Grapefruit Juice: Potential Functional Drink for Improved Antioxidant Capacity in Adults

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Abstract
Cardiovascular disease is attributed to sedentary lifestyle, diets high in fat and refined carbohydrates, and low in fruits and vegetables. The objective of this study was to determine whether drinking grapefruit juice positively influences cardiovascular risk markers, antioxidant status, and lipid peroxidation in adults not changing their eating habits. An experiment to test whether daily consumption of 20 fl. oz. of freshly squeezed grapefruit juice for two months affected lipid panels, C-reactive protein, insulin, body composition, blood pressure, antioxidant status, and malondialdehyde production was conducted. Fasting blood samples were collected pre-treatment and 60 days after drinking grapefruit juice post-treatment. Drinking grapefruit juice did not affect (P > 0.1) plasma total cholesterol, triglycerides, Apo A, Apo B, LDL, HDL, body fat percentage, BMI, blood pressure, plasma insulin, or C-reactive protein. Drinking grapefruit juice did not affect systolic and diastolic blood pressure. However, drinking grapefruit juice significantly (P < 0.05) increased total plasma antioxidant capacity and decreased (P < 0.05) plasma malondialdehyde concentration. Drinking grapefruit juice may be cardio-protective via increasing total antioxidant status and decreasing lipid peroxidation independent of any of the cardiovascular risk markers measured in the study.

Keywords: Antioxidant capacity; Grapefruit juice; Lipid peroxidation; Lipid panels; Blood pressure

Introduction
Cardiovascular disease (CVD) is the leading cause of death in the United States with an average mortality rate of 2,200 individuals per day, which is equivalent to one death every 39 seconds[8]. Cardiovascular disease is a multifaceted problem thought to arise from numerous factors including eating habits and diets high in saturated fats[9,10]. Oxidative stress is a free radical that may result in vascular damage, impaired endothelium vasodilatation, and atherogenesis[5-7]. Oxidative stress may result in the formation of oxidized LDL, a process largely recognized to be critical for atherosclerosis[8-11]. Oxidative stress are known to have detrimental effects on proteins, lipids, and nucleic acid, which may lead to subsequent cellular dysfunction and apoptosis[12]. Research has shown that oxidative stress also results in cardiomyocyte cell death[13]. Collectively, there seems to be an association between high levels of oxidative stress and increased incidence of CVD.

Among dietary sources, plant foods, in particular fruits...
and vegetables are considered to be the richest sources of anti-
oxidants. Plant sources are considered to have medicinal proper-
ties with antioxidative capabilities, which can inhibit oxidative
stress. This hypothesis stem from a study concluding that eating
diets rich in antioxidants protect cardiovascular by inhibiting
free radical formation[14]. Furthermore, epidemiological studies
have observed lower incidence of CVD in individuals who con-
sumed higher amount of fruits and vegetables than populations
who consume lower amounts of it[15-18].

The cardio-protection observed from these studies may be
partially attributed to high antioxidant contents of fruit and
vegetables.

Studies have attempted to assess how different antioxi-
dants found in fruits and vegetables (e.g. β-carotene, vitamin
C, vitamin E, resveratrol, and lycopene) reduce CVD risk[19-22].
Some antioxidants in fruits and vegetables appear to robustly
protect against CVD. For instance, polyphenols like hesperdin,
naringin, and monoterpenes, which are naturally occurring bio-
active compounds in citrus fruits, were able to inhibit LDL oxida-
tion in vitro[23-27]. In one study, subjects who drank fresh carrot
juice, naturally rich in β-carotene, for three months, showed an
improvement in antioxidant status and decreased lipid peroxi-
dation[28]. A recent study from our research group observed an
increase in total antioxidant status and decreased lipid peroxi-
dation in adults consuming 20 fl. oz. of fresh squeezed orange
juice for 90 consecutive days[29]. In several animal studies, rats
drinking orange juice or eating orange and grapefruit pulp re-
sulted in improving antioxidant status, reducing oxidative stress,
and decreasing lipid peroxidation in these animals[30-32]. Oranges
and grapefruits are rich sources of vitamin C, which can protect
the cardiovascular system against oxidative stress[33]. An epide-
mologic study found that subjects who consumed vitamin C
had lower CVD mortality rates[33]. Interestingly, vitamin C con-
tributes to as much as 66% to 77% of the antioxidant content in
grapefruit juice[33].

Dietary Guidelines for Americans based on a healthy
2000 calorie is 2 cups of fruit servings per day. According to
dietary guide line suggests 1 cup of 100% fruit juice equivalent
to 1 of whole fruit[34]. The goal of this study was to evaluate
whether drinking 20 fl oz (2 ½ cups) freshly squeezed grape-
fruit juice daily for 60 days increase total antioxidant capacity
while decrease lipid peroxidation in adults with elevated plasma
cholesterol and triglycerides levels. Additionally, we wanted to
determine if drinking grapefruit juice lowers plasma cholesterol
and triglycerides in these subjects.

Materials and Methods

Demographics of the Study Population

The research study was approved by the Texas
A&M University-Kingsville Institutional Review Board (TA-
MUK-IRB) prior to initiating the study. Participants in this
study were 12 males and 12 females, were diagnosed with el-
evated plasma cholesterol and triglyceride levels. Participants
age ranged from 45 to 55 years. Participants were asked not to
take multi-vitamins and minerals for the duration of the study.
Subjects did not use tobacco nor were they taking any prescribed
medication. Subjects were asked not to change their lifestyle or
dietary habits other than drinking grapefruit juice. Participants
agreed to drink the grapefruit juice daily and signed a consent
form to agree with the rules and regulations of the study. Grape-
fruit was juiced and delivered to research participants on a daily
basis. Each subject was asked to drink 20 fl oz of grapefruit juice
daily as a morning snack for the two months duration (60 days)
of the study. Weight and height were measured both pre-treat-
ment and post-treatment to calculate BMI. After 12 hours of
fasting, blood samples were collected and plasma was harvested
by a licensed nurse at the start of the study and after 60 days
when the study was concluded. Blood was centrifuged at 1,500x
g for 15 minutes to obtain the plasma. Lipid panels including
triglyceride, cholesterol, HDL, VLDL, LDL, Apo A, Apo B, and
Lp (a), were analyzed using Modular Analytics D 2400 system
and Cobas Integra 800 of Roche Diagnostics Corp. Indianapolis,
IN.

Blood Pressure Collection

Three blood pressure measurements from the left upper
arm were taken and the values were averaged using the OMRON
Model #HEM 711 (Omron Healthcare Inc, Vernon Hills, IL) at
the beginning and end of the study as described previously[28,29].
Blood pressure was taken from each subject and repeated if: 1) an
error occurred with the reading, 2) subjects seemed anxious
or nervous, or 3) blood pressure measurement was above the
normal range (120 mm Hg/80 mm Hg).

Bioelectrical Impedance Analysis

Body fat percent was assessed by bioelectrical Imped-
ance Analysis (BIA) using Quantum II (RJL Systems, 2006,
Clinton Twp., MI). BIA was performed while each subject
was lying supine with their arms and legs spread open. After
the electrode site was cleaned with isopropyl alcohol, electrode
patches with self-adhesive conducting gel were attached to the
dorsal surface of the right foot and right hand. The electrodes
introduced an alternating current (50 kHz) at the base of the toes
and fingers with the Quantum II measuring the voltage changes.
Body fat percentage was assessed at the beginning and end of
the study.

C-reactive Protein and Insulin

Plasma C-reactive protein was analyzed using a C-re-
active protein ELISA kit as an index for inflammation (Life Di-
agnostics, Westchester, PA, USA). Plasma insulin was evaluated
to assess insulin resistance (Linco Research, Inc. St. Charles,
MI).

Total Antioxidant Status and Malondialdehyde Production

After the plasma was obtained, an aliquot was refriger-
ated for total antioxidant status using a commercially available
kit (Calbiochem, San Diego, CA, USA) as a quantitative mea-
sure of circulating antioxidant status. Also, an aliquot of plasma
samples was used to evaluate malondialdehyde using a kit from
Northwest Life Science (Vancouver, WA, USA) as an indicator
of lipid peroxidation.

Statistical analysis

The study used a pre/post-test within gender and t-test
between genders to determine the effects of drinking 20 fl oz
fresh grapefruit juice daily as an independent variable on vari-
obles of interest at baseline and 60 days after the initiation of the
study, as described elsewhere[35].
Results

Drinking 20 fl oz of grapefruit juice daily for two months did not (P > 0.1) change subject weight, body fat percentage, BMI, or blood pressure (Table 1). Drinking grapefruit juice also did not affect fasting plasma triglycerides or total cholesterol, nor did it alter plasma HDL, LDL, VLDL, Apo A, Apo B, Lp (a), C-reactive protein, or insulin levels (Table 1). However, plasma antioxidant status significantly (P < 0.05) increased while plasma malondialdehyde concentrations significantly (P < 0.05) decreased with drinking grapefruit juice (Table 1).

Table 1: Effects of drinking orange juice on anthropometrics, blood pressure, lipid panel, insulin, inflammatory marker, and antioxidant status.

| Variables                  | Pre-Test | Post-Test |
|----------------------------|----------|-----------|
| Age (yrs)                  | 50 ± 2   | 50 ± 2    |
| Body weight (kg)           | 91 ± 3   | 92 ± 3    |
| BMI                        | 31 ± 1   | 31 ± 1    |
| Fat %                      | 31 ± 1   | 31 ± 1    |
| Systolic (mm Hg)           | 127 ± 6  | 128 ± 7   |
| Diastolic (mm Hg)          | 75 ± 4   | 75 ± 4    |
| TAG (mg/dl)                | 140 ±13  | 155 ±13   |
| Cholesterol (mg/dl)        | 216 ± 9  | 225 ± 9   |
| HDL (mg/dl)                | 51 ± 2   | 52 ± 2    |
| VLDL (mg/dl)               | 30 ± 1   | 31 ± 1    |
| LDL (mg/dl)                | 143 ± 6  | 152 ± 6   |
| Apo A (mg/dL)              | 145 ± 7  | 152 ± 7   |
| Apo B (mg/dL)              | 103 ± 4  | 106 ± 4   |
| Lp (a) (mg/dL)             | 12 ± 2   | 13 ± 2    |
| Insulin (μU/mL)            | 19 ± 3   | 20 ± 3    |
| CRP (mg/L)                 | 7.022 ± 0.50 | 7.194 ± 0.50 |
| Total antioxidant capacity (mM) | 1.21 ± 0.10 \* | 1.63 ± 0.10 \* |
| Malondialdehyde (μM)       | 43 ± 2 \* | 10 ± 2 \* |

\*Means ± SEM with unlike superscript are significantly (P ≤ 0.05) different from each other.

As in our previous studies, we also tested for potential differences on variables of interest between genders\(^{28,29}\). As expected, male participants weighed more than females and females had a significantly higher (P < 0.05) body fat percentage than males. Excluding HDL the other lipid panels, blood pressure, and plasma insulin were not significantly (P > 0.1) different between genders (Tables 2 and 3). Interestingly, percentage of body fat and C-reactive protein were significantly higher in females, while plasma concentrations of malondialdehyde and antioxidant capacity were significantly (P < 0.05) higher in males prior to drinking grapefruit juice. Drinking grapefruit juice, however, significantly (P < 0.05) lowered malondialdehyde and increased plasma antioxidant capacity in both genders (Table 3).

Table 2: Gender differences on anthropometrics, Blood Pressure and lipid panels.

| Variables                  | Female | Male |
|----------------------------|--------|------|
| Age (years)                | Pre 50 ± 5 | 50 ± 5 |
|                           | Post 50 ± 5 | 50 ± 5 |
| Body weight (Kg)          | Pre 79 ± 4  | 102 ± 5 |
|                           | Post 80 ± 4  | 104 ± 5 |
| BMI                       | Pre 31 ± 2  | 31 ± 2 |
|                           | Post 31 ± 2  | 31 ± 2 |
| Fat (%)                   | Pre 43 ± 2 \* | 19 ± 2 \* |
|                           | Post 46 ± 2 \* | 21 ± 2 \* |
| Systolic pressure (mm Hg) | Pre 124 ± 8 | 130 ± 8 |
|                           | Post 125 ± 8 | 131 ± 8 |
| Diastolic pressure (mm Hg)| Pre 73 ± 8  | 77 ± 8 |
|                           | Post 74 ± 7  | 75 ± 8 |
| TAG (mg/dl)               | Pre 125 ±16 | 156 ±16 |
|                           | Post 141 ± 19 | 169 ±19 |
| Cholesterol (mg/dl)       | Pre 220 ± 12 | 210 ±11 |
|                           | Post 230 ± 12 | 220 ±11 |
| HDL (mg/dl)               | Pre 56 ± 4 \* | 46 ± 4 \* |
|                           | Post 54 ± 4 \* | 47 ± 4 \* |
| VLDL (mg/dl)              | Pre 25 ± 3  | 32 ± 3 |
|                           | Post 28 ± 4  | 34 ± 4 |
| LDL (mg/dl)               | Pre 144 ± 9  | 139 ± 9 |
|                           | Post 151 ± 10 | 153 ±10 |

a,bMeans ± SEM with unlike superscript are significantly (P ≤ 0.05) different from each other.

Table 3: Gender differences on cardiovascular, insulin, inflammatory marker and antioxidant status.

| Variables              | Female | Male |
|------------------------|--------|------|
| Apo A (mg/dL)          | Pre 150 ± 10 | 133 ± 9 |
|                        | Post 155 ± 11 | 148 ± 11 |
| Apo B (mg/dL)          | Pre 102 ± 5  | 103 ± 5 |
|                        | Post 109 ± 5  | 103 ± 5 |
| Lp (a) (mg/dL)         | Pre 13 ± 3   | 11 ± 3 |
|                        | Post 13 ± 4   | 10 ± 4 |
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### Table 1: Antioxidant Capacity of Grapefruit Juice

|                          | Pre          | Post         |
|--------------------------|--------------|--------------|
| **Insulin (μU/mL)**      | 18.4 ± 5     | 19.8 ± 5     |
| **CRP (mg/L)**           | 22.0 ± 5     | 17.7 ± 5     |
| **Total antioxidant capacity (mM)** |            |              |
| Pre                      | 1.10 ± 0.10c | 1.31 ± 0.12c |
| Post                     | 1.40 ± 0.10b | 1.85 ± 0.12c |
| **Malondialdehyde (μM)** |              |              |
| Pre                      | 38 ± 3a      | 50 ± 3a      |
| Post                     | 8 ± 3c       | 12 ± 3c      |

*Means ± SEM with unlike superscript are significantly (P ≤ 0.05) different from each other.

**Discussion**

In the present study, we have examined the effect of drinking 20 fl oz of grapefruit juice on CVD risk markers independent to changes in diet and lifestyle. This method of evaluation on CVD risk markers provides a more realistic “real-life scenario” study, since most people have hard time to permanently change eating habits. Further, eating habits drastically differ among individuals.

In the current study, we took a simple, yet novel approach by testing whether adults drinking 20 fl oz of freshly squeezed grapefruit juice as a daily morning snack for two months would provide cardioprotective benefits without implementing any other changes to diet or lifestyle. Despite no changes in anthropometric values, lipid panels, insulin, and C-reactive protein, grapefruit juice contributed to a 35% increase in total antioxidant status. A 76% reduction in lipid peroxidation was also observed, which was determined by measuring plasma malondialdehyde levels. Vitamins and bioactive compounds in grapefruit juice likely contributed to improving antioxidant status and decreasing lipid peroxidation. These findings are consistent with our previous human and animal studies[28,31,32]. Similar to our previous work on drinking 20 fl oz daily of either freshly squeezed carrot juice or orange juice, drinking grapefruit juice did not affect plasma insulin and C-reactive protein.

**Conclusion**

The cardio-protective effect of grapefruit juice to decreased lipid peroxidation is directly related to increase in antioxidant status.

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