal diameter measurement that is taken from the brachial artery at its widest location where a clear endothelial layer could be observed. This procedure required from 2 to 5 minutes to complete and occasionally measurements could not be obtained.

For both methods, the probe was held at 60 degrees to the angle of laminar blood flow in the center of the vessel to obtain optimal images of a region of the brachial artery that was straight and had no bifurcations. The arm of the subject was marked with a black X to identify the exact position for all measurements. The subject's left arm was comfortably immobilized with a pillow in an extended position to give consistent

imaging of their brachial artery. The brachial artery was measured 1-10 cm above the antecubital space and located in the longitudinal plane for measurements of velocity of blood flow and diameter of the artery at the location providing the straightest appearance. All measurements taken before the cuff was placed on the participant were called pre-cuff. The diameter was measured from leading edge to leading edge, or intima, in centimeters for both methods.

The blood pressure cuff was placed over the left forearm and inflated to 50 mm Hg over baseline systolic pressure where it was maintained for 5 minutes to promote reactive hyperemia. Upon its release (maximum flow) and 1 minute after the cuff was removed, the velocity of blood flow was measured in meters/second. At 2 minutes after the cuff was removed, the velocity of blood flow and diameter of the vessel was again measured with the cross-sectional and M-mode method.

After these baseline measurements, subjects were asked to eat one half of a plain bagel (See Appendix C) and were randomly assigned to one of two treatment groups: an isocaloric sugar water control of 10% USP dextrose (no cranberry juice), or 3 mL/kg of cranberry juice. The same Doppler/Ultrasound procedure described above was repeated at 2, 4, and 6 hours following test beverage consumption.

All measurements were taken with subject information and treatment group remaining anonymous to the technician. The Doppler/Ultrasound images from the subjects were recorded on disks for further review by the investigators. Data was recorded on a standardized sheet (See Appendix D).

Resistance was measured as a function of mean arterial pressure (MAP), vessel diameter, and velocity of blood flow (Formula: $R = MAP/(3.14)(r^2)$(velocity) (personal communication, Dr. Ted Wilson, 04-01-02.)

Materials

The cranberry juice was prepared from a mixture of Ben Lear and Stevens berries grown in the 2000 season and collected from bogs near Warrens and Manitowosh Waters, WI. The berries were homogenized to create a cranberry juice that was pasteurized and bottled in 68 ounces containing 0.3 calories/mL. After bottling, the juice was refrigerated until use.

Statistical Analysis

A two-way ANOVA (group x time) with repeated measures was used to analyze the data. The two groups were the control group and CBJ group and were measured at four different time points. Alpha was set at 0.05 to achieve statistical significance.

## RESULTS

Eleven of the original 24 subjects had ultrasound measurements that were technically unsuitable for analysis. Descriptive characteristics of the 13 subjects whose data were analyzed are presented in Table 1. There were no significant differences in the mean age, height, weight, resting blood pressure, BMI, dose, or blood lipids between the two groups.

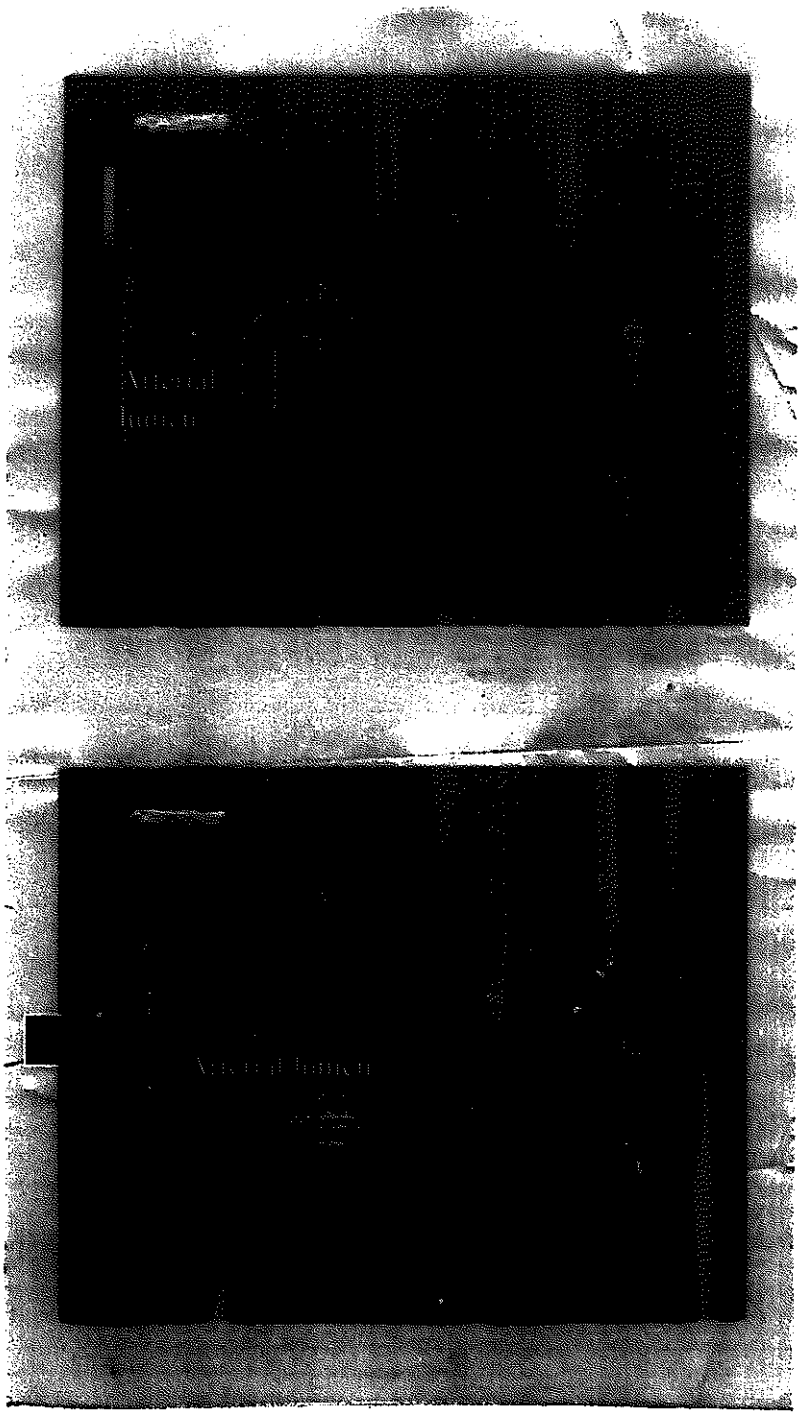
The two methods used to determine the diameter of the blood vessel were a cross-sectional method and a longitudinal method called the m-mode. Figure 1 shows representative examples of the ultrasound images obtained using the two methods.

Table 1. Subject Characteristics: CBJ Group (n = 6); Control Group (n = 7)

| Group | Age (years) | Height (inches) | Weight (kilograms) | |
|---|---|---|---|---|
| Control | 59.1±5.1 | 71.4±2.2 | 101.0±14.2 | |
| CBJ | 61.3±3.4 | 71.0±2.8 | 92.2±10.4 | |
| Total | 60.2±4.4 | 71.2±2.4 | 96.9±12.9 | |
| | **RSBP (mmHg)** | **RDBP (mmHg)** | **BMI ($kg/m^2$)** | **Dose (3 mL/ kg)** |
| Control | 133.0±14.0 | 82.3±7.0 | 30.6±3.3 | 303.2±42.5 |
| CBJ | 133.1±14.8 | 78.7±6.8 | 28.4±3.1 | 276.3±31.2 |
| Total | 132.4±13.7 | 80.6±6.9 | 29.6±3.3 | 290.0±38.6 |
| | **T Chol** | **LDL** | **HDL** | **Tri** |
| Control | 153.2±22.6 | 81.8±35.2 | 35.8±4.8 | 175.4±82.1 |
| CBJ | 163.2±23.4 | 93.7±15.8 | 49.3±13.7 | 103.3±68.2 |
| Total | 159.1±22.5 | 88.9±24.3 | 43.9±12.7 | 139.4±79.9 |

Values represent mean ± standard deviation

RSBP = Resting Systolic Blood Pressure
RDBP = Resting Diastolic Blood Pressure
Dose = Amount of Cranberry Juice or Sugar Water consumed
T Chol = Total Cholesterol in mg/dL
LDL = Low-Density Lipoprotein in mg/dL
HDL = High-Density Lipoprotein in mg/dL
Tri = Triglycerides in mg/dL



A. Cross-Sectional method of measuring diameter at pre-cuff. The two values (distance 1 and distance 2) were averaged to create a single value for use in all statistical analyses.

B. M-mode method of measuring diameter at pre-cuff. The two values (distance 1 and distance 2) were averaged to create a single value for use in all statistical analyses.

**Figure 1. Cross-sectional method and M-mode method of measuring diameter at pre-cuff.**

These data are presented in Table 2. and overall, there was a significant difference between the methods ($p < 0.05$), with the cross-sectional method averaging 18% higher values than the m-mode method. This trend was similar at all measurement time points.

When analyzing the change in diameter over time, there was no significant difference between the control and CBJ groups. At pre-cuff, there was a tendency for diameters to decrease over time for both methods. However, this decrease was not statistically significant. At 2 minutes after cuff removal, diameters decreased significantly ($p < 0.05$) over time. For the m-mode method, values from both groups at Hour 2, Hour 4, and Hour 6 were significantly ($p < 0.05$) lower than at Hour 0, but were not different from each other. For the cross-sectional method, only Hour 6 values were significantly lower ($p < 0.05$) than Hour 0. One other observation was that overall diameter of the brachial artery 2 minutes after cuff removal was larger than the values at pre-cuff.

Data for velocity of blood flow are presented in Table 3. Peak systolic, end systolic, and end diastolic velocities were measured at pre-cuff, maximum flow, 1 minute after cuff removal, and 2 minutes after cuff removal. Velocity decreased significantly ($p < 0.05$) over time, however, there were no differences between the control and CBJ groups at any time point. In general, the Hour 2, Hour 4, and Hour 6 measurements were significantly different ($p < 0.05$) than Hour 0 values. Representative Doppler/ultrasound images of blood flow at pre-cuff, maximum hyperemia, and post maximum are shown in Figure 2.

Table 4 represents data for mean arterial pressure (MAP) and resistance to blood flow. Mean arterial pressure decreased significantly over time ($p < 0.05$) with Hour 2, Hour 4, and Hour 6 values significantly different ($p < 0.05$) than Hour 0. However, there was no significant difference between the CBJ and control group. Resistance followed an identical significant ($p < 0.05$) trend, with Hour 2, Hour 4, and Hour 6 values being significantly lower ($p < 0.05$) than Hour 0. Once again, there was no significant difference between the CBJ group and the control group.

Table 2. M-mode Versus Cross-Sectional Diameters (Centimeters) at Pre-Cuff and 2 Minutes After Cuff Removal. Measurements Were Conducted Prior to Beverage Consumption and 2, 4, and 6 Hours After Beverage Consumption

| | Hour 0 | Hour 2 | Hour 4 | Hour 6 |
|---|---|---|---|---|
| **Pre-cuff (cm)** | | | | |
| M-mode | | | | |
| Control | .445±.061 | .402±.053 | .391±.046 | .412±.050 |
| CBJ | .401±.064 | .405±.074 | .402±.067 | .394±.060 |
| Total | .425±.064 | .404±.061 | .396±.055 | .404±.053 |
| Cross-sectional | | | | |
| Control | .493±.055 | .490±.059 | .481±.057 | .460±.046 |
| CBJ | .488±.051 | .476±.037 | .475±.049 | .467±.041 |
| Total | .491±.051* | .483±.049* | .479±.051* | .463±.042* |
| **2 Minutes After Cuff Removal (cm)** | | | | |
| M-mode | | | | |
| Control | .452±.043 | .417±.035 | .415±.027 | .414±.045 |
| CBJ | .437±.053 | .439±.060 | .399±.067 | .424±.041 |
| Total | .445±.046 | .428±.048# | .406±.051# | .418±.041# |
| Cross-Sectional | | | | |
| Control | .502±.073 | .500±.053 | .472±.052 | .453±.039 |
| CBJ | .500±.060 | .494±.040 | .500±.054 | .471±.084 |
| Total | .501±.065* | .497±.046* | .483±.052* | .461±.062*# |

Values represent mean ± standard deviation

* Significantly different than M-mode ($p < 0.05$)

# Significantly different than Hour 0 ($p < 0.05$)

Table 3. Peak Systolic, End Systolic, and End Diastolic Velocities (Meters/Second) at Pre-Cuff, Maximum Flow, 1 Minute After Cuff Removal, and 2 Minutes After Cuff Removal at Hours 0, 2, 4, and 6

| | Hour 0 | Hour 2 | Hour 4 | Hour 6 |
|---|---|---|---|---|
| **Pre-Cuff (m/sec)** | | | | |
| Peak Systolic | | | | |
| Control | .912±.485 | .749±.165 | .726±.143 | .676±.240 |
| CBJ | .827±.224 | .739±.170 | .716±.140 | .726±.120 |
| Total | .873±.375 | .744±.160 | .721±.135 | .699±.188 |
| End Systolic | | | | |
| Control | .181±.053 | .175±.079 | .140±.046 | .132±.050 |
| CBJ | .162±.032 | .155±.078 | .188±.045 | .171±.059 |
| Total | .172±.044 | .166±.076 | .162±.050 | .150±.056 |
| End Diastolic | | | | |
| Control | .123±.099 | .043±.028 | .026±.020 | .020±.012 |
| CBJ | .059±.035 | .030±.020 | .020±.007 | .015±.008 |
| Total | .093±.081 | .037±.025 | .023±.015 | .018±.010 |
| **Maximum Flow (m/sec)** | | | | |
| Peak Systolic | | | | |
| Control | 1.781±.735 | 1.482±.455 | 1.473±.538 | 1.450±.494 |
| CBJ | 1.910±.591 | 1.444±.313 | 1.491±.457 | 1.574±.302 |
| Total | 1.843±.648 | 1.461±.380 | 1.482±.482 | 1.514±.404 |
| End Systolic | | | | |
| Control | 1.013±.445 | .759±.335 | .770±.356 | .805±.315 |
| CBJ | 1.072±.278 | .643±.329 | .688±.332 | .885±.181 |
| Total | 1.041±.364 | .705±.324 | .732±.333 | .842±.255 |
| End Diastolic | | | | |
| Control | .687±.354 | .584±.267 | .521±.253 | .521±.178 |
| CBJ | .648±.181 | .472±.261 | .463±.242 | .535±.162 |
| Total | .669±.277 | .532±.260 | .494±.239 | .528±.164 |

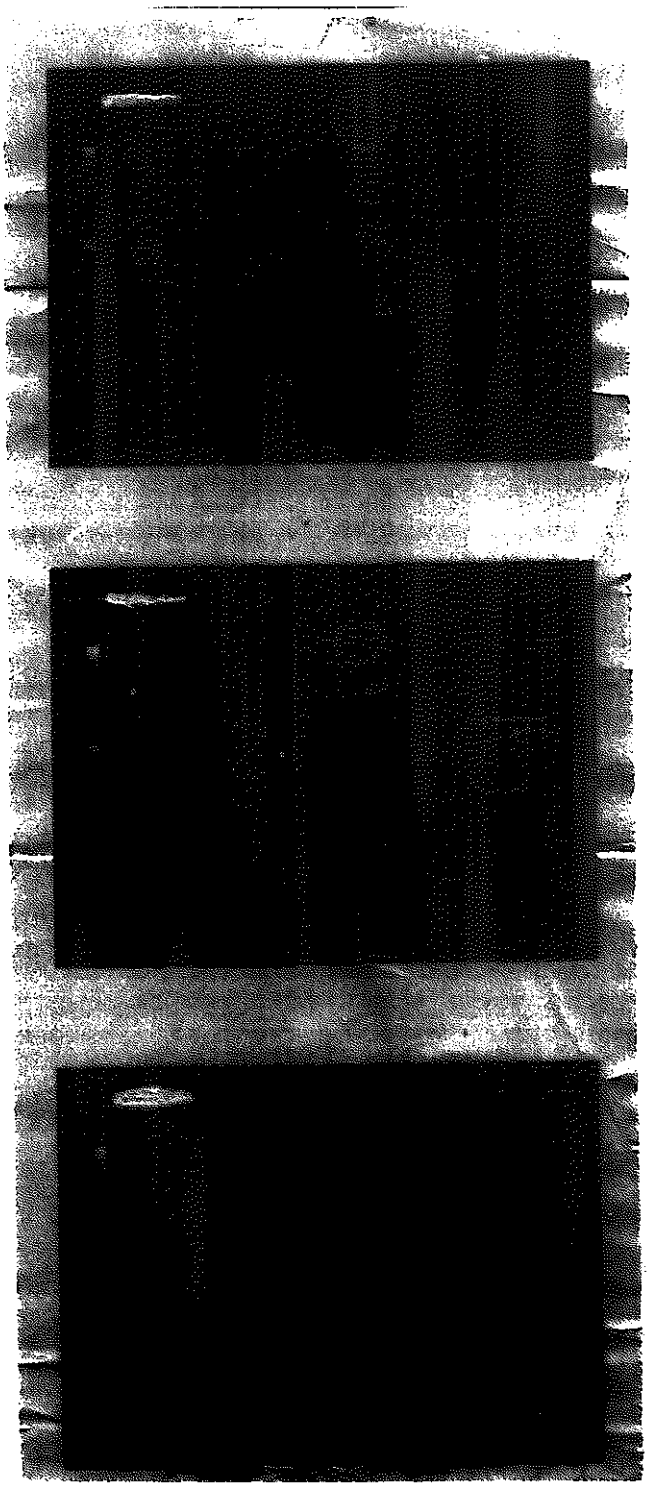
**1 Minute After Cuff Removal (m/sec)**

| | | | | |
|---|---|---|---|---|
| Peak Systolic | | | | |
| Control | 1.031±.391 | .965±.308 | 1.002±.232 | .827±.245 |
| CBJ | 1.070±.314 | 1.063±.180 | 1.111±.095 | 1.010±.188 |
| Total | 1.054±.343 | 1.013±.252 | 1.051±.184 | .914±.233 |
| End Systolic | | | | |
| Control | .347±.200 | .264±.141 | .206±.115 | .145±.067 |
| CBJ | .357±.216 | .332±.214 | .250±.116 | .293±.093 |
| Total | .352±.199 | .295±.174 | .226±.113 | .213±.108 |
| End Diastolic | | | | |
| Control | .171±.092 | .094±.051 | .052±.057 | .025±.034 |
| CBJ | .130±.071 | .104±.071 | .060±.054 | .057±.043 |
| Total | .152±.082 | .099±.058 | .055±.053 | .040±.040 |

**2 Minutes After Cuff Removal (m/sec)**

| | | | | |
|---|---|---|---|---|
| Peak Systolic | | | | |
| Control | .977±.361 | .906±.306 | .896±.177 | .802±.266 |
| CBJ | .998±.306 | .922±.107 | .981±.174 | .869±.136 |
| Total | .986±.323 | .913±.228 | .935±.174 | .833±.210 |
| End Systolic | | | | |
| Control | .203±.088 | .168±.092 | .177±.098 | .108±.066 |
| CBJ | .196±.075 | .171±.048 | .193±.109 | .155±.102 |
| Total | .200±.079 | .169±.072 | .184±.099 | .130±.084 |
| End Diastolic | | | | |
| Control | .062±.040 | .055±.031 | .045±.030 | .016±.015 |
| CBJ | .067±.055 | .036±.027 | .035±.020 | .016±.005 |
| Total | .064±.045 | .047±.030 | .040±.025 | .016±.011 |

Values represent mean ± standard deviation



A. Velocity of blood flow at pre-cuff. Peak systolic velocity is the highest peak (1), followed by end systolic velocity at the next peak (2), and end diastolic velocity at the end of the cardiac cycle (3).

B. Velocity of blood flow at maximum flow. Peak systolic velocity is the highest peak (1), followed by end systolic velocity at the next peak (2), and end diastolic velocity at the end of the cardiac cycle (3).

C. Velocity of blood flow at 1 minute after cuff removal. Peak systolic velocity is the highest peak (1), followed by end systolic velocity at the next peak (2), and end diastolic velocity at the end of the cardiac cycle (3).

**Figure 2. Velocity of blood flow at pre-cuff, maximum flow, and 1 minute after cuff removal.**

Table 4. Mean Arterial Pressure (MAP) and Resistance at Hours 0, 2, 4, and 6

| | Hour 0 | Hour 2 | Hour 4 | Hour 6 |
|---|---|---|---|---|
| **MAP (mm Hg)** | | | | |
| Control | 99.0±8.4 | 93.5±9.7 | 93.3±10.4 | 94.1±11.2 |
| CBJ | 96.8±8.9 | 94.1±8.3 | 90.7±8.3 | 88.2±9.7 |
| Total | 98.0±8.4 | 93.8±8.7* | 92.1±9.2* | 91.4±10.6* |
| **Resistance** | | | | |
| Control | 1.7±0.8 | 1.4±0.6 | 1.3±0.4 | 1.1±0.5 |
| CBJ | 1.5±0.5 | 1.3±0.5 | 1.2±0.3 | 1.1±0.2 |
| Total | 1.6±0.7 | 1.4±0.5* | 1.2±0.4* | 1.1±0.3* |

Values represent mean ± standard deviation

*Significantly different than Hour 0 ($p < 0.05$)

## DISCUSSION

The current study evaluated the acute effects of CBJ on brachial artery diameter and velocity of blood flow in males aged 40-69 with known CHD. This population was chosen because a treatment effect should be the most observable and beneficial to this group. This participant population had characteristics that were also similar to the participants in previous tea (7) and PGJ studies (21) that used Doppler/ultrasound methods similar to those used in the current study.

Diameter of the brachial artery was measured using two different methods: one simple cross-sectional method and one more time-consuming called the M-mode. Although it takes more time, ultrasonographers at the Mayo Clinic in Rochester,

Minnesota suggested the M-mode method be used in the current study because they feel it is easier to see the endothelial lining (intima) of the blood vessel, thus making it easier to measure the diameter of the vessel. Diameter values obtained by the m-mode method from hour 0 to 6 were typically 18% less than those obtained by the cross-sectional method, however the trends across time were virtually identical. This suggests that future studies could obtain reliable data using the less time-consuming cross-sectional method. However, the investigator should be aware of potential diameter differences depending on the methodology used, and take this into account when making comparisons among studies.

There were no changes in diameter between the control group or the CBJ group at pre-cuff or two minutes after maximum flow at Hours 0, 2, 4, or 6. This could be because CBJ may have no effect on blood flow, or that the methods to measure diameter and blood flow are not accurate enough to detect very small treatment differences.

The system did appear to be sensitive enough to detect a change in diameter within each treatment group over time with 2 minutes after cuff removal having significantly higher values than pre-cuff values. Paradoxically, diameter values in both the control and the CBJ groups decreased over time in a similar manner. This was in contrast to what was observed by Stein et al. (21) who observed that two weeks of PGJ consumption caused significant increases in diameter.

Velocity of blood flow also decreased over time, however, there were no differences between the control and CBJ groups at any time point. Measurements were characterized by very large standard deviations. Large standard deviations were probably

due to the difficulty in obtaining the exact same section of the artery for each measurement. If velocity was measured in a non-straight segment, non-laminar flow would be created and this could also be the source of this error.

Unlike the current study that used CAD patients, a study by Agewall et al. (1) involved 12 healthy subjects that consumed red wine and underwent brachial artery diameter and velocity of blood flow measurements with similar methods. Blood samples were also drawn to determine plasma glucose and cholesterol. Subjects were randomized to red wine with or without alcohol groups, and the procedure was repeated in one week using the opposite condition. It was found that the red wine group had a significant increase in resting brachial artery diameter, resting blood flow, heart rate, and plasma ethanol 30-60 minutes after drinking. These measurements were unchanged in de-alcoholized wine. Unlike the current study, FMD was significantly greater after drinking de-alcoholized red wine than with red wine or before consumption, which supports the hypothesis that the antioxidants in red wine, not the ethanol, have cardioprotective qualities.

Unlike the current study that observed participants for 6 hours, Stein et al., used similar methods, but observed subjects for a longer consumption period (21). They found that FMD of the brachial artery improved after consumption of PGJ for 14 days. It was also found that PGJ reduces the susceptibility of LDL cholesterol to oxidation. Unlike the current study, there was no control group used, and the participants were able to continue ingesting their antioxidants or vitamins during the study. Also, nitroglycerin-

induced vasodilation was studied and found to be improved following consumption of PGJ.

Duffy et al. (7), also used similar methods to measure the brachial artery, however, their study did include a control group. Endothelial function was assessed at six times: baseline, 2 hours after consumption of 450 mL of tea (short-term tea), after consumption of 900 mL of black tea daily for 4 weeks but none the morning of the study (long-term tea), 2 hours later that day after 450 mL of tea (short-on-long-term tea), after 900 mL of water daily for 4 weeks (long-term water), and 2 hours after 450 mL of water (short-term water). Using water as a placebo prevented the blinding of participants to treatment. Unlike the current study, participants were allowed to use antioxidant vitamin supplements as long as they were not greater than the recommended dietary allowance. However, participants were given a food-frequency questionnaire to estimate their dietary flavonoid intake. Like the study done by Stein et al., (21) FMD was evaluated and found to be improved after short- and long-term ingestion of tea. Tea had no effect on baseline arterial diameter, maximal hyperemia, diastolic blood pressure, or heart rate. The short-term tea ingestion increased systolic blood pressure by 5 mm Hg, but long-term tea ingestion had no effect. Nitroglycerin-induced vasodilation was studied similar to Stein et al. (21), but no effect of tea consumption on this value was found.

Differences between the findings of our study and others could be due to a variety of factors. The researchers had limited control over the participants' medication use. Although the participants were instructed which medications to avoid and which were permitted, non-compliance to these instructions may have had some impact on our

results. In at least one case it is believed that a participant took their vasodilatory medication during the experiment, though this could not be verified by speaking with the participant. The temperature in the room where the measurements were conducted was controlled by the hospital, not the investigators. If a participant was cold, it is possible that vasoconstriction of the blood vessels could have occurred affecting blood flow as well.

Also, the methods of measuring diameter of the brachial artery and velocity of blood flow in the brachial artery are difficult to master and reproduce. A few participants were analyzed twice (data not shown) and the pre-cuff diameter and velocity of blood flow values differed by approximately 30%. The technician who conducted the measurements in the current study was inexperienced with the method used and had difficulty making measurements. Additionally, because no precise way to measure blood flow has been standardized, many different trials were run. The angle of the probe across the artery was also an issue because after hyperemia, the vessel often times moves within the arm, which makes it difficult for the technician to measure the exact angle at the exact same area on the artery.

The hypothesis of the current study was that the diameter of the brachial artery would increase to improve blood flow over time after CBJ ingestion. However, this hypothesis was not supported. In fact, the diameter of the vessel decreased over the 6 hours, as did velocity of blood flow. It was speculated that this may have occurred because of an increase in the resistance to blood flow because the participants' blood pressures remained unchanged over time. Resistance was measured as a function of

mean arterial pressure (MAP), vessel diameter, and velocity of blood flow. However, resistance to blood flow and MAP significantly decreased over time, even though there was no difference between the groups. Therefore, there is no logical explanation for the results found in the current study. This study revealed consistent comparisons of the two methods, which may be useful for others wishing to study brachial artery diameters.

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# APPENDIX A

# INFORMED CONSENT

INFORMED CONSENT

Changes in Brachial Artery Diameter and Velocity of Blood Flow During the Six Hours Following Consumption of Cranberry Juice

I,_____, give my informed consent to participate in a study to test the effects of cranberry juice on forearm blood flow. I have been informed that I have documented cardiovascular disease as evidence by one or more of the following, and I can only participate if I have had: myocardial infarction, coronary artery bypass graft, angioplasty or stent, or coronary artery stenosis of 50% or more as documented by angiography. I am age 40-69. I have been informed that I may not participate in this study if I smoke, if I have Raynauds disease, if I have a preexisting heart condition exceeding these limits (Clinical terms: unstable angina pectoris, uncontrolled hypertension, or an ejection fraction less than 40%.) I may not participate if I have diagnosed diabetes, kidney disease, liver disease, or other endocrine disorders. I have been informed that I must abstain from calcium channel-blocker use and long acting nitrates for 24 hours prior to analysis. Because of the effects on blood flow, I am also required to abstain from fruits and antioxidant or vitamin supplements for a 5-day period prior to enrolling in and during these studies to provide a washout of antioxidants from these sources.

I have been informed that the blood flow in my arm will be examined briefly. A blood pressure cuff will be placed over my left forearm where it will be inflated and maintained for 5 minutes. Upon deflation, blood flow will be measured by a painless method called Doppler/Ultrasound three times at one-minute intervals. I have been informed that I will then be placed by chance into one of two groups: a sugar water control (no cranberry juice), or 3 mL/kg of cranberry juice. The same procedures for blood flow are then repeated 2, 4, and 6 hours following the consumption of the assigned beverage. I have been informed that I will be involved in testing on a single day in November 2001. I have been informed that I will need to arrive approximately between 6:00-7:00 AM and remain for approximately six hours at the Cardiology Department at Franciscan Skemp Healthcare.

I have been informed that my participation in this study involves the consumption of pure cranberry juice, which has a bitter taste. I have been informed that my blood pressure will be taken, and I may experience minimal discomfort from the blood pressure cuff. I have been informed that there is experienced, medical staff using sterile and safe equipment. Also, I have been informed that I must fast overnight for nine hours, which may produce an uncomfortable hunger feeling. I have been informed that I will be given 1/2 bagel to eat when I arrive and am otherwise unable to eat or drink any other beverage besides water until completion of the study.

I have been informed that the results of this study may be beneficial to persons with heart disease and to the body of scientific knowledge. I have also been informed that my compensation for participating in this study is $50.

I have been informed that I will be randomly assigned to one of two treatment groups, and that participant treatment group identity will be kept unknown to myself and the technician responsible for collection of the blood flow data. I have been informed that all my individual data will be kept confidential and referred to by identification number only. Group data will be used in publications or presentations with each individual's confidentiality maintained.

I have been informed that my participation in this study is completely voluntary. I am free to stop at any time for any reason without penalty and without loss of benefits to which I am otherwise entitled.

I have been informed that my participation in this study will cost approximately six hours of my time. I am also responsible for travel to and from Franciscan Skemp Healthcare the day of testing.

I have been informed that this study is of minimal risk.

Question or concerns about this study may be referred to Carey Weise at (608) 783-4355, Dr. Ted Wilson at (608) 785-8177, or thesis advisor, Dr. John Porcari at (608) 785-8684. Questions regarding the protection of human subjects may be addressed to the Chair of the UW-La Crosse Institutional Review Board for the Protection of Human Subjects, (608) 785-8124.

Participant's Signature Date

_____ _____

Researcher's Signature Date

_____ _____

Please write your address below for your check and a copy of the results of the study.

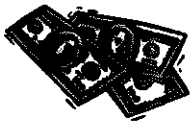
Name_____
Street and Number_____
City, State, and Zip Code_____
Phone_____

# APPENDIX B

# INFORMATIVE HANDOUT

# ***Please help with a Cranberry Juice Study and earn $50!***

We want to show that Cranberry Juice helps protect people from heart disease.



-24 participants are needed to participate in my cranberry juice study. It takes just one long morning. Pick one study day: November 13,14,15,16,18, or 19.

-Participants must be ages 40-69 years, have known heart disease, and cannot be smokers or diabetic.

-Participants must refrain from using vitamins, or other herbal supplements for 5 days prior to the study.

-Study will be done at Franciscan Skemp Healthcare and last about six hours. In this study, blood flow in the arm will be measured by a painless, non-invasive method. (Doppler/Ultrasound)

-Participants will have to drink one glass of cranberry juice or sugar water at 7:00 AM and will finish the study at 1:00 PM.

**For questions or to sign up please see Carey Weise or call 783-4355.**

***Thank You! Your time will really help people with heart disease!***

# APPENDIX C

# BAGEL NUTRITION INFORMATION

Lenders Frozen Original Plain Bagel

Information from Nutrition Facts Label:
Serving Size: 1 bagel/ 57g
150 Calories

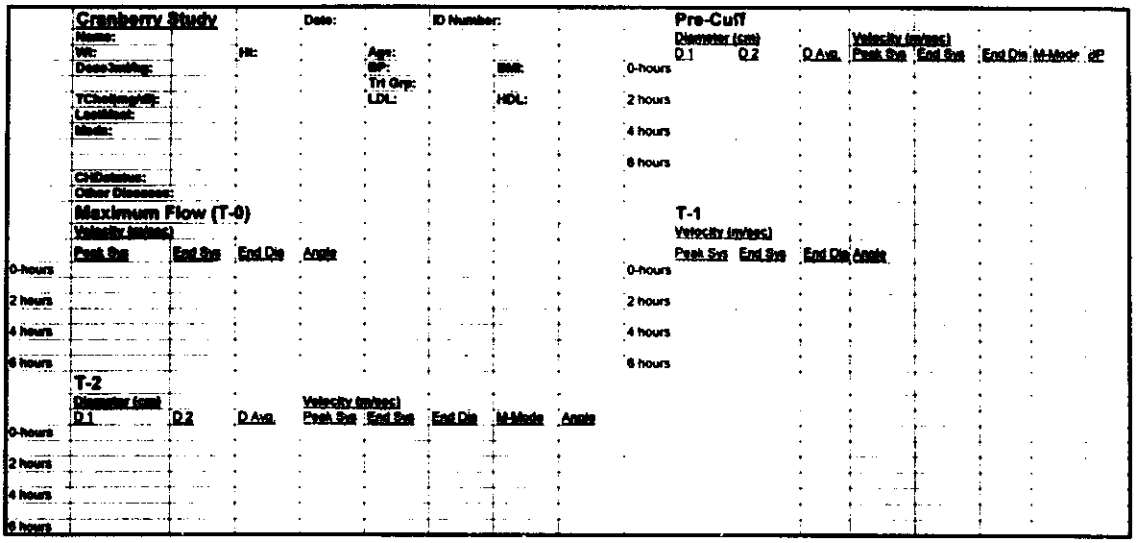
Grams and Percent Daily Value
Total Fat: 0.5g; 1%
Saturated Fat: 0g; 0%
Cholesterol: 0g, 0%
Sodium: 320 mg; 13%
Total Carbohydrate: 30g; 10%
Dietary Fiber: 1g; 4%
Sugars: 2g
Protein: 6g

Percent RDA based on a 2000 calorie diet:
Vitamin A 0%
Vitamin C 0%
Calcium 0%
Iron 10%
Thiamin 15%
Riboflavin 10%
Niacin 10%
Folate 10%

Ingredients:
Enriched flour, malted barley flour, enzyme, niacin, reduced iron, thiamin monoitrate, riboflavin, folic acid, ascorbic acid (dough conditioner), water, high fructose corn syrup, yeast, salt, calcium sulfate, L-cysteine, monohydrochloride (dough conditioner), and yellow corn meal

# APPENDIX D

# DATA COLLECTION SHEET



Cranberry Study

Date:

ID Number:

Name:

Wt:

Ht:

Age:

BP:

Trt Grp:

BMI:

LDL:

HDL:

Maximum Flow (T-0)

Velocity (m/sec)

Peak Sys | End Sys | End Dia | Angle

0-hours

2 hours

4 hours

6 hours

T-2

Diameter (cm) | Velocity (m/sec)

D 1 | D 2 | D Avg. | Peak Sys | End Sys | End Dia | M-Mode | Angle

0-hours

2 hours

4 hours

6 hours

Pre-Cuff

Diameter (cm) | Velocity (m/sec)

D 1 | D 2 | D Avg. | Peak Sys | End Sys | End Dia | M-Mode | dP

0-hours

2 hours

4 hours

6 hours

T-1

Velocity (m/sec)

Peak Sys | End Sys | End Dia | Angle

0-hours

2 hours

4 hours

6 hours

# APPENDIX E

# REVIEW OF RELATED LITERATURE

## REVIEW OF RELATED LITERATURE

### Introduction

Coronary heart disease (CHD) is the number one killer among Americans today (29). For this reason, researchers are studying new ways to prevent it or stop its progression. The French are unusual because even with their smoking habits and higher-fat meals, they tend to have a lower incidence of heart disease than Americans (31). This idea has come to be known as the French paradox, and is thought to be due to the greater amounts of red wine consumed by the French people (26). Consequently, researchers began to study the effects of red wine *in vitro* and *in vivo* and found scientific evidence supporting the French paradox hypothesis. This led to the idea of studying grape juice, since red wine is made from grapes. The flavonoids and polyphenols found in grapes are considered to have cardioprotective qualities (13), which have been confirmed in several other studies (11, 28). Many of these same polyphenols can be found in cranberry juice; and indeed preliminary *in vitro* and *in vivo* studies have suggested that cranberry juice has similar properties as grape juice (21, 33, 34).

A precise way to measure the effects of these compounds on the human body is through a method called Doppler/Ultrasound. This method shows the images of vessels (in this case the brachial artery) on a screen along with sound to interpret blood flow. Blood velocity and diameter of the vessel are used to determine blood flow measurements. Researchers use this method because it is easier to observe the brachial artery compared to the coronary arteries of the heart. Because it is felt that blood flow

through the brachial artery may represent blood flow patterns in the heart, the results from the effects of a test compound on the brachial artery will then be used to extrapolate effects of the same test compound on the coronary arteries.

Studies began with alcohol to observe its effects on the cardiovascular system, and support that it is beneficial to consume 1-2 drinks per day. One study that investigated this theory was conducted by Rimm et al. (27). They examined the existance of an inverse relationship between alcohol consumption and the risk of coronary artery disease (CAD). This study began in 1986 with a detailed food-frequency questionnaire. The subjects included 44,059 men aged 40-75 years. The subjects provided information on medical history, heart disease risk factors, and dietary changes during the previous 10 years. They were then given follow-up questionnaires in 1988 to provide information on newly diagnosed coronary artery disease. The results showed that average daily alcohol intake was inversely related to risk of non-fatal and fatal myocardial infarction. Coronary artery bypass grafting, (CABG), and angioplasty, (PTCA), were also less frequent with increasing alcohol consumption. For all total coronary artery events combined, there was a highly significant inverse relationship between risk of CAD and alcohol consumption. Increasing consumption of alcohol from each beverage type was inversely related to risk of CAD. However, alcohol from spirits had the most pronounced inverse association.

Red Wine Studies

There are many studies that have demonstrated the cardioprotective effects of red wine. However, many still wonder what makes red wine more beneficial to health than other alcoholic beverages. A review by Burns et al., (5) examined alcohol consumption

and mortality to see if wine is different from other alcoholic beverages. The review concluded that both clinical and experimental evidence suggests that red wine does offer a greater protection to health than other alcoholic beverages. The protection is attributed to grape-derived antioxidant polyphenolic compounds found in red wine.

Flavonoids, or derivatives of polyphenols, are the compounds found in fruits, nuts, and berries that are shown to have cardioprotective qualities. Hertog et al. (16) studied dietary antioxidant flavonoids and the risk of CHD. They assessed the flavonoid intake of 805 men aged 65-84 in 1985 by a cross-check dietary history. The men were followed for 5 years. Their major sources of flavonoids included tea, onions, and apples. Flavonoid intake was inversely associated with mortality from coronary heart disease and also showed an inverse relation with incidence of myocardial infarction. The intake of tea, onions, and apples was also inversely related to CHD mortality, but the associations were not as strong. Therefore, this study showed that flavonoids in regularly consumed foods may reduce the risk of death from CHD in elderly men.

Red wine can be beneficial to our health for many reasons. In 1993, Frankel et al. (12) looked at *in vitro* studies with phenolic substances in red wine and human low-density lipoprotein, (LDL), also known as the "bad cholesterol." Elevated LDL may contribute and is a risk factor for heart disease. It was found that red wine inhibits the copper-catalyzed oxidation of LDL and that the non-alcoholic components of red wine have antioxidant properties toward oxidation of LDL.

The effect of red wine consumption on LDL oxidation has also been studied in humans (2). Eighteen males received 400 mL of red wine per day for two weeks. Red

wine consumption resulted in a 20% reduction in the propensity of whole plasma to undergo lipid peroxidation as determined by the thiobarbituric acid reactive substances (TBARS) assay. Wine also reduced the propensity of the LDL to undergo lipid peroxidation induced by copper ions. There was also a significant increase in plasma high-density lipoprotein (HDL) cholesterol and in plasma apolipoprotein A-I concentrations of up to 26% and 12%, respectively.

Miyagi et al. in 1997 (22) investigated the effects of the flavonoids in red wine, white wine, beer, and PGJ on LDL oxidation. The study showed that red wine significantly inhibited LDL oxidation, but white wine and beer did not. The red wine contained a large amount of flavonoids, while the white wine and beer did not. Purple grape juice was found to suppress LDL oxidation, while ethanol alone did not. The study concluded that the *in vitro* antioxidant effect on LDL of red wine, as well as PGJ, is closely associated with flavonoids. However, *in vivo* antioxidant activity was also demonstrated in humans after drinking red wine but not PGJ. This suggests that flavonoids in red wine may be absorbed from the intestine more efficiently than those in PGJ.

An important factor in the progression of atherosclerosis is the shear stress on the endothelium, or the inner lining of the walls of the arteries. Flow-mediated vasodilation, FMD, is a term used to describe the diameter of the blood vessel at baseline compared to the diameter after reactive hyperemia as a percent change (28). When a blood pressure cuff is placed on the arm for 5 minutes, carbon dioxide ($CO_2$) is trapped and cannot be removed. Reactive hyperemia, or a rush of blood into the vessel, is caused by $CO_2$ and

vasodilation of the peripheral arterioles from the $CO_2$ (4). Carbon dioxide causes the smooth muscle around these vessels to dilate, and their internal diameters will widen (4). When the cuff is removed, $CO_2$ can now escape because blood is once again moving through the arteries and veins of the arm, and the vessels are dilated because of the presence of $CO_2$ during the flow obstruction (14). This creates a system where the blood flow into the arm is easier because resistance is now lower than pre-cuff. As a result, more blood moves into the arm and the velocity of the blood in the brachial artery is increased (32).

Velocity of blood flow increases because blood is no longer shut off from the area, and the arm turns red. Increased velocity also causes an increase in the sheer stress put on the endothelial lining of the blood vessel because of viscous drag of the blood against the vascular walls (6). The sheer stress turns on the enzyme nitric oxide synthase (NOS) (24). Nitric oxide synthase releases nitric oxide (NO), which vasodilates the vessels by relaxing smooth muscle in the local arterial wall (18). Nitric oxide is released from the endothelium following its cleavage from arginine (23). These events lead to what is called flow-mediated vasodilation (FMD).

Flow-mediated vasodilation thus reduces resistance even further than originally occurred in response to the $CO_2$ and makes it even easier for blood to move into the arm and wash out the $CO_2$ (19). The effect is transient however, because the $CO_2$ eventually gets washed out, the arm goes from hyperemic (red) to pink and back to normal (4). Blood flow and vessel diameter also return to normal values.

Nitric oxide synthase is stimulated by flavonoids (10), which are compounds found in nuts, vegetables, seeds, and fruit (17). Flavonoids in these beverages may help the vessel make more NO when $CO_2$ or FMD occurs to improve endothelial function (28).

Agewall et al. (1) studied the acute effect of red wine and de-alcoholized red wine on endothelial function. High frequency ultrasound was used to measure blood flow and percentage brachial artery dilation after reactive hyperaemia induced by placing a cuff over their forearm. The subjects drank 250 mL of red wine with or without alcohol over 10 minutes, in random order. The brachial artery was measured again 30 and 60 minutes after drinking. They were then studied within a week using the opposite condition. After the red wine with alcohol, the resting brachial artery diameter, resting blood flow, heart rate, and plasma ethanol increased significantly, however, these were not changed in persons consuming the de-alcoholized red wine. In contrast, flow-mediated dilation of the brachial artery was significantly higher after drinking the de-alcoholized red wine than after the red wine with alcohol. This supports the hypothesis that the antioxidant qualities of red wine, not ethanol, may protect against cardiovascular disease. After drinking red wine with alcohol, the brachial artery dilated and the blood flow increased, and these changes were not seen after the de-alcoholized red wine.

Hashimoto et al., (15) observed the effect of acute intake of red wine on FMD of the brachial artery. The study included 11 healthy men and each drank water, Japanese vodka, red wine, and red wine without alcohol on four different evenings. The ultrasound measurements were taken before intake and at 30 and 120 minutes after

drinking each beverage. Flow-mediated dilation improved 120 minutes after red wine consumption. It also improved 30 and 120 minutes after ingesting red wine without alcohol. It significantly decreased 30 and 120 minutes after Japanese vodka. This again helps to illustrate that the constituents of red wine, not alcohol, improve endothelial function.

Purple Grape Juice Studies

Several investigators have suggested that purple grape juice (PGJ) may provide the same benefits as red wine. Folts et al. (11) conducted a series of studies involving red wine and grape juice. In one study, an *in vivo* animal model was developed to study the interaction of platelets with stenosed and damaged canine coronary arteries. They also showed that cyclic flow reductions (CFRs) can be observed by measuring arterial blood flow with peripheral vascular disease (PVD) and CAD. Cyclic flow reductions can cause acute ischemia and are directly related to *in vivo* platelet activity. The CFRs can be abolished with platelet inhibitors.

Folts et al. (11) studied platelet activity with red wine and grape products. First red versus white wine was studied in the animal model, with the wine given through a stomach tube. Within 90 minutes after the red wine was given, the CFRs were abolished, which suggests that the red wine caused significant *in vivo* platelet inhibition. A similar study was done with white wine, which showed no significant effect on the CFRs, indicating that the white wine did not inhibit platelet activity. The blood alcohol content remained the same in both groups.

Folts et al. (11) studied the effects of French red versus white wine on platelet activity of humans. The subjects were not taking any known platelet inhibitors and refrained from drinking alcoholic beverages. Blood samples were taken four different times. The red wine decreased platelet activity by 39±11%, while white wine did not significantly decrease platelet activity. The blood alcohol content was 0.05 g/dl one hour after both wines. This showed that the red wine inhibited human platelet activity, but the white wine did not.

Because the grapes used for purple grape juice have many of the polyphenolic compounds found in red wine, Folts et al. (11) studied the possible platelet inhibitory effects of Welch's purple grape juice in the animal model. The dogs were given the PGJ through a stomach tube. Again, the CFRs were abolished within 90 minutes. This showed that something in PGJ could also inhibit platelet activity.

Stein et al. conducted an important study in the evolution of PGJ as cardioprotective (28). It was found that PGJ improved endothelial function and reduced the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. Fifteen adults with angiographically documented CAD drank PGJ for 14 days. Flow-mediated vasodilation was measured using high-resolution brachial artery ultrasonography. At baseline, FMD was impaired, whereas after drinking PGJ, FMD increased. In a linear regression model that included age, lipid values, artery diameter, and the use of lipid-lowering and antioxidant therapies, the positive effect of PGJ on FMD was significant. Therefore, the improved endothelium-dependent vasodilation and prevention of LDL oxidation are potential mechanisms by which flavonoids in PGJ may

prevent cardiovascular events, independent of alcohol content. This study, however, did not use a control group.

In 2001, Chou et al. (7) also observed the effect of ingestion of PGJ on endothelial function in CAD patients. They felt that the Stein et al. study was unclear because most subjects also took vitamin E. Therefore, it was unclear if the results were due to PGJ or vitamin E and PGJ combined. This study involved 22 adults with documented heart disease who ingested either 4 or 8 mL/kg/day of PGJ for 56 days. After 28 days, they added vitamin E to their intake of PGJ. After 4 weeks of ingesting PGJ, FMD increased. When vitamin E was added for 4 weeks, FMD did not improve any further. Therefore, both high and low doses of PGJ had similar effects on endothelial function, and adding vitamin E had no further effect.

Because acute cardiac events are associated with decreased platelet-derived nitric oxide (NO) release, Freedman et al. (13) studied the effects of PGJ and PGJ-derived flavonoids on platelet function and platelet nitric oxide production. Twenty healthy subjects consumed PGJ for 14 days. Platelet aggregation was inhibited, platelet-derived NO production increased, and superoxide release decreased after PGJ consumption. Alpha-tocopherol levels also increased significantly after PGJ consumption, and the plasma protein-independent antioxidant activity increased by 50%. Incubation of platelets with select flavonoid fractions isolated from PGJ consistently attenuated superoxide levels, but had variable effects on whole-blood aggregation, platelet aggregation, and NO release. It was determined that both in vitro incubation and oral

supplementation with PGJ decreased platelet aggregation, increased platelet-derived NO release, and decreased superoxide production.

Cranberry Juice Studies

It is known that cranberry juice (CBJ) helps prevent the development of urinary tract infections (UTI) because it reduces the ability of the bacteria to adhere to the urinary tract wall (3). Because CBJ contains flavonoids like red wine and PGJ, researchers started to investigate the cardioprotective effects of this beverage.

Avorn et al. (3) found that there is a reduction of bacteriuria and pyuria after ingestion of cranberry juice. One hundred and fifty-three subjects were tested, and they contributed at least 1 urine sample after baseline testing. The effect was not seen in the first study month; it appeared only after 4-8 weeks of use of the cranberry beverage and persisted at the same level. This study demonstrated that active cranberry compounds are absorbed into the human circulatory system, where they remain active.

This led investigators to study other effects cranberry compounds on the circulatory system. Wilson et al. (33) found that cranberry extract inhibits low density lipoprotein oxidation. The authors collected blood from 5 male volunteers aged 22-33 years old after an overnight fast. This was the first study to show that *in vitro* LDL oxidation can be inhibited by cranberry extracts in a way similar to red wine and PGJ studies and was supported by a further study (34).

The effects of CBJ on vasodilation have also been studied (21). The study examined vascular smooth muscle vasodilatory responses to CBJ were mediated in an endothelium-dependent manner similar to red wine. The results showed that CBJ

vasodilates isolated rat aorta *in vitro*. It also showed that CBJ infusion transiently reduces blood pressure in anesthetized rats. The CBJ infusion into anesthetized rats produced a hypotensive/cardioacceleratory effect.

Endothelial dysfunction and its effect on blood flow

As mentioned earlier, endothelial dysfunction has been associated with coronary heart disease and increased oxidative stress (8). An improvement in endothelial function would mean an improvement in blood flow to the heart, which would be especially beneficial to patients with CAD.

Lerman et al. (20) studied the relationship between endothelial dysfunction and atherosclerosis. Twenty subjects had their coronary and circulating endothelin concentrations measured at baseline and with acetylcholine administration while undergoing diagnostic coronary angiography. They were divided into 2 groups: group 1 had a normal vasodilatory response, while group 2 had coronary vasoconstriction. The result was that the baseline concentrations of endothelin were higher in those with coronary vasoconstriction. Thus in people with coronary endothelial dysfunction, endothelin concentrations are increased, which can be associated with coronary vasoconstriction.

In 2000, Suwaidi et al. (30) conducted a long-term follow-up study on patients with mild coronary artery disease and endothelial dysfunction. Follow-up was obtained in 157 patients with mildly diseased coronary arteries. They were divided into three groups based on their response to acetylcholine: group 1 included patients with normal endothelial function, group 2 included patients with mild endothelial dysfunction, and

groups 3 included patients with severe endothelial dysfunction. During the 28-month follow-up period, none of the patients in group 1 or 2 had cardiac events. However, 6 patients from group 3 had 10 cardiac events, which included myocardial infarction, percutaneous or surgical coronary revascularization, and/or cardiac death. The study concluded that severe endothelial dysfunction without the presence of obstructive coronary artery disease is associated with increased cardiac events. This supports the idea of how endothelial dysfunction plays a role in the progression of coronary atherosclerosis.

Plotnik et al. (25) looked at the effects of antioxidant vitamins on vasoactivity of the brachial artery after a high-fat meal. Twenty subjects fasted overnight, had their blood drawn, and had brachial artery vasodilation, blood pressure, and heart rate assessed. They then ate a high-fat meal followed by oral ingestion of the antioxidants vitamin C and vitamin E, in random order. Ten of the twenty subjects also had a low-fat meal immediately following similar vitamin intake. The studies were separated by one week. Lipoprotein and glucose determinations were repeated 2 and 4 hours after eating. No significant changes in flow-mediated vasodilation occurred after the low-fat meal, high-fat meal with vitamins, or low-fat meal with vitamins. The study did determine, however, that a single high-fat meal transiently reduces endothelial function for up to 4 hours in healthy subjects with normal cholesterol levels. This is due probably to the accumulation of triglyceride-rich lipoproteins. The decrease in endothelial function is blocked by pretreatment with antioxidant vitamins C and E, which suggests an oxidative mechanism.

Duffy et al. (9) studied the effects of tea on endothelial function because it too contains antioxidant flavonoids. Sixty-six patients with documented CAD consumed tea or water. They made 3 visits, each 4 weeks apart. The patients fasted overnight, didn't smoke for 24 hours, withheld vasoactive medications for 12 or more hours, and withheld long-acting vasoactive medications for 24 or more hours. They maintained their normal diet, but excluded red wine and tea consumption during the study. Endothelial function was assessed at six times: baseline, 2 hours after consumption of 450 mL of tea (short-term tea), after consumption of 900 mL of black tea daily for 4 weeks but none the morning of study (long-term tea), 2 hours later that day after 450 mL of tea (short-on-long-term tea), after 900 mL of water daily for 4 weeks (long-term water), and 2 hours after 450 mL of water (short-term water). The study concluded that both short-and long-term tea consumption improved FMD of the brachial artery, whereas water had no effect. In addition, consumption of short-term tea after four weeks of tea (short-on-long-term) produced further improvement of FMD compared with long-term tea. Tea had no effect on baseline arterial diameter, maximal hyperemia, diastolic blood pressure, or heart rate. Short-term tea ingestion increased systolic blood pressure by 5 mm Hg; however, this effect did not persist with long-term tea consumption.

Summary

Many studies have been done to observe the effects of various beverages on the cardiovascular system. While it is proven that beverages containing flavonoids are beneficial to health, more research is needed to determine exactly how flavonoids are cardioprotective and the dose is needed for an effect. An improvement in blood flow to

the heart due to flavonoid-induced vasodilation would mean less work for the heart and better health for the patient.

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