Acute Effects of Passion Fruit Juice Supplementation on Cardiac Autonomic Function and Blood Glucose in Healthy Subjects

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ABSTRACT: Ascorbic acid supplementation provides beneficial effects on enhancing cardiac autonomic function in patients with heart failure. Ascorbic acid also reduces blood sugar levels and enhances insulin activity, and encourages cardiac autonomic function. Passion fruit is rich in ascorbic acid and potential antioxidants. This study aimed to evaluate the acute effects of passion fruit juice (PFJ) supplementation primarily on cardiac autonomic function and secondary on blood glucose in healthy subjects. A randomized cross-over trial was conducted in 14 healthy subjects aged 21.29±0.73 years. Subjects were supplemented with either 50% PFJ, or glucose and fructose solution as a placebo (PLA) at 3.5 mL/kg body mass with a 1-week washout between treatments in a single-dose design. Short-term heart rate variability and blood glucose levels were evaluated prior to supplementation (T0) and following supplementation for 30, 60, 90, and 120 min (T30, T60, T90, and T120, respectively). Indexes of cardiac autonomic function at T30, including high frequency power ($P=0.03$) and total power ($P=0.01$), were significantly higher and the ratio of low frequency/high frequency power was significantly lower ($P=0.01$) in the PFJ group compared to the PLA group. Blood glucose levels significantly increased at T30 in both PLA ($P=0.00$) and PFJ ($P=0.00$) groups. However, there were no significant differences between groups. A single administration of PFJ enhanced cardiac autonomic function through augmentation of parasympathetic activity, although it did not attenuate postprandial hyperglycemia. PFJ may be potentially recognized as beverage able to prevent cardiovascular disease.

Keywords: vitamin C, passion fruit, autonomic nervous system, cardiovascular disease, diabetes mellitus

INTRODUCTION

Large datasets provide insights into the epidemiology of cardiovascular disease (CVD) worldwide, which is the leading cause of disease burden and deaths (Hinton et al., 2018; India State-Level Disease Burden Initiative CVD Collaborators, 2018). Risk factors for CVD include aging, high blood pressure, hypercholesterolemia, and obesity, and are among the most essential contributors to disability-adjusted life years (Hinton et al., 2018). Dietary and nutritional approaches are the most paramount modifiable factors in the prevention and management of CVD. These factors can affect CVD directly by contributing to the accumulation of vascular plaques, and indirectly by regulating the rate of aging, the major risk factor for CVD (Brandhorst and Longo, 2019). Diet composition is one of the preeminent approach to improve human health, and which may be helpful in preventing development of CVD, such as through decreasing oxidative stress, inflammation, atherosclerosis, and insulin resistance (Casas et al., 2018). A high consumption of vegetables and fruits is widely recommended as an excellent source of dietary fiber, antioxidants, and polyphenols (Slavin and Lloyd, 2012).

Passion fruit is mostly grown in tropical and sub-tropical parts of the world (Zas and John, 2016). It is widely consumed due to its pleasant flavour and acidic aroma (Fernandes et al., 2011). Furthermore, passion fruit is considered an important source of minerals and vitamins, such as ascorbic acid, phyto-constituents, flavonoids, and phenolic compounds (Ramaiya et al., 2013; Zas and John, 2016). Ascorbic acid has been reported to improve cardiac autonomic nervous system in previous studies, which suggest that ascorbic acid supplementation enhances parasympathetic (vagal) nervous activity (Buttros et al., 2009; Monahan et al., 2004) and attenuates sympathetic nervous activity (Bruno et al., 2012; Leuenberger et al., 2012). Maintaining regular activities of these systems plays a
crucial role in the prevention of cardiovascular pathology and dysfunction, including hypertension, ischemic heart disease, arrhythmias, and congestive heart failure (Bairey Merz et al., 2015). Previous studies have demonstrated that consuming yellow passion fruit for 5 days decreases systolic blood pressure and oxidative stress in spontaneously hypertensive rats (Zhao et al., 2017). In addition, intake of passion fruit seed extracts containing piceatannol for 16 weeks improves blood lipids, platelet aggregation, and cardiac function in CVD-induced rats (Ishihata et al., 2016). A clinical trial in type 2 diabetic subjects demonstrated a reduction in systolic blood pressure and blood glucose following administration of purple passion fruit for 16 weeks (Raju et al., 2013). Studies in diabetic rats have also revealed that passion fruit extract effectively reduces blood glucose, increases antioxidants, and improves lipid profiles (Barbalho et al., 2011; Kandandapani et al., 2015; Uchida-Maruki et al., 2015). These data demonstrate the feasibility of passion fruit to exhibit beneficial effects for enhancing cardiac autonomic function and preventing of diabetes mellitus. However, knowledge obtained in the aspect of medical nutrition therapy is limited and broader preventive strategies could reduce the burden of CVD. Accordingly, this study, aimed to explore the effects of a single-dose passion fruit juice (PFJ) supplementation primarily on cardiac autonomic function and secondary on blood glucose in healthy subjects.

**MATERIALS AND METHODS**

**Study design and subjects**

This was a randomized cross-over study conducted in Mueang, Chonburi, Thailand. Fourteen healthy male and female subjects aged between 20 to 22 years (21.29±0.73 years) with body mass index (BMI) 20.65±1.26 kg/m² were enrolled. Inclusion criteria included: (a) male or female; (b) aged between 20 to 30 years; (c) normal BMI (18.5~23.0 kg/m²); and (d) healthy of body and mind. Exclusion criterion included regular smokers or drinkers. Withdrawal criteria were as follows: (a) presented abnormal symptoms including nausea, vomiting, dizziness, or syncope; (b) participation in another intervention; and (c) requested to cease participation from the study.

**Power calculation**

Sample size was calculated using a cross-over study formula generated by Machin and Campbell (2005). Basu and colleagues (2010) studied the effect of strawberry supplementation on decreases in atherosclerotic markers in subjects with metabolic syndrome. Accordingly, the authors reported a mean difference in decreasing serum glucose between treatment and control groups of 0.1 mmol/L [standard deviation (SD) 0.1]. Hence, with α error of 0.05 and β error of 0.10, the sample size in this study was 14 subjects including a 10% drop-out rate.

**Ethics statement**

All subjects signed a consent form prior to screening and enrollment in the study. Subjects were informed of the study protocols, details, risks, and their role in the study, both in writing and verbally before signing the consent form. This study was conducted under the approval of the Human Ethics Committee of Burapha University (approval no. 175/2560), and in accordance with the ethical standards of the Declaration of Helsinki. This study is registered with the Thai Clinical Trials Registry (identification no. TCTR20180223005).

**Recruitment and screening of subjects**

This study recruited 14 healthy subjects at Burapha University, Mueang, Chonburi, Thailand from January to February 2018. Placards containing the study details were posted in the main areas of the University, such as the library, cafeteria, and student dormitories. Subjects interested in participating in the study contacted a research assistant by phone. After making an appointment, subjects were screened through health questionnaires used to examine their general information, medical illness, exercise participation history, supplementation intake history, and mental health, in addition to a physical examination, which measured body mass (BM), height, BMI, blood pressure (BP), and heart rate (HR). During the week following screening, subjects who were selected based on the inclusion and exclusion criteria and provision of informed consent participated in the initial installment of the study.

**Experimental protocol**

During the first visit, anthropometric measurements were taken. Subjects were then randomly supplemented with either 50% PFJ, or glucose and fructose solution as a placebo (PLA) at 3.5 mL/kg BM in a single-dose design. Short-term HR variability (HRV), blood glucose levels, HR, and BP were evaluated before supplementation (T0) and following supplementation for 30, 60, 90, and 120 min (T30, T60, T90, and T120, respectively). Subjects participated in the second visit the following week during which they underwent additional treatment. A 1-week interval was established as a wash-out period between treatments. All measurements were carried out at the same time of day, and under similar environmental conditions.

**Supplements**

The PFJ used in this study was a commercially available PFJ produced from purple passion fruits at Doi Kham Food Products Co. Ltd., (Chiang Rai, Thailand). The concentration of PFJ was 50% (50 g/100 mL) according to a
Blood glucose levels was measured using a Accu-Chek Measurement of blood glucose and Ginsberg, 2017).}

**Assessment of cardiac autonomic function**

Short-term HRV was analyzed to assess cardiac autonomic function following HR and BP measurements. HRV data was generated from lead II electrocardiography (Rm perLab 4/30, AD Instruments, Bella Vista, NSW, Australia) was generated from lead II electrocardiography (Pow-erLab 4/30, AD Instruments, Bella Vista, NSW, Australia). The subjects’ HRV data were collected over 5 periods: 5-min before supplementation, and post-supplementation at 25 ~ 30 min, 55 ~ 60 min, 85 ~ 90 min, and 115 ~ 120 min. Analysis of HRV data accounted for the time domain and frequency domain. The time domain consisted of the SD of normal beat-to-beat (R-R) intervals (SDNN) and the root-mean-square of successive R-R (RMSSD). The frequency domain comprised of the total power (TP), very low frequency (VLF, DC to 0.04 Hz), low frequency (LF, 0.04 to 0.15 Hz), and high frequency powers (HF, 0.15 to 0.4 Hz), and the LF/HF ratio. HRV data reveal sympathetic and parasympathetic nervous activities as well as baroreceptor activity (Shaffer and Ginsberg, 2017).

**Measurement of blood glucose**

Blood glucose levels was measured using a Accu-Chek® Guide blood glucose monitoring system (Roche Diabetes Care Inc., Indianapolis, IN, USA) consisting of lancets, test strips, and a glucometer. Upon lancing a subject’s fingertip, a drop of capillary blood (approximately 0.6 μL) was obtained and the test strip was inserted into the glu-cometer. Values were reported by the glucometer moni-tor as mg/dL.

**Measurements of HR and BP**

Subjects’ HR, systolic BP (SBP), and diastolic BP (DBP) were measured after resting in the supine position for 15 min using a digital automatic BP monitor (Rossmax CF155f, Rossmax Swiss GmbH, Berneck, Switzerland).

**Anthropometry**

Subjects’ height was measured using a stadiometer (Health o meter®, Chicago, IL, USA) in the standing position during inspiration. BM, BMI, fat distribution (waist and hip circumferences and their ratio), and body composition (body fat percentage, fat mass, fat-free mass, muscle mass, protein mass, mineral mass, water mass, and basal metabolic rate) were measured in the standing position, while subjects were wearing minimal clothing, using a body composition analyzer (InBody270, InBody Co., Ltd., Seoul, Korea).

**Data analyses**

Normality of data was analyzed and confirmed using Shapiro-Wilk tests. Differences in variables within each group at T0, T30, T60, T90, and T120 and between groups at T30, T60, T90, and T120 were analyzed using one-way repeated measures analysis of covariance (ANCOVA) by adding T0 as a covariate and using the Bonferroni post hoc test for multiple comparisons. Differences between groups at baseline (T0) were analyzed using paired t-test. All analyses were carried out using IBM SPSS Statistics for Windows (IBM Inc., Armonk, NY, USA). Data are presented as mean±SD. A P-value of <0.05 was consid-ered statistically significant.

**RESULTS**

**Physical and physiological characteristics**

A total of 14 subjects were eligible, enrolled, and com-pleted the study. Table 1 shows the physical and physi-ological characteristics of subjects during the study period. There were no significant differences in age, gen-der, height, BM, BMI, body fat percentage, fat mass, fat-free mass, muscle mass, protein mass, mineral mass, water mass, basal metabolic rate, waist and hip circum-ferences, and waist/hip ratio between subjects in the PLA and PFJ groups.

**Cardiac autonomic nervous activity**

Compared with the PLA group, SDNN (F=4.85, P=0.04, effect size=0.17), RMSSD (F=5.32, P=0.04, effect size=0.24) was also significant-
P=0.01, effect size=0.18), HF power (F=5.23, P=0.03, effect size=0.23), and HF power in normalized unit (F=7.09, P=0.01, effect size=0.23) were significantly higher at T30 in the PFJ group [SDNN, 73.16±40.77 vs. 63.79±48.45 ms; RMSSD, 81.72±14.24 vs. 63.79±16.99 ms; TP (F=7.19, P=0.03, effect size=0.17), and HF power in normalized unit (F=7.09, P=0.01, effect size=0.23) were significantly higher at T30 in the PFJ group compared with the PLA group [LF power (normalized unit), 61.31±14.24 vs. 48.16±16.99]. The LF/HF ratio value (F=7.74, P=0.01, effect size=0.24) was also significant-ly lower at T30 in the PFJ group compared with the PLA group (0.66±0.50 vs. 1.52±2.31). Moreover, the LF pow-er in normalized unit (F=5.72, P=0.03, effect size=0.19) and LF/HF ratio (F=5.60, P=0.03, effect size=0.19) were significantly lower at T120 in the PFJ group compared with the PLA group [LF power (normalized unit), 34.18
Table 1. Physical and physiological characteristics of subjects in the two treatment groups

|                          | Placebo group       | PFJ group        | P-value |
|--------------------------|---------------------|-----------------|---------|
| Age (yrs)                | 21.29±0.73          | 21.29±0.73      | 1.00    |
| Gender (M/F, %)          | 7/7 (50/50)         | 7/7 (50/50)     | 1.00    |
| Height (m)               | 1.65±0.11           | 1.65±0.11       | 0.79    |
| BM (kg)                  | 56.16±9.31          | 58.48±10.10     | 0.34    |
| BMI (kg/m²)              | 20.64±1.30          | 20.70±1.35      | 0.88    |
| Body fat (%)             | 22.54±7.07          | 22.31±6.92      | 0.85    |
| Fat mass (kg)            | 12.16±2.53          | 12.14±2.57      | 0.93    |
| Fat-free mass (kg)       | 44.01±10.80         | 44.33±10.45     | 0.78    |
| Muscle mass (kg)         | 24.20±6.64          | 24.35±6.45      | 0.80    |
| Protein mass (kg)        | 8.67±2.19           | 8.73±2.11       | 0.83    |
| Mineral mass (kg)        | 3.09±0.69           | 3.11±0.65       | 0.81    |
| Water mass (kg)          | 32.24±7.93          | 32.50±7.69      | 0.64    |
| Waist circumference (cm) | 68.93±4.20          | 68.93±4.34      | 0.73    |
| Hip circumference (cm)   | 86.43±4.47          | 85.64±4.99      | 1.00    |
| W/H ratio                | 0.79±0.05           | 0.79±0.05       | 0.53    |
| BMR (kcal/d)             | 1,318.07±233.32     | 1,327.57±226.31 | 0.96    |

Data are mean±SD (n=14).
Differences between groups were analyzed using paired-t-test.
PFJ, passion fruit juice; BM, body mass; BMI, body mass index; W/H, waist to hip circumference ratio; BMR, basal metabolic rate.

Fig. 1. Standard deviation of normal beat-to-beat intervals (SDNN) in passion fruit juice and placebo groups. Data are mean±SD (n=14). *Significantly different from placebo group (P<0.05).

Fig. 2. Root-mean-square of successive beat-to-beat (RMSSD) in passion fruit juice and placebo groups. Data are mean±SD (n=14). *Significantly different from placebo group (P<0.05).

Fig. 3. High frequency (HF) power in normalized unit in passion fruit juice and placebo groups. Data are mean±SD (n=14). *Significantly different from placebo group (P<0.05).

Analysis of HRV data did not indicate a significant change in HRV values between time points in either treatment group (Fig. 1 ~ 4).

Analysis of changes in blood glucose levels in the PLA
Passion Fruit Juice and Cardiac Autonomic Function

Fig. 4. Ratio of low frequency (LF) to high frequency (HF) power in passion fruit juice and placebo groups. Data are mean±SD (n=14). *Significantly different from placebo group (P<0.05).

Fig. 5. Blood glucose level in passion fruit juice and placebo groups. Data are mean±SD (n=14). Significantly different from *T0, †T30, ‡T60, and ‡T90 (P<0.05).

Table 2. Heart rate variability of subjects in the two treatment groups (unit: ms²)

|                | Placebo group | PFJ group     | P-value |
|----------------|--------------|---------------|---------|
| **Total power**|              |               |         |
| T0             | 3,174.01±3,237.22 | 10,566.44±19,140.31 | 0.18    |
| T30            | 4,678.06±7,430.06 | 7,346.33±8,464.80 | 0.01    |
| T60            | 2,852.35±1,484.42 | 7,099.00±8,329.05 | 0.24    |
| T90            | 2,959.20±1,670.50 | 4,958.96±3,336.26 | 0.51    |
| T120           | 3,398.82±1,791.84 | 6,130.39±5,392.74 | 0.38    |
| **VLF power**  |              |               |         |
| T0             | 1,021.29±803.60 | 2,501.52±3,152.49 | 0.12    |
| T30            | 1,146.02±586.20 | 1,488.87±1,229.85 | 0.09    |
| T60            | 1,225.34±1,155.67 | 1,581.16±1,088.19 | 0.15    |
| T90            | 1,216.06±721.96 | 1,878.97±2,082.10 | 0.40    |
| T120           | 1,427.02±1,281.06 | 1,544.59±1,423.43 | 0.91    |
| **LF power**   |              |               |         |
| T0             | 832.39±765.98 | 2,433.17±4,199.59 | 0.18    |
| T30            | 1,237.00±1,449.89 | 1,807.90±2,368.16 | 0.16    |
| T60            | 805.87±720.25 | 2,003.31±2,781.03 | 0.34    |
| T90            | 799.12±766.84 | 1,013.31±1,092.58 | 0.87    |
| T120           | 980.48±705.59 | 1,246.73±1,310.39 | 0.97    |
| **HF power**   |              |               |         |
| T0             | 1,110.77±7,736.86 | 4,806.91±10,776.95 | 0.22    |
| T30            | 1,849.10±4,002.78 | 3,752.29±5,138.10 | 0.03    |
| T60            | 741.32±635.35 | 2,762.18±4,003.99 | 0.12    |
| T90            | 863.30±1,147.54 | 1,436.21±986.28 | 0.08    |
| T120           | 880.02±903.31 | 2,406.57±2,585.70 | 0.49    |

Data are mean±SD (n=14). Differences between groups were analyzed utilising one-way repeated measures ANCOVA.

PFJ, passion fruit juice; VLF, very low frequency; LF, low frequency; HF, high frequency; T0, before supplementation; T30, after supplementation for 30 min; T60, after supplementation for 60 min; T90, after supplementation for 90 min; T120, after supplementation for 120 min.

Heart rate and blood pressure

One-way repeated measures ANCOVA revealed that HR and BP were not significant differences between interventions at any time point (Table 3).

There were no significant alterations in HR and BP between time points in both the PLA and PFJ groups (Table 3).

DISCUSSION

CVDs are often accompanied by disparity of the sympathetic-vagal discharge to the heart, resulting in chronic adrenergic stimulation and decreased HRV (Kubota et al., 2017; La Rovere and Christensen, 2015). However, higher vagus nerve activity promotes greater HRV and favorable cardiovascular outcomes (Olshansky et al., 2008). This randomized cross-over study evaluated the effect of PFJ supplementation on enhancing cardiac autonomic function and attenuating postprandial blood glucose in healthy subjects. Our findings reveal beneficial effective-

from those at baseline (Fig. 5).

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ness of acute PFJ supplementation on cardiac autonomic responses.

PFJ is the main product derived from passion fruit pulp (Laboissière et al., 2007). PFJ contains numerous essential nutrients, including amino acids, fiber, sugar (glucose, fructose, and sucrose), organic acids, phenolic compounds, flavonoids, antioxidants, ascorbic acid, vitamin A, minerals (calcium, phosphorus, sodium, potassium, and magnesium), and trace elements (ferrous, copper, manganese, zinc, and selenium) (Ramaiya et al., 2013; Ramaiya et al., 2014; Singh and Das, 2013; Zhu et al., 2017). Studies investigating the anti-inflammatory and antioxidant effects of passion fruit reported that patients with knee osteoarthritis, asthma, and hypertension experience improvements following supplementation with purple passion fruit peel extract (Farid et al., 2010; Christie-David et al., 2015; Dakhale et al., 2011; Kotb and Al Azzam, 2015) and serum insulin (Afkhami-Ardekani and Shojaoddiny-Ardekani, 2007; El-Aal et al., 2018) in type 2 diabetic patients. Oxidative stress can disturb glucose metabolism by damaging enzymes and cellular machinery, and by increasing insulin resistance (Asmat et al., 2016). Hence, antioxidant supplementation, such as with ascorbic acid, vitamin A, vitamin E, and glutathione, can be beneficial for reducing blood glucose and hyper-

| Table 3. Heart rate and blood pressure of subjects in the two treatment groups |
|-------------------------------|-------------------------------|-----------------|
|                               | Placebo group                 | PFJ group       | \(P\)-value   |
| Heart rate (beats/min)        |                               |                 |               |
| T0                            | 69.37±10.51                   | 68.16±10.57     | 0.49          |
| T30                           | 70.24±7.99                    | 66.56±10.58     | 0.63          |
| T60                           | 70.91±6.66                    | 68.37±10.00     | 0.73          |
| T90                           | 70.28±9.15                    | 68.87±9.20      | 0.22          |
| T120                          | 68.98±8.59                    | 66.92±10.94     | 0.35          |
| Systolic BP (mmHg)            |                               |                 |               |
| T0                            | 113.14±10.27                  | 116.07±11.21    | 0.42          |
| T30                           | 112.79±11.66                  | 115.07±10.45    | 0.97          |
| T60                           | 112.79±10.98                  | 114.29±11.39    | 0.74          |
| T90                           | 115.64±11.86                  | 118.86±10.73    | 0.11          |
| T120                          | 116.00±10.48                  | 116.07±10.06    | 0.43          |
| Diastolic BP (mmHg)           |                               |                 |               |
| T0                            | 70.50±6.73                    | 71.64±4.94      | 0.62          |
| T30                           | 68.00±7.16                    | 71.07±8.97      | 0.42          |
| T60                           | 70.29±7.77                    | 68.14±4.82      | 0.11          |
| T90                           | 71.57±8.81                    | 68.71±5.78      | 0.13          |
| T120                          | 70.29±7.68                    | 71.00±5.55      | 0.95          |

Data are mean±SD (n=14).
Differences between groups were analyzed utilising one-way repeated measures ANCOVA.
PFJ, passion fruit juice; BP, blood pressure; T0, before supplementation; T30, after supplementation for 30 min; T60, after supplementation for 60 min; T90, after supplementation for 90 min; T120, after supplementation for 120 min.
glycemia (Khan et al., 2015; Li et al., 2017; Palekar and Ray; 2017). Kandandapani et al. (2015) reported a significant increase in superoxide dismutase and catalase, and a decrease in thiobarbituric acid reactive substances (TBARS) levels in the vital organs of diabetic rats treated with passion fruit *Passiflora edulis* extracts. Moreover, studies have shown supplementation of PFJ for 28 days significantly reduces plasma TBARS in normal Wistar rats (Kandandapani et al., 2015). In the present study, we did not observe an additional effect of PFJ on attenuating postprandial hyperglycemia. This may be due to an insufficient amount or duration of ascorbic acid. In the previous studies, rats were chronically administered high doses of ascorbic acid (i.e., 1,000 mg/d for 6 weeks to 3 months) (Afkhami-Ardekani and Shojaoddiny-Ardekani, 2007; Kotb and Al Azzam, 2015), whilst we used a single administration at a low dose. However, we showed that PFJ is beneficial for maintaining blood glucose levels. Moreover, at T120 blood glucose levels were significantly decreased from baseline in the PLA group compared with the PFJ group.

Our data did not reveal a significant change in either HR or BP. PFJ may have raised parasympathetic nervous activity resulting in reduced HR and BP, whereby a latter reduction leads to decreased baroreceptor activity (Gronda et al., 2017). Then, the inhibitory signal from the baroreceptor to the sympathetic nervous control at the vasomotor center is diminished (Kougias et al., 2010). Hence, the sympathetic nervous activity is stimulated to raise BP. Finally, a constant BP and HR is present during the experiment to maintain cell nourishment. These mechanisms are clearly observed, particularly in healthy subjects.

This study has some limitations. Firstly, it was designed to explore acute PFJ supplementation. Further investigation on chronic PFJ supplementation is needed to confirm the favorable effects of PFJ on cardiac autonomic function and blood glucose levels in the context of medical nutrition therapy. Secondly, we did not investigate insulin concentrations. Therefore, it is not possible to explain certain essential mechanisms involved in controlling blood glucose, such as changes in insulin levels or insulin sensitivity.

The present study suggests that single-dose PFJ supplementation enhances cardiac autonomic function, but does not attenuate postprandial hyperglycemia in healthy subjects. Accordingly, this study shows PFJ is advantageous for human health, and that PFJ may be employed as a potential beverage for prevention of CVD and to encourage a healthy heart.

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**AUTHOR DISCLOSURE STATEMENT**

The authors declare no conflict of interest for all potential sources of bias, including affiliations, funding sources, and financial or management relationships.

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