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Prevention of tea-induced extrinsic tooth stain

Abstract: *Objectives:* The objectives of this study were to determine whether the addition of milk to tea reduces the ability of tea to stain extracted human teeth and, if so, to ascertain the component of milk that is responsible for milk's stain reducing properties. *Methods:* Extracted human teeth were immersed in a tea solution, with the addition of 2% milk, 5.26% lactose, 2.7% casein or 10% fat-free milk for 24 h at 37°C. A dental spectrophotometer (VITA Easyshade Compact) was used to evaluate the colour of the teeth both before and after immersion in the tea solutions. Commission internationale de l'éclairage (CIE) $L^*a^*b^*$ colour space values were recorded, and the change in colour (ΔE^*) was calculated. A two-tailed *t*-test or one-way analysis of variance (ANOVA) was used to determine whether there were statistical differences between groups. *Results:* Milk significantly reduces the ability of tea to stain teeth ($P = 0.0225$), specifically in the L^* and a^* dimensions ($P = 0.0182$ and $P = 0.0124$, respectively) of the colour sphere. Casein, which makes up 80% of the protein content in bovine milk, is the component of milk that is responsible for significantly reducing tea's ability to stain teeth ($P < 0.0001$). *Conclusions:* The addition of milk to tea significantly reduces the tea's ability to stain teeth. Casein was determined to be the component of milk that is responsible for preventing tea-induced staining of teeth to a similar order of magnitude that can be obtained by vital bleaching treatments.

Key words: dental hygiene; diet; extrinsic stain; staining; tooth discoloration

Introduction

Tea is one of the most common drinks in the world, second only to water (1). As research uncovers its health benefits, tea is becoming increasingly popular (2) and patients are concerned about the effects of staining on their dentition (3).

The increase in market share of commercially available surface whiteners and vital bleaching agents is indicative of consumer concern about oral aesthetics (4). In fact, 81% of surveyed dentists in the United Kingdom indicated that take-home vital bleaching services were offered and 32% provided bleaching services in their offices in 2008 (5), compared with a similar survey performed 4 years previously, which showed that only 35% of surveyed dentists provided home-based vital bleaching and 18% provided in-office bleaching (6). This dramatic increase in whitening/bleaching options that are offered professionally is likely a reflection of consumer demand.

The perception of tooth colour is dependent on both the intrinsic and extrinsic tooth stain. While intrinsic tooth stains are integrated into the

mineral structure of enamel and/or dentin during development (e.g. tetracycline treatment, excess fluoride ingestion) and therefore difficult to remove, extrinsic tooth stain is generally the result of consumption of chromogenic substances in the diet. Consequently, extrinsic tooth stain is more readily treatable compared with intrinsic stain. The extrinsic discoloration of teeth by tea is believed to be a result of the deposition of tannins on the tooth surface (7). The binding of tannins or other polyphenols to the hydroxyapatite component of enamel can be further exacerbated by the presence of salivary proteins (8).

Other proteins, specifically those found in milk, have also been shown to bind to the tannins in tea (9). Whether this binding could result in altered tooth stain intensity has not been demonstrated. Oral health professionals and tea aficionados concerned about tooth stain as a result of tea consumption often advocate the addition of milk to tea in an attempt to reduce stain; however, the efficacy of this strategy has not been scientifically demonstrated. This study is a covariate adaptively randomized, blinded, *in vitro* study conducted in the laboratory. The aims of this study were to (i) determine whether the addition of milk to tea can affect enamel staining by tea and (ii) elucidate the component of milk that is responsible for the reduction in the ability of tea to stain enamel.

Materials and methods

This study has been approved by the University of Alberta Health Ethics Research Board and conforms to Canada's Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans. The Ethics Research Board waived the need for consent.

Sample inclusion/exclusion criteria

Extracted permanent human teeth were collected from the University of Alberta, School of Dentistry Clinics between September 2011 and June 2012. For privacy and ethical reasons, the reason for tooth extraction and identifying characteristics (e.g. age, health, gender) of patients were not recorded. Teeth with amalgam restorations, carious lesions, obvious cracks or fractures, and/or insufficient crown height for spectrophotometric analysis were excluded from the study.

A total of 42 teeth that met inclusion criteria were collected for both studies. In both studies, $N = 6$ per group was used. Each tooth was used for only one study.

Sample preparation

All teeth were processed in accordance with the guidelines outlined by the Center for Disease Control and Prevention for using extracted teeth as research material (10). In brief, the extracted teeth were cleaned of blood and visible debris prior to being stored in normal saline. The teeth were then heat-sterilized by autoclaving (40 min, 121°C, 776 mmHg) and placed in 1 M sodium hydroxide (NaOH) for 48 h to remove the pellicle (11). Each tooth was assigned to a group using covariate adaptive randomization to account for tooth anatomy (i.e. molar versus incisor). The teeth were stored in double-distilled, deionized water (ddH_2O) until used.

Staining procedure

A tea solution was prepared by boiling 36 g of orange pekoe tea (Red Rose, Unilever, Toronto, ON, Canada) in 800 ml of water for 10 min. The following 20% (volume/volume) solutions were made to represent the various components of 2% bovine milk: 5.26% lactose (12) (Sigma-Aldrich, Oakville, ON, Canada), 2.7% casein (12) (Sigma-Aldrich, Oakville, ON, Canada), 10% fat-free milk (Carnation, Smucker Foods, Markham, ON, Canada), and 2% milk (Dairyland, Burnaby, BC, Canada) or ddH_2O.

To investigate whether milk reduces tea-induced staining on teeth, two groups of teeth were soaked in (i) tea + water (to control for dilution) or (ii) tea + milk. To determine the component of milk that was responsible for reducing staining, teeth were divided into the following groups: (i) tea + water, (ii) tea + casein, (iii) tea + lactose, (iv) tea + fat-free milk and (v) tea + milk.

Teeth were divided into groups using covariate adaptive randomization and individually incubated in each solution ($N = 6$ per group) at 37°C for 24 h. Following incubation, the teeth were immersed in 5 ml of ddH_2O for 10 min to remove any debris and allowed to dry on a laboratory bench for 10 min prior to spectrophotometric evaluation.

Colour evaluation

All teeth were evaluated using a dental spectrophotometer (VITA Easyshade Compact, VITA, Bad Säckingen, Germany) according to the instrument directions. The spectrophotometer was placed perpendicular and flush to the tooth surface, and the centre of the cervical third of the tooth crown was used for measurements and to ensure reproducibility. Commission Internationale de l'éclairage (CIE) $L^*a^*b^*$ colour space values of each tooth were recorded prior to staining and compared with values obtained following tea staining using the following formula (13):

$$\Delta E^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

The CIEL$^*a^*b^*$ system measures colour on a three coordinate scale: L^* – lightness of colour ($L^* = 0$ is black, while $L^* = 100$ is white); a^* – colour in the red and green dimension ($a^* > 0$ is red, while $a^* < 0$ is green); and b^* – colour in the yellow and blue axis ($b^* > 0$ is yellow while $b^* < 0$ is blue) (13).

All measures were performed in triplicate and under identical lighting conditions by at least two independent, blinded investigators.

Statistics

Data were analysed using GraphPad Prism v. 5.0 (GraphPad, La Jolla, CA, USA). A two-tailed *t*-test (unpaired when

comparing different groups, paired when comparing final versus baseline readings) or one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* using the tea alone group as control was used as appropriate. A Bartlett's test for equal variances was also used following one-way ANOVA. $P \leq 0.05$ was deemed statistically significant. Ninety-five percent confidence intervals (95% CI) were also calculated.

Results

All values are expressed as mean ± standard error of the mean (SEM) [95% confidence interval (CI)].

The addition of milk significantly reduces the ability of tea to stain teeth

The addition of milk to tea [ΔE^* = 5.38 ± 1.23 (2.97–7.79)] significantly reduces ΔE^* when compared with control [ΔE^* = 10.05 ± 1.11 (7.87–12.23)] (P = 0.0225) (Fig. 1). Specifically, the L^* and a^* values of the group of teeth exposed to the tea + milk solution [L^* = 72.40 ± 0.83 (70.77–74.03); a^* = 3.13 ± 0.42 (2.31–3.95)] were significantly lower than that of the group exposed to tea alone [L^* = 64.97 ± 2.37 (60.32–69.62); a^* = 6.70 ± 1.03 (4.68–8.72)] (P = 0.0182 for L^*; P = 0.0124 for a^*). There were no statistically significant changes in b^* values between the two groups (Table 1).

The casein in milk is responsible for milk's ability to reduce tea staining

Bartlett's test for determining homoscedasticity showed that the variance between groups was not significant. One-way ANOVA followed by *post hoc* Dunnett's tests of ΔE^* values reveals that casein [ΔE^* = 4.80 ± 0.77 (3.29–6.31)] significantly reduces the staining of enamel compared with control [ΔE^* = 12.73 ± 0.83 (11.1–14.36)] ($P < 0.0001$), while the addition of lactose [ΔE^* = 11.71 ± 1.76 (8.26–15.16)] did not affect tea's ability to

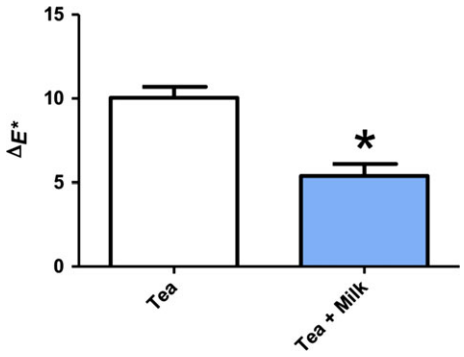

Fig. 1. The addition of milk reduces the ability of tea to stain teeth. The addition of 20% (volume/volume) of 2% bovine milk to a tea solution significantly reduces the ability of the tea to stain the enamel of extracted teeth when compared to control (20% v/v, ddH_2O) (*P = 0.0225 using t-test). N = 6/group.

Table 1. **Spectrophotometric values of teeth obtained using the VITA Easyshade dental spectrophotometer before and after staining with tea or tea + milk solutions. Values are mean ± standard error of the mean (95% confidence interval)**

	L^*		a^*		b^*		
	Baseline	Final	Baseline	Final	Baseline	Final	ΔE^*
Tea (control)	73.69 ± 1.53 (70.69–76.69)	64.97 ± 2.37‡ (60.32–69.62)	2.57 ± 0.70 (1.2–3.94)	6.70 ± 1.03‡ (4.68–8.72)	34.25 ± 1.66 (31–37.5)	35.18 ± 1.04 (33.14–37.22)	10.05 ± 1.11 (7.87–12.23)
Tea + Milk	75.37 ± 0.85 (73.7–77.04)	72.40 ± 0.83†‡ (70.77–74.03)	2.10 ± 0.48 (1.16–3.04)	3.13 ± 0.42†‡ (2.31–3.95)	34.42 ± 1.58 (31.32–37.52)	33.90 ± 0.98 (31.98–35.82)	5.38 ± 1.23† (2.97–7.79)

†$P \leq 0.05$ when compared with tea control (unpaired t-test).
‡$P \leq 0.05$ when compared with baseline reading (paired t-test).

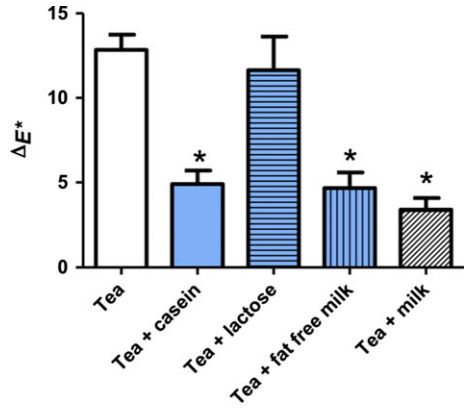

Fig. 2. Casein is the milk component that is responsible for reducing tea's ability to stain teeth. The addition of casein and fat-free milk to a tea solution reduces tea's ability to stain enamel ($P < 0.0001$) to a level similar to that of 2% milk. Lactose does not alter tea's ability to stain enamel. *indicates $P \leq 0.05$ when compared with tea group using one-way ANOVA followed by Dunnett's *post hoc*. $N = 6$/group.

stain teeth ($P \leq 0.05$) (Fig. 2). To evaluate putative role of fat in enamel staining, fat-free milk was added to tea and compared with 2% milk. Results show that the absence of fat does not significantly alter ΔE^* values [$\Delta E^* = 4.64 \pm 1.03$ (2.62–6.66)] (Fig. 2).

Similar to the results with milk, casein was found to significantly reduce L^* values ($P \leq 0.05$) while having minimal effects on a^* and b^* values (Table 2).

Discussion

The results from this study suggest that the addition of milk to tea may be an effective way of reducing extrinsic staining of enamel. Tea stains teeth both darker (L^* dimension) and redder (a^* dimension), and the addition of milk is capable of reducing both of these values, leading to a total reduction in ΔE^* by approximately 4.7. The colour difference between teeth stained with tea and the solution containing tea + milk is of particular significance as the human eye is able to detect differences as small as ΔE^* of 2.3 (13).

The magnitude of stain reduction is similar to the reduction, which is obtained by commercially available whitening products. Vital bleaching solutions can result in ΔE^* changes of 4.7 –6.0 (14) and is more effective than whitening toothpastes (15), which makes the addition of milk to tea an effective, viable and economical solution to the prevention of staining, without the additional risk of increased enamel roughness that is observed with many abrasive dentifrices (16). Whether the addition of milk to tea will reduce staining in the *in vivo* oral cavity is yet to be determined.

Although both the pellicle and salivary proteins have been removed from the experiments presented here and therefore do not identically mimic the *in vivo* oral cavity environment, this study examined the propensity of milk to reduce the staining capacity of tea, rather than the role of the pellicle

Table 2. **Spectrophotometric values of teeth obtained using the VITA Easyshade dental spectrophotometer before and after staining with tea or tea + components of milk solutions. Values are mean ± standard error of the mean (95% confidence interval)**

	L^*		a^*		b^*		
	Baseline	Final	Baseline	Final	Baseline	Final	ΔE^*
Tea	73.23 ± 0.77 (71.72–74.74)	63.58 ± 1.09‡ (61.44–65.72)	3.81 ± 0.47 (2.89–4.73)	10.41 ± 0.54‡ (9.35–11.47)	37.02 ± 1.53 (34.02–40.02)	40.89 ± 0.97‡ (38.99–42.79)	12.73 ± 0.83 (11.1–14.36)
Tea + casein	76.54 ± 1.61 (73.38–79.7)	73.27 ± 1.63†‡ (70.08–76.46)	1.67 ± 0.63* (0.44–2.9)	3.92 ± 0.87†‡ (2.21–5.63)	32.51 ± 1.69 (29.2–35.82)	33.76 ± 1.63†‡ (30.57–36.95)	4.80 ± 0.77† (11.1–14.36)
Tea + lactose	74.84 ± 1.26 (72.37–77.31)	65.75 ± 2.62‡ (60.61–70.89)	2.71 ± 0.64 (1.46–3.96)	8.29 ± 1.25‡ (5.84–10.74)	36.69 ± 1.52 (33.71–39.67)	40.31 ± 1.51‡ (37.35–43.27)	11.71 ± 1.76 (8.26–15.16)
Tea + fat-free milk	73.17 ± 1.43 (70.37–75.97)	73.5 ± 1.31† (70.93–76.07)	2.89 ± 0.48 (1.95–3.83)	2.68 ± 0.38† (1.94–3.42)	34.23 ± 1.37 (31.54–36.92)	34.41 ± 1.23† (32–36.82)	4.64 ± 1.03† (2.62–6.66)
Tea + milk	76.84 ± 1.12 (75.94–77.74)	75.91 ± 0.84*‡ (74.26–77.56)	2.37 ± 0.58 (1.23–3.51)	3.68 ± 0.66†‡ (2.39–4.97)	34.08 ± 1.37 (31.39–36.77)	35.95 ± 1.81‡ (32.4–39.5)	3.95 ± 0.71† (2.56–5.34)

†$P \leq 0.05$ when compared with control (one-way ANOVA with Dunnett's *post hoc* using tea as control column).
‡$P \leq 0.05$ when compared with baseline reading (paired *t*-test).

and/or salivary proteins in mediating stain formation. A number of other researchers have already investigated the role of the pellicle and/or salivary proteins in stain formation (8, 17, 18) and are in agreement that the presence of pellicle/salivary proteins serves to exacerbate stain formation on hydroxyapatite (the primary mineral component of enamel).

The results presented here strongly suggest that the addition of milk to tea is an effective way of reducing enamel staining; however, the addition of milk to tea may also affect the putative health benefits of tea, primarily its antioxidant, anticaries and antibacterial properties. Tea polyphenols have been shown to interact with and bind with casein, the most abundant protein in cow's milk (19). This interaction is believed to result in the precipitation of the polyphenol/casein complex, thereby preventing the tannin from binding to the tooth surface and causing staining. However, this precipitation can also have significant effects on the beneficial antioxidant and anticariogenic properties of tea. Casein has been found to bind to epigallocatechin gallate (EGCG), a tea polyphenol, and lead to its aggregation and precipitation (20). EGCG has been shown to be an effective anticaries agent (21) via the suppression of *Streptococcus mutans* growth (22). It is not known whether the aggregation of EGCG by casein would reduce its anticariogenic or antioxidant properties.

The precipitation of EGCG by milk may also have effects on periodontal disease. EGCG has been shown to prevent collagen degradation (23), a significant destructive component of periodontal disease. EGCG has also been shown to reduce the production of the inflammatory mediator prostaglandin E2 by *Porphyromonas gingivalis* stimulated human gingival epithelial cells (24), and whether aggregated EGCG would have the same effect remains to be determined.

The systemic effects of adding milk to tea has been controversial. At least one group has found that the addition of milk to tea prevents the protective cardiovascular effects of tea on the vascular endothelium (25), although the study lacked statistical power and a balanced control group. Another group has found that the addition of milk to black tea does not significantly affect plasma levels of antioxidants from consumed tea (26).

Conclusion

Thirty-four percent of the adult population in the United States is dissatisfied with their current tooth colour (27), and half of surveyed patients in the United Kingdom perceive that they have some sort of tooth discoloration (28). The results of this study reveal that the addition of milk to tea is a simple and effective way of reducing the impact of tea consumption on tooth coloration. However, it is important to keep in mind that the addition of milk to tea is a culture-specific phenomenon and that before clinicians recommend the addition of milk to tea to their patients to reduce the impact of enamel stain, it is important to assess whether the patients are consuming tea for health benefits, as these benefits may also be affected.

Clinical relevance

Scientific rational for study

Patients are increasingly concerned about the effect of stain-inducing beverages such as tea.

Principal findings

This study demonstrates that the addition of milk to tea effectively reduces the ability of tea to stain teeth, and casein, the primary protein found in milk, is responsible for this reduction in staining.

Practical implications

This manuscript shows that addition of milk to tea may be a cost-effective, easily implemented home care option to reduce tea-induced extrinsic tooth stain.

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