

January 19, 2006

The acute effects of *citrus aurantium* extract
on blood pressure and heart rate

by

John G. Seifert, PhD, Aaron Nelson, MS, Julia Devonish, MS,
and Edmund R. Burke, PhD

¹ Human Performance Laboratory
St. Cloud State University
720 4th Avenue South
St. Cloud State University
320-308-3637 (phone)
320-308-5399 (fax)
jseifert@stcloudstate.edu

and

² Dept of Biology
Colorado University – Colorado Springs
Colorado Springs, CO

Running title: *Citrus aurantium* and cardiovascular responses

This study was funded by a research grant from Enforma Natural Products
Status: currently under review for publication

ABSTRACT

Ephedrine-type compounds may lead to increases in heart rate and blood pressure. Citrus aurantium (CA; sometimes called bitter orange) is a thermogenic compound that contains synephrine. Synephrine is an alkaloid compound that shares a similar chemical structure to ephedrine, but has a shorter half-life than ephedrine.

PURPOSE: To investigate the cardiovascular effects of CA ingestion, in an herbal blend, on mildly overweight individuals.

METHODS: Fourteen females and nine males (Mean \pm SD) age 24.7 ± 7.4 yrs, BMI: 26.6 ± 3.8) volunteered in this placebo crossover, double-blind designed study. Subjects refrained from exercise for 24 hours before their trials and entered the laboratory following an overnight fast. Subjects ingested four capsules in the experimental treatment for a total of 52 mg synephrine and 704 mg caffeine over a 24 hour period or a non-caloric placebo. Subjects sat quietly for 60 min. Heart rate and BP were recorded at 60 min. Expired air was analyzed for the next 10 min of that session.

RESULTS: No between group or within group differences were observed for HR and BP during the CA and PL trials when data were analyzed for gender and caffeine usage. RER decreased significantly for the low caffeine users when subjects ingested CA, from 0.84 ± 0.05 to 0.81 ± 0.04 .

CONCLUSIONS: The results of this study indicate that acute CA ingestion did not lead to increased cardiovascular stress and that fat oxidation increased in certain populations.

Key words: Citrus aurantium, synephrine, blood pressure, heart rate, bitter orange

Introduction

Roughly 60% of the American population is overweight with about 21% being obese¹³. Billions of dollars are spent each year in efforts to lose weight and change body composition⁴. Some diet aids work by suppressing appetite, others stimulate the central nervous system, while others work through the generation of body heat (thermogenesis). This slight rise in body temperature increases metabolic rate within the tissues. As metabolic rate increases, caloric expenditure and fat metabolism increase as well.

When the central nervous system is stimulated, sympathetic nervous system function may be accelerated, too. Alpha- and beta- adrenergic receptors make up part of the sympathetic system. When the alpha-adrenergic sympathetic system is stimulated, blood pressure increases via vasoconstriction. When the beta₁ adrenergic receptors are stimulated, heart rate increases. Glycogenolysis and metabolism increase when the beta₂ and beta₃ receptors are stimulated^{9,14}. The body's primary adrenergic stimulants are epinephrine and norepinephrine. However, ephedrine, pseudophedrine, ephedra, and caffeine may also stimulate the adrenergic system. Although these stimulants show some effectiveness for weight loss, they also exert considerable influence over the cardiovascular system^{11,15}. In one study, when subjects ingested 20 mg of ephedra alkaloids and 150 mg of caffeine, heart rate and blood pressure increased significantly over a placebo condition¹⁵. Additionally, Haller et al.¹¹ reported that heart rate and blood pressure increased by 9.4 bpm and 11.5 mm Hg, respectively, when subjects ingested 50 mg ephedra alkaloids and 400 mg caffeine.

There is widespread interest in moving from those diet aids that lead to cardiovascular stimulation to those which are generally considered safe and effective. One such product that has generated much interest, because of its positive effects on weight loss and thermic effect of food, is citrus aurantium, also known as bitter orange^{7,13}. Citrus aurantium is a citrus fruit (typically immature) from which extracts are derived. These derived extracts contain the alkaloid synephrine and small amounts of tyramine, octopamine, hordenine, and n-methyltyramine. Synephrine has six possible isomers (para, meta, and ortho each in d- and l- forms)¹ and is structurally similar to epinephrine. It is well known that epinephrine can raise heart rate and blood pressure. Due to the structural relationship of synephrine to epinephrine, many concerns have been raised as to the safety of citrus aurantium^{1,6,8,10,12}. Penzak et al.¹² and Haller et al.¹¹ suggested that citrus aurantium may lead to increases in heart rate, blood pressure, and adverse cardiovascular events in humans.

What is not known is if citrus aurantium will stimulate the cardiovascular system when given over a 24 hour time period compared to a single dose feeding. Therefore, the purpose of this study was to investigate the cardiovascular effects of acute ingestion of citrus aurantium on mildly overweight individuals.

Methods

Twenty-three subjects volunteered to participate in this double blind, placebo controlled cross-over study. Fourteen subjects were female and nine were male, see Table 1 for subject characteristics. The Saint Cloud State University Institutional Review Board approved this study prior to data collection. All subjects completed a health history questionnaire and provided informed consent.

Table 1. Subject characteristics

	Age (yrs)	Height (m)	Weight (kg)	BMI
Overall	24.5 (7.4)	1.74 (.09)	81.1 (17.1)	26.6 (3.8)
Females (n=14)	25.4 (9.3)	1.68 (.07)	72.4 (11.8)	25.5 (3.2)
Males (n=9)	23.1 (2.5)	1.82 (.06)	94.5 (15.7)	28.3 (4.1)

Mean (\pm SD)

Two treatments were tested in this study, the experimental intervention which contained the active ingredients of citrus aurantium and guarana (Acceleron®; Advantra Z®, Nutratch Inc., Wayne, NJ) and a non-caloric placebo. Each capsule of the experimental treatment contained active ingredients of citrus aurantium (6% synephrine concentration yielding 13 mg synephrine) and 176 mg caffeine (see Table 2). While on the experimental treatment, each subject consumed four capsules for a total of 52 mg synephrine and 704 mg caffeine over a 24 hour period.

Table 2. Ingredients list (per capsule).

<u>Ingredient</u>	<u>Amount</u>
Citrus Aurantium	
P-Synephrine	13 mg
Guarana (caffeine)	176 mg
Green Tea Powder extract	55.5 mg
Bee Pollen	1 mg
White Willow Bark Powder	1 mg
Panax Ginseng Root	2 mg
Garcinia Cambogia extract	2 mg
Vanadium	0.15 mcg

On a given treatment, day 1 was used to collect baseline data. All subjects were instructed to refrain from exercise 24 hours before their trial. Dietary intake was not controlled, but all subjects were instructed to maintain a consistent intake from trial to trial. Subjects entered the laboratory in the morning following an overnight (12 hour) fast. They sat quietly for 30 minutes in a comfortable chair in a room with dim light.

Expired air was collected for 10 minutes from 20 to 30 minutes of this session as subjects breathed through a one-way breathing valve. Expired air was collected in Douglas bags and contents measured by Ametek Oxygen and Carbon Dioxide Analyzers (Thermox, Pittsburgh, PA) while volume was measured by a Tissot. Indirect calorimetry was used to determine oxygen uptake (VO_2) and carbon dioxide production (VCO_2). The non-protein respiratory exchange ratio (RER) value was calculated from VO_2 and VCO_2 . The RER was used as an index of fat and carbohydrate oxidation. Heart rate (Polar HR monitor, Stamford, CT) and blood pressure were collected at 30 minutes.

Following baseline data collection, subjects were given three treatment capsules and instructed to ingest one capsule with each meal during that day. Subjects entered the laboratory again on the next day following an overnight fast and were given the fourth treatment capsule with water. Subjects then sat quietly for 60 minutes. The 60 min period was estimated to give maximal blood concentration. Expired air was then collected and analyzed during the next 10 minutes of the session. Heart rate and blood pressure were recorded at the end of expired air collection. Subjects repeated the protocol one week later with the other treatment.

Three analyses of the data were undertaken using 2x2 ANOVA. First, analysis of the data was undertaken in which treatment and time were the

independent factors using all 23 subjects. The second analysis was done to separate and analyze the data according to gender. The third analysis was used to analyze the data for differences in caffeine and non-caffeine users. Fourteen subjects were classified as non-caffeine users and nine as caffeine users. Classification was based on the breakpoint of 150 mg caffeine per day. Statistical significance was established at $p < .05$. All data are listed as mean \pm standard deviation.

Results

All 23 subjects completed the study. Based on self-report and questionnaire, all subjects ingested the given capsules on time as noted in the Methods section. No adverse treatment effects were observed or reported by the subjects.

Table 3 contains the cardiovascular data from the entire subject population. No statistical differences were observed within groups or between groups for any of the measures. All variables remained constant from the baseline to the post ingestion collection period. Three subjects (1 male and 2 females) were pre-existing hypertensives (systolic blood pressure > 140 mmHg). Blood pressure, for all three, changed similarly regardless of treatment.

Table 3. The effects of supplementation on cardiovascular and metabolic parameters (n=23)

Treatment	Heart Rate	Systolic BP	Diastolic BP	MAP	RER
PL pre	62.4 (11.8)	119.9 (12.0)	77.7 (8.9)	91.8 (9.1)	0.85 (.07)
PL post	60.7 (12.5)	118.7 (10.2)	76.7 (7.8)	90.7 (8.5)	0.86 (.05)
CA pre	63.5 (14.2)	119.2 (14.3)	76.9 (8.4)	91.0 (9.8)	0.85 (.06)
CA post	60.9 (12.4)	118.9 (11.1)	79.2 (6.4)	92.4 (7.3)	0.83 (.07)

PL: Placebo; CA: Citrus aurantium; Mean \pm SD; Heart rate (bpm); BP: Blood Pressure (mmHg); MAP: Mean Arterial Pressure; RER: Respiratory Exchange Ratio

Table 4 contains data separated by gender. No significant differences were observed when data were broken into gender categories. All changes were not statistically significant (effect sizes were below 0.2 for all variables). Likewise, males did not demonstrate a significant cardiovascular response to citrus aurantium ingestion.

Table 4. The effects of gender and supplementation on physiological function.

Females (n=14)

Treatment	Heart Rate	Systolic BP	Diastolic BP	MAP	RER
PL pre	65.9 (13.2)	117.9 (12.6)	76.3 (10.5)	90.1 (10.4)	0.86 (.08)
PL post	65.1 (13.1)	117.2 (12.6)	76.1 (8.9)	89.8 (9.5)	0.85 (.05)
CA pre	70.2 (13.8)	112.8 (12.4)	74.2 (8.8)	87.1 (9.4)	0.84 (.05)
CA post	65.5 (12.4)	116.2 (10.3)	79.4 (5.9)	91.7 (7.0)	0.82 (.09)

Males (n=9)

Treatment	Heart Rate	Systolic BP	Diastolic BP	MAP	RER
PL pre	56.9 (6.3)	123.0 (11.0)	80.0 (5.2)	94.3 (6.4)	0.85 (.05)
PL post	53.9 (8.0)	120.9 (11.9)	77.7 (6.3)	92.1 (7.2)	0.86 (.05)
CA pre	53.1 (7.0)	128.1 (11.4)	91.0 (6.2)	97.1 (7.3)	0.85 (.06)
CA post	53.8 (8.8)	123.0 (11.6)	78.8 (7.5)	93.5 (8.0)	0.83 (.05)

PL: Placebo; CA: Citrus aurantium; Mean \pm SD; Heart rate (bpm); BP: Blood Pressure (mmHg); MAP: Mean Arterial Pressure; RER: Respiratory Exchange Ratio

The final analysis included data separated by caffeine and non-caffeine users can be found in Table 5. As with the previous analysis, no significant differences were observed for the cardiovascular data. There was a significant change in RER data for the non-caffeine users. After ingestion of citrus aurantium, non-caffeine users demonstrated a significant decrease in RER indicating greater fat oxidation.

Table 5. The effects of caffeine use and supplementation on physiological responses.

Low-caffeine users (n=14)

Treatment	Heart Rate	Systolic BP	Diastolic BP	MAP	RER
PL pre	63.4 (11.9)	120.6 (12.1)	79.8 (9.6)	93.4 (9.7)	0.84 (.06)
PL post	63.4 (13.1)	117.9 (12.7)	78.1 (8.2)	91.3 (8.9)	0.84 (.04)
CA pre	65.8 (15.2)	119.4 (13.6)	77.9 (9.2)	91.8 (10.3)	0.84 (.05)
CA post	64.7 (13.7)	120.6 (11.1)	80.6 (6.3)	94.0 (7.3)	0.81 (.04) *

*: Significantly different from pre-ingestion value

High Caffeine users (n=9)

Treatment	Heart Rate	Systolic BP	Diastolic BP	MAP	RER
PL pre	60.9 (12.1)	118.8 (12.5)	74.6 (7.0)	89.3 (8.1)	0.87 (.09)
PL post	56.7 (10.9)	119.8 (12.2)	74.7 (7.2)	89.7 (8.3)	0.88 (.06)
CA pre	60.0 (12.6)	118.9 (16.2)	75.2 (7.2)	89.8 (9.5)	0.86 (.06)
CA post	55.0 (7.3)	116.1 (11.1)	76.9 (6.2)	90.0 (7.0)	0.86 (.10)

PL: Placebo; CA: Citrus aurantium; Mean \pm SD; Heart rate (bpm); BP: Blood Pressure (mmHg); MAP: Mean Arterial Pressure; RER: Respiratory Exchange Ratio

Discussion

The results of this study indicate that acute ingestion of citrus aurantium did not lead to negative cardiovascular effects, regardless of subject grouping (by gender or caffeine usage). Subjects in the present study ingested 39 mg of synephrine and 528 mg of caffeine the day before testing followed by ingestion of an additional 13 mg synephrine and 176 mg caffeine the next morning following an overnight fast. It was thought that this administration protocol would establish an environment where tissues would be saturated before the post-test phase. Haller et al.¹¹ noted that blood synephrine levels remained elevated for approximately 6-8 hours after ingesting a single dose of 46.9 mg of synephrine. Although not tested, by giving three doses in one day, it was expected that blood levels would remain elevated throughout the day.

Citrus aurantium contains the adrenergic amines of synephrine, octopamine, tyramine, hordenine, and n-methyltyramine. These amines can be found in the m- and p- isomer forms. Brown et al.³ reported the m- isomers were significantly more potent than the p- isomer forms for both synephrine and octopamine. Numerous researchers have reported on the influence, or potential influence, of citrus aurantium on the cardiovascular system^{8, 11, 12}. However, these researchers do not make the distinction whether the m- or p- isomers were used in their respective studies and how that would affect physiological and

pharmacokinetic responses. For example, Haller et al.¹¹ mentions that phenylephrine is used to treat nasal congestion and hypotension implying that similar physiological responses would be expected by ingesting dietary supplements containing citrus aurantium, failing to distinguish that it is only the m-isomer of synephrine which bears resemblance to phenylephrine. In a technical note obtained from Nutratech, Inc, the citrus aurantium used in the present study contained 100% of the p-synephrine isomer.

Brown et al.³ noted that the m-synephrine isomer, specifically, was a selective alpha adrenergic agonist. Vasoconstriction is one result of alpha adrenergic stimulation. It is this vasoconstriction that can lead to increased blood pressure. Although Penzak et al.¹² reported that synephrine stimulates the alpha-1 adrenergic receptors and could lead to adverse cardiovascular events, their data indicate that citrus aurantium results in similar blood pressure and heart rate response as plain water. In contrast, Preuss et al.¹³ and Gougeon et al.⁷ reported that citrus aurantium leads to beta adrenergic stimulation. Although receptor results from the previously mentioned studies are seemingly in contrast, the differences are easily explained by the fact that the different isomers stimulate different receptors. The alpha receptors bind with m-synephrine while p-synephrine stimulates the beta receptors.

Haller et al.¹¹ noted that citrus aurantium ingestion led to significant increases in blood pressure when 5.5 mg of synephrine was ingested with 239 mg caffeine and 5.7 mg octopamine (in the form of Xenadrine EFX®). In a second treatment administration, subjects ingested 46.9 mg synephrine (from Advantra Z®) with no caffeine and trace amounts of octopamine. Interestingly, blood pressure did not change when subjects ingested 46.9 mg synephrine. Our data is in agreement with the 46.9 mg trial, no significant changes were observed for blood pressure and heart rate when subjects ingested 52 mg of

synephrine with 704 mg of caffeine. Given the mix of ingredients in Xenadrine, one can only theorize what compound or combination could have caused the increased BP. It is plausible that the high level of octopamine, and not the citrus aurantium, in the Xenadrine EFX® treatment, may have exerted significant influence on blood pressure. Octopamine has been reported to be a significant stimulant to both the alpha- and beta- adrenergic receptors^{3,5}. Heart rate will increase if the Beta₁ receptors are stimulated while metabolism will increase if Beta₂ and Beta₃ receptors are activated.

It has been reported that citrus aurantium ingestion may lead to increased heart rate^{11,12}. Haller et al.¹¹ monitored heart rate and blood pressure over a six hour period following ingestion of control, Advantra Z® (citrus aurantium), pressure over a six hour period following ingestion of control, Advantra Z® (citrus aurantium), Xenadrine EFX® treatments. Subjects ingested a meal three hours after treatment ingestion. All three treatments, including the placebo condition, resulted in increased heart rate after eating. Given the control group responded similarly to the Advantra Z® group after the meal at the 4 and 8 hour measurements, the apparently significant difference seen at only 6 hours must be questioned. Unfortunately, no explanation is given for this HR change. It would be expected that the thermic effect of food (TEF) increased heart rate, that the redistribution of blood to the digestive system may have led to increased heart rate, or that there were outliers that influenced this value. The TEF is plausible since Gougeon et al.⁷ reported that the TEF increased by 29% in 17 females after they ingested 26 mg synephrine.

To our surprise, the low caffeine group, in the present study, did not have any adverse reactions to the supplement. Ordinarily, these subjects ingested <150 mg/day of caffeine, but ingested 700 mg over a 24 hr period with the supplement. Heart rate and blood pressure were maintained at baseline levels

throughout the testing period. There was, however, a significant decrease in RER values indicating greater fat oxidation for the low caffeine group (Table 5). There were also strong trends ($p=.08$ and $p=.1$) for increased fat oxidation in males and females when data were separated by gender (Table 4).

The citrus aurantium/synephrine dose given in the present study (52 mg synephrine) is similar to that of Haller et al.¹¹ (46.9 mg), twice that of Gougeon et al.⁷ (26 mg), and four times that of Penzak et al.¹² (13 mg). Regardless of dosing, data from all four studies have similar findings that citrus aurantium presented no significant influence on blood pressure and heart rate. At this point, there seems to be little or no scientific justification to attribute a cardiovascular risk to ingestion of citrus aurantium, and p-synephrine specifically. Evidence is emerging that p-synephrine is a thermogenic agent that may have significant weight management benefits when combined with physical activity and caloric moderation. It is recommended, however, that further studies be undertaken that investigate long term use and risks of citrus aurantium as well as synergistic effects with other compounds. Since different companies will vary the amounts and forms of synephrine, tyramine, and octopamine in their products, knowing what isomer is present is critical in understanding the pharmacological action of the given compound.

Acknowledgement: This article is dedicated to Dr. Ed Burke, who passed away before the completion of this paper.

References

1. Allison DB, Cutter G, Poehlman ET, Moore DR, Barnes S. Exactly which synephrine alkaloids does citrus aurantium (bitter orange) contain? *Int J Obesity*. 2005;1-4.
2. Bent S, Padula A, Neuhaus J. Safety and efficacy of citrus aurantium for weight loss. *Am J Card*. 2004;94:1359-1361.
3. Brown CM, McGrath JC, Midgley JM, Muir AGB, O'Brien JW, Thonoor CM, Williams CM, Wilson VG. Activities of octopamine and synephrine stereoisomers on α -adrenoceptors. *Br J Pharmacol*. 1988;93:417-429.
4. Colditz GA. Economic costs of obesity. *Am J Clin Nutr*. 1992;55:503S-507S.
5. Fontana E, Morin N, Prevot D, Carpenne C. Effects of octopamine on lipolysis, glucose transport and amine oxidation in mammalian fat cells. *Comp Biochem Physiol*. 2000;125:33-44.
6. Fugh-Berman A, Myers A. Citrus aurantium, an ingredient of dietary supplements marketed for weight loss: current status of clinical and basic research. *Exp Biol Med*. 2004;229:698-704.
7. Gougeon R, Harrigan K, Tremblay JF, Hedrei P, Lamarche M, Morais JA. Increase in the thermic effect of food in women by adrenergic amines extracted from citrus aurantium. *Obesity Res*. 2005;13:1187-1194.
8. Gray S, Woolf AD. Citrus aurantium used for weight loss by an adolescent with anorexia nervosa. *J Adol Hlth*. 2005;37:415-416.
9. Guyton AC, Hall JE, editors. *Textbook of Medical Physiology*, 10th edition. WB Saunders, Philadelphia, PA. Pp 700-702.
10. Haller CA, Benowitz NL, Jacob III P. Hemodynamic effects of ephedra-free weight-loss supplements in humans. *Am J Med*. 2005;118:998-1003.
11. Haller CA, Jacob P, Benowitz NL. Short-term metabolic and hemodynamic effects of ephedra and guarana combinations. *Clin Pharmacol Ther*. 2005;77:560-571.

12. Penzak SR, Jann MW, Cold JA, Hon Yi Yuen, Desai HD, Gurley BJ. Seville (sour) orange juice: synephrine content and cardiovascular effects in normotensive adults. *J Clin Pharmacol.* 2001;41:1059-1063.
13. Preuss HG, DiFerdinando D, Bagchi M, Bagchi D. Citrus aurantium as a thermogenic, weight-reduction replacement for ephedra: an overview. *J Med.* 2002;33:247-264.
14. Scheidegger K, Robbins DC, Danforth E Jr. Effects of chronic beta receptor on glucose metabolism. *Diabetes.* 1984;33:1144-1149.
15. Vukovich MD, Schoorman R, Heilman C, Jacob P 3rd, Benowitz NL. Caffeine-herbal ephedra combination increases resting energy expenditure, heart rate and blood pressure. *Clin Exp Pharmacol Physiol.* 2005;32:47-53.