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Caffeine as a flavor additive in soft-drinks

Russell SJ Keast & Lynnette J Riddell

The corresponding author may be contacted at the following address:
Dr. RSJ Keast
Senior Lecturer
School of Exercise and Nutrition Sciences
Deakin University
221 Burwood Highway, Burwood, Victoria 3125 Australia.
Phone: 03 9244 6944 International: +61 3 9244 6944
Fax: 03 9244 6017 International: +61 3 9244 6017
e-mail: russell.keast@deakin.edu.au

The second author address is:
Dr. LJ Riddell
Lecturer
School of Exercise and Nutrition Sciences
Deakin University
221 Burwood Highway, Burwood, Victoria 3125 Australia.
Phone: 03 9241 7270 International: +61 3 9241 7270
Fax: 03 9244 6017 International: +61 3 9244 6017
e-mail: lynn.riddell@deakin.edu.au
ABSTRACT

Over 60% of soft-drinks sold in the United States contain caffeine, a mildly addictive psycho-active chemical, as a flavor additive. Using sweeteners as controls, we assessed whether caffeine has flavor activity in a cola soft-drink. A forced-choice triangle discrimination methodology was used to determine detection thresholds of caffeine in sweeteners and a cola beverage. The subjects (n=30, 28 female, 23±4 years old) were trained tasters and completed over 1600 discrimination tests during the study. The mean detection thresholds for caffeine in the sweet solutions were: 0.333±0.1 mM sucrose; 0.467±0.29 mM aspartame; 0.462±0.3 mM sucralose, well below the concentration in common cola beverages (0.55-0.67mM). A fixed concentration of caffeine, corresponding to the concentration of caffeine in a common cola beverage (0.67mM) was added to the sweeteners and a non-caffeinated cola beverage. Subjects could distinguish between caffeinated and non-caffeinated sweeteners (p<0.001), but all subjects failed to distinguish between caffeinated and non-caffeinated cola beverage (p=1.0). Caffeine has no flavor activity in soft-drinks yet will induce a physiologic and psychologic desire to consume the drink.
INTRODUCTION

Caffeine is a widely-consumed mildly-addictive chemical occurring naturally in coffee, tea, and chocolate, and as an additive in soft-drinks. Dependence on a psychoactive chemical is defined as ‘a pattern of behaviour focused on repetitive and compulsive seeking and taking of a psychoactive drug’ (Heishman & Henningfield, 1992). Using the Diagnotistic and Statistical Manual of Mental Disorders (IV) criteria for substance dependence, three independent studies found between 15-25% of the caffeine drinking population were classified as dependent on caffeine (Hughes, Oliveto, Bickel, & Higgins, 1993; Oberstar, Bernstein, & Thuras, 2002; Strain, Mumford, Silverman, & Griffiths, 1994). The remaining 75% of caffeine consumers are not classified as dependent, however may suffer symptoms including headaches, feelings of drowsiness, fatigue and work difficulty, depression and irritability, when they stop consuming caffeine (Richardson, Rogers, Elliman, & O'Dell, 1995; Silverman, Evans, Strain, & Griffiths, 1992; Strain et al., 1994). These physiologic and psychologic withdrawal symptoms can occur 3-6 hours after last caffeine consumption, last up to one week, and are independent of the amount of caffeine ingested (for a review on caffeine withdrawal see (Juliano & Griffiths, 2004)).

The consumption of a food during childhood results in preference for that flavor of the food that may last a lifetime (Mennella & Beauchamp, 2005), and influence the flavours of foods within a culture (Keast & Lau, 2006). The development of flavor preferences and aversions are established when individuals associate (unconsciously) a food/flavor with its post-ingestive consequences. The mode of action of caffeine in developing flavour preference is not immediate (Yeomans et al., 2000) as, for example, we experience with a sucrose solution (sweet and appetitive). Caffeine may elicit no perceived flavour or bitterness in the mouth.
depending on concentration (Keast & Roper, 2007), but the positive affects occur
post-consumption with increased vigilance and attention, enhanced mood and arousal
as well as enhanced motor activity. Behavioural studies have shown that the
consumption of caffeine promotes a physiologic and psychologic dependence that is
reinforced with repeat consumption (Garrett & Griffiths, 1998; Hughes et al., 1993;
Schuh & Griffiths, 1997). The common method of repeat caffeine consumption is via
caffeinated beverages such as coffee, tea, cocoa, and soft drinks which are
hedonically pleasant to drink. In addition, when caffeine is co-consumed with a
source of sugar (glucose), there appears to be a synergy of cognitive modulating
effects (Scholey & Kennedy, 2004). This is of particular concern for soft-drinks sold
to children and adolescents as the dose of caffeine required to modify behaviour in
humans is low (>50 mg) (Nehlig, 1999), similar to the dose delivered in 500 ml
common cola soft-drinks (~53-65 mg, 0.55-0.67 mM). Such doses of caffeine may
cause children and adolescents to consume more of the sugar sweetened soft-drink
due to physiologic and psychologic modes of action (Myers & Sclafani, 2006).
Moreover, the consumption of sugar sweetened soft-drinks has been linked to
increasing body mass index and risk of obesity in children and adolescents (Ebbeling
et al., 2006; Ludwig, Peterson, & Gortmaker, 2001; Striegel-Moore et al., 2006), and
the prevalence of childhood obesity is an emerging epidemic that increases the
personal and economic burden of diet related diseases (Drewnowski & Darmon,
2005). As a result, some school boards and districts have banned sales of soft-drinks
(Fried & Nestle, 2002).

Manufacturers claim caffeine is added as a flavor enhancer in soft drinks
(PepsiCo, 1981), however any flavor effect of caffeine will be a function of its
concentration in the soft-drink (Griffiths & Vernotica, 2000) i.e., the concentration of
caffeine in soft-drinks may be below its flavor detection threshold. The aim of this research was to investigate the detection threshold of caffeine in sweet solutions, and the flavor activity of caffeine in soft-drinks (e.g., Colas). If caffeine is included as a flavor additive in soft-drinks, by definition it must have flavor activity within the soft-drink, otherwise it may be viewed as an ingredient to modify consumer behaviour.
METHODS

Subjects

Subjects (n=30, 23±4 years old, 28 female) between the ages of 18 and 38 were University students in Melbourne, Australia. All subjects agreed to participate and provided informed consent on an approved Institutional Review Board form. The participants were asked to refrain from eating, drinking or chewing gum for one hour prior to testing. Participants were initially trained in the use of the general Labelled Magnitude Scale (gLMS) following the published standard procedures (Bartoshuk, 2000; Green, Shaffer, & Gilmore, 1993). Participants were trained to identify each of the five taste qualities by presenting them with exemplars. Salty taste was identified as the predominant taste quality from 150 mM NaCl, bitterness as the predominant quality from 0.05mM quinine HCl, sweetness as the predominant quality from 300 mM sucrose, sourness as the predominant quality from 3 mM citric acid, and umami from the predominant quality from a mixture of 100 mM glutamic acid monosodium salt and 50mM inosine 5’-monophosphate. To help subjects understand a stimulus could elicit multiple taste qualities, 300 mM sucrose and 0.1 mM quinine-HCl (sweet and bitter) and 50 mM NH4Cl (salty, bitter, and slightly sour) were also employed as training stimuli.

Stimuli

Sucrose was purchased from USB (Cleveland, OH), aspartame was purchased from APS Cotter (Victoria, Australia), sucralose was obtained from Johnson & Johnson (NSW, Australia), and caffeine was purchased from Sigma Chemical (St. Louis, MO). The sweeteners were chosen as they are representative of common sweeteners used in soft-drinks. Coca-Cola, non-caffeinated diet Coca-Cola, and
Nobles Ultra Pure Filtered water were purchased from a local supermarket. Aqueous solutions were freshly prepared every day, using distilled-filtered (df) water, several hours in advance of testing. Water (df) was used as the blank stimulus and Nobles Ultra Pure water as rinsing agent in all experiments. All stimuli were served at room temperature (20°C±3°C).

All testing took place in specialized sensory-testing facility comprising of seven individual computerized booths. Each subject was isolated from other subjects by vertical dividers and there was no interaction between subjects.

**Intensity matching of sweeteners to degassed cola beverage**

The threshold of caffeine was determined at a level of sweetness common to all sweeteners and the cola beverage, because variation in sweetness will affect the threshold concentration of caffeine. The cola beverage was degassed to minimise irritant sensory inputs during the intensity matching phase (Cowart, 1998). The intensity matching procedure involved adjusting the concentrations of sucrose, aspartame, and sucralose solutions until the intensity was rated equi-intense to degassed cola beverage on the gLMS (Keast & Breslin, 2002). Equi-intense sweetener concentrations compared to degassed cola beverage were: 204 mM sucrose, 1.5 mM aspartame, 0.42 mM sucralose.

**Detection threshold determination of caffeine in sweeteners**

A triangle forced-choice initially-ascending procedure was used to determine detection threshold of caffeine for each subject. The range of concentration used was modified from ISO method for investigating sensitivity of taste (Table 1). Starting at a 0.33 mM caffeine concentration (step 7), solutions (10 ml) were presented in 30 ml plastic medicine cups in groups of three. Subjects were instructed to hold the sample in their mouth for three seconds, then expectorate. Within each set of three solutions,
two were the sweetener and the third was the sweetener + caffeine and subjects had to identify which one was different (triangle test). The order of presentation was randomised and could have been any of three possible orders (A/non-caffeinated and B/caffeinated): AAB, ABA, BAA. If subjects failed to correctly identify the odd sample, the concentration was increased one step. If subjects correctly identified the sample on two occasions, the concentration was decreased one step. The level at which the sequence changed from ascending to descending or descending to ascending was termed a reversal. Four reversals were required and the best estimate threshold for each subject was the geometric mean of the concentration where the last miss occurred and the next higher step. There was an interstimulus interval of approximately 60 sec, during which time the subject was required to rinse with water at least four times. The detection threshold method was repeated in a separate session to check reproducibility of detection thresholds.

Flavor activity of 0.67mM caffeine in cola beverage and sweeteners

An aliquot (9.3 ml) of chilled (8°C) diet non-caffeinated Coca-Cola (1 l) was removed and discarded. The cola soft-drink (990.7 ml) was spiked with 9.3 ml of 72 mM caffeine to yield a cola soft-drink with 0.67 mM caffeine, the same caffeine concentration in a common cola soft-drink. A diet non-caffeinated cola soft-drink was spiked with 9.3 ml of water and used as the control. The same method was applied to the sweeteners. A forced-choice triangle test was performed in triplicate and used to determine if subjects could discriminate between the control and caffeinated samples. To minimise the risk of a type 1 error (erroneously concluding there is a difference), subjects were asked for their certainty in selecting the odd sample during the cola soft-drink test. Subjects (2/30) who stated they were not confident at picking the odd sample, yet correctly identified (2/3 or 3/3 trials) the odd sample were retested.
Statistical Analysis

Significance of difference for triangle test (p<0.001) was assessed using Triangle Test for difference table (Meilgaard, Civille, & Carr, 1991). Statistical analyses of threshold results were determined with Freidman Test. The analyses were conducted with the SPSS 13.0 package. P values <0.05 were considered statistically significant.
RESULTS

Caffeine detection thresholds were determined for all subjects in three sweeteners commonly used in soft-drinks. The mean detection thresholds for caffeine in the sweet solutions were: 0.333±0.1 mM sucrose; 0.467±0.29 mM aspartame; 0.462±0.3 mM sucralose, below the concentration in common cola beverages. The caffeine threshold in sucrose was below the concentration of caffeine in common cola beverages for all subjects. Five subjects (17%) had caffeine thresholds in high intensity sweeteners greater than the concentration of caffeine in common cola beverages. A Freidman test revealed a significant difference in the mean detection threshold of caffeine in sucrose compared with the high intensity sweeteners (p<0.05).

To test if caffeine has flavor activity in soft-drinks, a fixed concentration of caffeine, corresponding to a concentration used in a common cola soft-drink (0.67 mM), was added to a commercially available non-caffeinated cola soft-drink and the equi-intense sweeteners. The equi-intense sweeteners were positive controls because subjects should discriminate between caffeinated and non-caffeinated samples as the fixed concentration of caffeine is significantly greater than the mean detection threshold concentration in sweeteners. As predicted, subjects (28 out of 30) were able to identify 0.67 mM caffeine in the equi-sweet solutions (p<0.001). However, when 0.67 mM caffeine was added to the soft-drink, subjects were unable to discriminate the difference between the caffeinated or non-caffeinated samples (p=1.0) (Figure 1). A Freidman Test revealed no significant difference in discrimination between the caffeinated and non-caffeinated equi-sweet solutions, but there was a significant difference in the subjects’ ability to discriminate caffeinated solutions between the sweeteners and soft-drink (p<0.001). Only two of 30 subjects selected above chance...
(both 2/3 correct trials) during the cafffeinated soft-drink test and were subsequently retested to minimise the risk of a type 1 error. During retesting, both subjects performed at chance (1/3 correct), and when questioned, stated there was no difference between the soft-drink samples presented. In summary, of the thirty highly trained subjects, none were able to discriminate between the soft-drink with caffeine and the soft-drink without caffeine.
DISCUSSION

The flavor activity of caffeine in soft drinks is dependent on its concentration (Keast & Roper, 2006), but independent of the type of sweetener used or whether the subjects have a preference for caffeinated soft-drinks (Griffiths & Vernotica, 2000). In this study, highly trained and experienced subjects were able to detect subtle differences in flavor when 0.67 mM caffeine was added to a sweet solution, but unable to detect 0.67 mM caffeine in a soft-drink. The soft-drink activates multiple sensory systems (e.g., taste, smell, chemesthesis) resulting in much sensory information being sent to the flavor processing regions of the brain. Any subtle effects caffeine may have on flavor were effectively masked and discrimination proved a cognitively impossible task. It is possible that interactions with other chemicals in the soft-drink matrix, or interactions between chemicals at taste receptors in the oral periphery were also involved in masking any subtle flavor effects of 0.67mM caffeine in soft-drink (Keast & Breslin, 2003). Subjects ability to discriminate caffeine will improve as the concentration of caffeine is increased (Griffiths & Vernotica, 2000), but the sub-threshold dose of caffeine (<0.67 mM) delivered in 500 ml soft-drinks are enough to have both physiologic and psychologic effect on the consumer.

During the development of flavor preferences, the inclusion of a mildly addictive compound such as caffeine to a food will increase the dependence and liking of that food, which will in turn increase consumption (Myers & Sclafani, 2006). As the consumption of soft-drinks has been associated with the increase in childhood and adolescent obesity, there are public health reasons to remove caffeine from sugar sweetened soft-drinks (Ebbeling et al., 2006; Ludwig et al., 2001; Striegel-Moore et al., 2006). Moreover, as this study has shown, there is no flavor based rationale to
add caffeine to soft-drinks. We pose the question: Should a moderately addictive agent such as caffeine be an additive in sugar-sweetened soft-drinks marketed to children and adolescents?
ACKNOWLEDGMENTS:

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REFERENCES


Table 1 Concentrations and dilution steps used to determine subject detection threshold for caffeine in water. Concentration series was adapted from ISO3970, Method of investigating sensitivity of taste.

<table>
<thead>
<tr>
<th>Caffeine concentration [mM]</th>
<th>Dilution step</th>
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<tr>
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</tr>
<tr>
<td>1.3</td>
<td>13</td>
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</table>
Figure 1: Flavor modification of 0.67mM caffeine in equi-sweet solution or caffeine-free Diet Cola beverage determined by forced choice triangle test. The x-axis represents the different solutions containing a fixed concentration of caffeine (0.67mM). The y-axis represents the percent correct discrimination. The two horizontal lines on the figure indicates the percentage at which subjects score if guessing (i.e. chance) and the level of significance at p<0.001 (upper bar). Bars above the upper line indicate subjects can easily discriminate solutions containing caffeine. n=30 subjects performing triplicate ratings.