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Addition of whole, semiskimmed, and skimmed bovine milk reduces the total antioxidant capacity of black tea

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Abstract

Epidemiological studies have shown that populations consuming fruits, vegetables, tea, cocoa, and red wine have lower incidences of cardiovascular disease, certain cancers, and eye disease. These health effects have largely been attributed to the polyphenol content of the foods and drinks studied. Black tea is rich in a range of polyphenolic compounds that could potentially have health-promoting properties. The scale of consumption of tea in the United Kingdom means that it could be an appropriate vehicle for increasing the antioxidant activity and polyphenol content of human plasma. However, it is common practice in the United Kingdom to add milk to tea, and some studies have suggested that this may decrease the overall antioxidant capacity. The objective of the present study was to analyze and compare the antioxidant capacity of 5 brands of tea and to test the hypothesis that the addition of different volumes of whole milk, semiskimmed, and skimmed milk may affect the antioxidant capacity. Each of the teas analyzed was a significant source of antioxidants. The addition of 10, 15, and 20 mL of whole, semiskimmed, and skimmed bovine milk to a 200-mL tea infusion decreased the total antioxidant capacity of all the brands of tea. Skimmed milk decreased the total antioxidant capacity of the tea infusion significantly ($P < .05$) more than either whole milk or semiskimmed milk. We conclude that black tea is a valuable source of antioxidants and that the effect of milk on the total antioxidant capacity may be related to the fat content of the milk.

Keywords: Total antioxidant capacity; Black tea; Milk; FRAP; Antioxidants
Abbreviations: FRAP, ferric iron reducing antioxidant power.

1. Introduction

Tea (*Camellia sinensis*) is one of the most commonly consumed beverages in the world; therefore, the potential beneficial effects of tea may be important for public health. Moreover, black tea is consumed in western countries, whereas consumption of green tea is more particular to Asia and the Middle East. As black tea is widely consumed in the United Kingdom (3-4 cups of tea per day compared to 1-2 cups of tea per day for other countries) [1], the antioxidant components of black tea may contribute significantly to the overall antioxidant intake in the United Kingdom diet.

Several epidemiological studies suggest that black and green tea consumption is associated with a reduced risk of coronary heart disease [2-5], stroke incidence [6], chronic inflammation [7], and cancer incidence [8,9]. Black tea is rich in a range of compounds such as catechins, flavonoids, theaflavins, thearubigins, and phenolic acids that could potentially have health-promoting properties. Many studies have confirmed that black tea possesses considerable antioxidant capacity [10-13]. Antioxidants are known to protect tissues against damage caused by oxygen free radicals and lipid peroxidation, and it is proposed that the

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protective effect of tea against cardiovascular disease may be attributed to its antioxidant components.

Furthermore, recent public health research has focused on the use of diet-based interventions to decrease the risk of certain chronic diseases (cardiovascular diseases, diabetes, obesity, cancer). There is a need for studies to identify dietary components that may be used as part of this strategy. The identification of beverages commonly consumed, such as tea, with a high total antioxidant capacity could help both in dietary advice and in overall health outcome.

In the United Kingdom, milk is commonly added to black tea, which may lead to the formation of polyphenol-milk protein complexes [14]. Some authors have proposed that the formation of these complexes may decrease the bioavailability and the antioxidant potential of polyphenols in vivo [15,16]. We hypothesized that different volumes of milk and milk of varying fat content (whole milk, 3.5%-3.7%; semiskimmed milk, 1.7%-2.0%; and skimmed milk, <0.5% fat) may affect the total antioxidant capacity of tea. Therefore, the objectives for the present study were as follows:

1. To analyze and compare the antioxidant capacity of 5 brands of commercially available tea
2. To determine the effect of infusion time on the antioxidant capacity
3. To examine the effect of different volumes of bovine milk (10, 15, and 20 mL) and milk of varying fat content (whole, semiskimmed, and skimmed) on the total antioxidant capacity of the 5 teas

2. Methods and materials

2.1. Chemicals

All chemicals and reagents were of analytical grade and were purchased from Sigma-Aldrich (Poole, United Kingdom). Black tea and milk were purchased from a local cooperative supermarket.

2.2. Protocol

Five brands of commercially available tea were chosen. The weight of each tea bag was approximately 3.125 g (range, approximately 2.7-3.3 g of tea per tea bag). In all experiments, tea preparations were made using a standard protocol. Each tea was infused in 200 mL of boiling water (90°C). All experiments were carried out on a minimum of 3 separate occasions. Samples were analyzed in triplicate for each experiment.

2.3. Experiment 1: effect of infusion time

To investigate the effect of infusion time on the antioxidant capacity of the 5 teas, 6 infusions of each tea were prepared and analyzed after 1, 2, 3, 4, 5, and 10 minutes of brewing. After the appropriate brewing time, the tea bag was removed, and samples were taken for analysis. The concentration effect of squeezing the tea bag was also examined. Teas were prepared and allowed to brew for 1, 2, 4, and 8 minutes. After the appropriate time, the tea bag was squeezed for 10 seconds, removed, and samples were taken for analysis.

2.4. Experiment 2: effect of the tea bag

Analysis of the effect of the tea bag on total antioxidant capacity was assessed by comparing tea brewed with the tea bag to tea brewed using black leaf tea. The black leaf tea was obtained by removing the tea leaves from the tea bag for each of the 5 brands of tea.

2.5. Experiment 3: analysis of bovine milk

Three different types of the same brand of bovine milk were analyzed as follows: whole milk (3.5%-3.7% fat), semiskimmed milk (1.7%-2.0% fat), and skimmed milk (<0.5% fat). Each sample was taken immediately after opening the milk carton to ensure that values reflected the antioxidant capacity of fresh milk. The 3 different types of milk were again analyzed 7 days and 14 days after opening. The effect of varying temperature on the total antioxidant capacity of milk was also analyzed (4°C-60°C).

2.6. Experiment 4: the effect of the addition of different types of milk

Before analyzing the effect of the addition of different types of milk, cold water (10, 15, and 20 mL) was added to each tea to analyze the dilution effect of an additional 10, 15, and 20 mL of liquid. To investigate the impact of milk of varying fat content on the total antioxidant capacity of tea, 10, 15, and 20 mL of bovine milk (whole, semiskimmed, and skimmed) were added to each tea. Each milk sample was taken immediately after opening the milk carton.

2.7. Total antioxidant capacity

The total antioxidant capacity of the tea samples was determined using a modification of the ferric iron reducing antioxidant power (FRAP) assay of Benzie and Szeto [17]. Briefly, FRAP reagent was prepared from 300 mmol/L of acetate buffer, pH 3.6, 20 mmol/L of ferric chloride, and 10 mmol/L of 2,4,6-tripryridyl-s-triazine made up in 40 mmol/L of hydrochloric acid. All 3 solutions were mixed together in the ratio 10:1:1. The FRAP assay was performed by incubating the sample and reagent at 37°C for 4 minutes. Absorbance at 593 nm was determined relative to a reagent blank also incubated at 37°C. The total antioxidant capacity of samples was determined against a standard of known FRAP value, ferrous sulfate (1000 μmol/L).

2.8. Statistical analyses

All data are presented as means (±SEM) of at least 3 independent experiments, each of which had a minimum of 3 replicates of sample. To determine the effect that a simple dilution may have, tea infusions (200 mL) with different

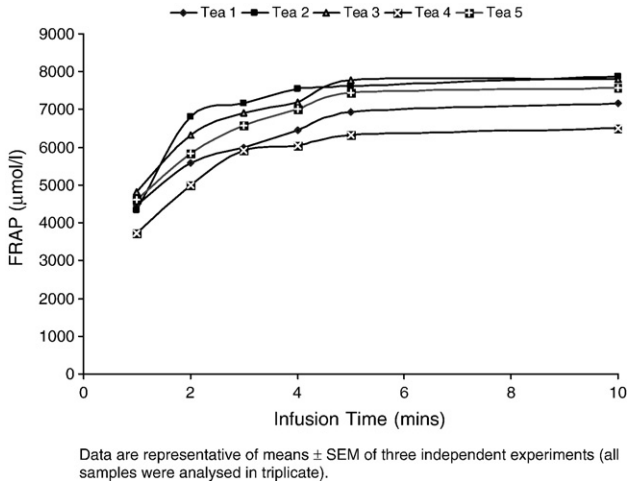


Fig. 1. The effect of infusion time on the total antioxidant capacity (FRAP; micromol per liter) of 5 different brands of tea. Teas were infused for 1, 2, 3, 4, 5, and 10 minutes.

volumes of cold water added (10, 15, and 20 mL) were compared to a standard 200 mL of tea infusion. To investigate the effect of the addition of whole, semiskimmed, or skimmed bovine milk (10, 15, and 20 mL), tea infusions with the various milk additions were compared to tea infusions with equal amounts of cold water added (to ensure that any effects noted were not simply due to dilution). For comparisons between samples, data were analyzed by analysis of variance and Tukey multiple comparison test (SPSS, version 17; SPSS Inc, Chicago, Ill). A probability of 5% or less was accepted as statistically significant.

3. Results

3.1. Experiment 1: effect of infusion time

As shown in Fig. 1, the antioxidant potential improved gradually according to the infusion time. All teas followed a similar trend. A maximal value was obtained after 10 minutes; however, more than 50% of the maximum value was obtained after only 1 minute of brewing. Moreover, more than 95% of the antioxidant capacity was available after 5 minutes and more than 90% available after 4 minutes for all of the teas. Tea 4 achieved the lowest maximum antioxidant capacity when compared to the other teas. For all of the teas analyzed, the maximum FRAP value (micromol per liter) was achieved at a faster rate when the tea bag was squeezed (10 seconds) before removal (results not shown).

3.2. Experiment 2: effect of the tea bag

Infusions of tea using either the tea bag or tea leaves removed from the bag were compared (Table 1). For all the teas, there was a significant difference ($P < .05$) between the 2 preparations. Tea infused using tea leaves obtained a higher total antioxidant capacity compared to tea infusions prepared with the tea bag. Furthermore, the maximum total antioxidant capacity using tea leaves was obtained after 2 minutes compared to 10 minutes when using tea bags. The recommended brewing time (according to the manufacturer's instructions) is between 3 and 4 minutes. For all further studies, a standard protocol for tea infusions (using tea bags) was used with an infusion time of 4 minutes. At this time, approximately 90% to 95% of the maximum antioxidant capacity had been achieved.

3.3. Experiment 3: total antioxidant capacity of bovine milk

The FRAP values (Fig. 2) indicate that the antioxidant capacity of bovine milk may be related to the fat content. The total antioxidant capacity of whole milk was significantly ($P < .05$) greater than both semiskimmed and skimmed milk. Furthermore, the general trend was that the antioxidant capacity decreased in all of the milk samples after 7 days and 14 days from opening with a significant ($P < .05$) decrease seen in the semiskimmed and the skimmed milk (after 14 days only). Variations in temperature of the milk did not affect the total antioxidant capacity (results not shown).

Table 1
Each tea was infused for 1, 2, 3, 4, 5, and 10 minutes

	Tea 1		Tea 2		Tea 3		Tea 4		Tea 5	
	A	B	A	B	A	B	A	B	A	B
1 min	4458 ± 247	7826 ± 155 *	4326 ± 91	7923 ± 55 *	4817 ± 118	7899 ± 38 *	3735 ± 91	6845 ± 154 *	4626 ± 44	7813 ± 161 *
2 min	5581 ± 208	8155 ± 59 *	6811 ± 146	8247 ± 68 *	6326 ± 132	8188 ± 37 *	4989 ± 167	7498 ± 93 *	5845 ± 231	8244 ± 93 *
3 min	5993 ± 297	8190 ± 81 *	7158 ± 235	8289 ± 88 *	6919 ± 128	8258 ± 11 *	5918 ± 13	7732 ± 49 *	6589 ± 233	8277 ± 66 *
4 min	6463 ± 242	8244 ± 85 *	7545 ± 102	8317 ± 86 *	7190 ± 98	8238 ± 10 *	6031 ± 42	7769 ± 31 *	7009 ± 250	8269 ± 69 *
5 min	6944 ± 146	8177 ± 131 *	7622 ± 98	8309 ± 83 *	7771 ± 94	8291 ± 48	6317 ± 25	7807 ± 110 *	7456 ± 159	8263 ± 47 *
10 min	7155 ± 174	8084 ± 131 *	7866 ± 77	8194 ± 74	7812 ± 83	8197 ± 19	6502 ± 47	7578 ± 101 *	7572 ± 46	8237 ± 56 *

Tea infusions were made from 200 mL of boiling water and tea bags (as sold) or tea leaves removed from bag. Column A for each tea illustrates the FRAP values (micromol per liter) of tea bag infusions. Column B for each tea illustrates the FRAP values (micromol per liter) of tea leaf infusions. Data are representative of means ± SEM of 3 independent experiments (all samples were analyzed in triplicate).

* $P < .05$, significantly different from tea bag infusion at same infusion time.

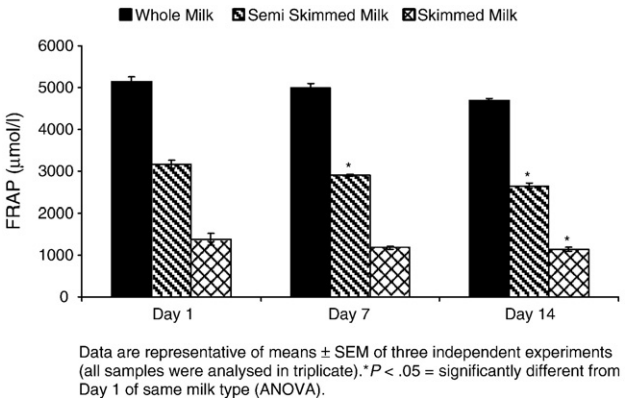


Fig. 2. Ferric iron reducing antioxidant power (micromol per liter) values of whole, semiskimmed, and skimmed milk on day of opening (day 1), 7 days after opening (day 7), and 14 days after opening (day 14).

3.4. Experiment 4: the effect of the addition of different types of milk

All teas were prepared as previously stated, and the effect of the addition of 10, 15, or 20 mL of water, whole milk, semiskimmed milk, or skimmed milk was investigated. For all of the brands of tea, the addition of 10, 15, and 20 mL of semiskimmed or skimmed milk significantly ($P < .05$) decreased the total antioxidant capacity compared to tea with the same volume of water added (Fig. 3A-E). The addition of whole milk to each of the teas also decreased the total antioxidant capacity but to a lesser extent.

4. Discussion

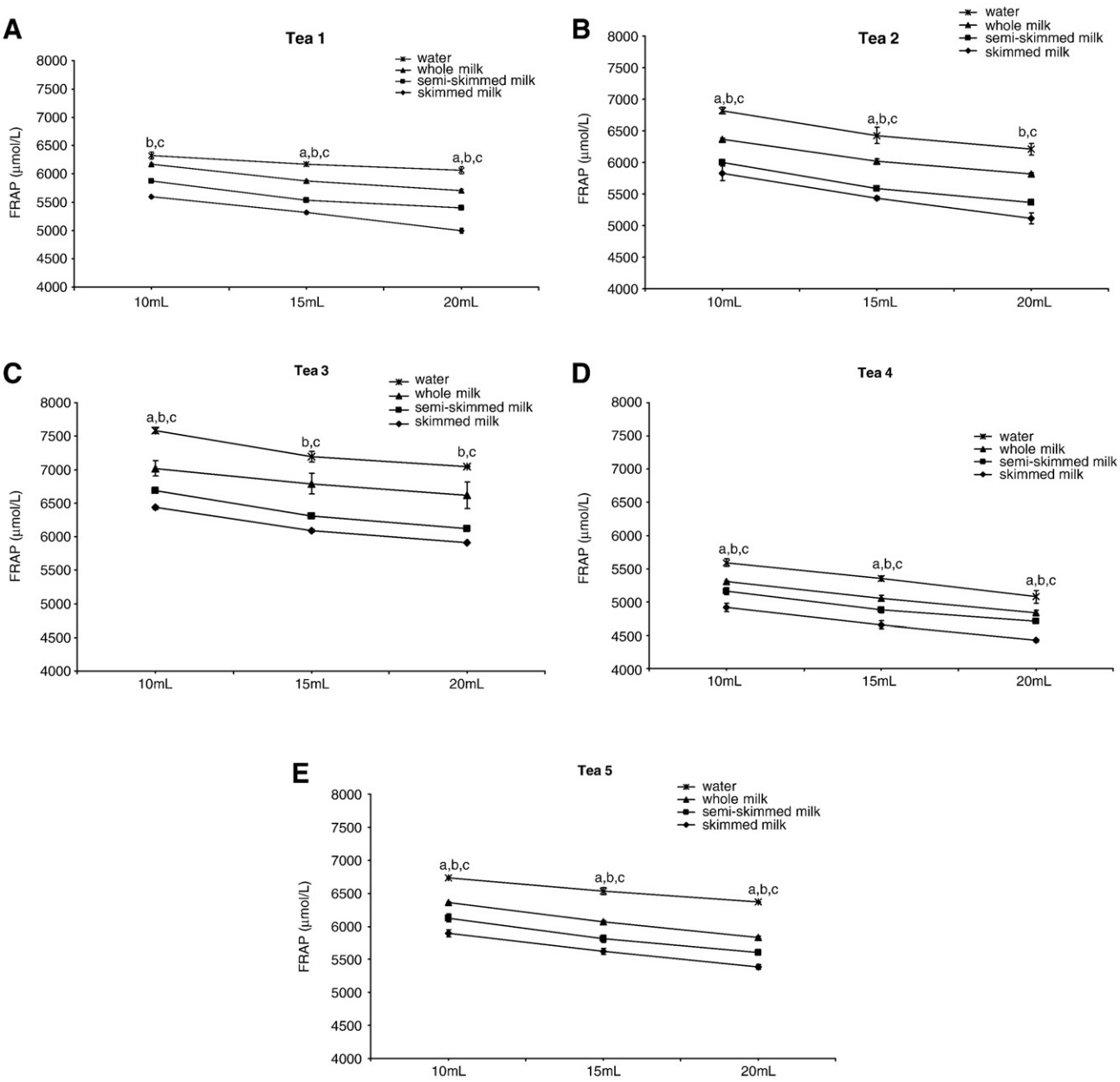
The present study analyzed the total antioxidant capacity of 5 different brands of tea, regularly consumed in the United Kingdom. Although tea is often reported to have a very high level of antioxidant activity, few researchers have looked at tea that is commercially available in most stores throughout the United Kingdom. Because of the high level of consumption of black tea in the United Kingdom, tea may be a significant contributor to the overall antioxidant status of certain individuals that may have beneficial health implications.

The 5 brands of tea were analyzed both as they are sold (tea leaves in tea bags) and also by analyzing infusions of the tea leaves after being removed from the bag. There was no significant ($P < .05$) difference in the total antioxidant capacity between the teas when the tea leaves were analyzed. Kyle et al [14] found similar results, they studied 6 brands of tea and observed no significant differences for total phenolics, catechins, and FRAP value after 7 minutes of infusion. For all of the brands of tea, the total antioxidant capacity was lower when tea was infused with the tea bag compared to the tea leaves. This may indicate an interaction of the tea antioxidant properties with the tea bag material as also reported by Langley-Evans [16]. Antioxidant concentrations of tea are known to be influenced by many factors including infusion time, stirring duration and intensity, leaf size, and tea bag porosity [18,19].

The FRAP values of whole, semiskimmed, and skimmed bovine milk obtained in the present study were 4 times greater than those reported by Langley-Evans [16] suggesting that the antioxidant capacity of milk can vary considerably between brands. Previous studies have mentioned that milk antioxidants may play an important role in preventing lipid peroxidation and peroxyl/superoxide radical generation and also in maintaining milk quality [20,21]. Several dairy products and various fractions from them have previously been shown to exhibit strong antioxidant activity [22-25]. Furthermore, in the present study, the greatest antioxidant potential was noted in whole milk. Milk is known to contain a number of fat-soluble antioxidants—tocopherols, carotenoids, and retinols. Therefore, decreasing the fat content of the milk may eliminate a number of the fat-soluble antioxidant components and therefore decrease its antioxidant potential.

Significantly in the present study, it appeared that the addition of bovine milk to tea did reduce the antioxidant potential, as observed values were between 2% and 15% lower than tea with similar quantities of cold water added and between 7% and 25% lower than a standard tea infusion without any addition. This decrease was not observed by Kyle et al [14] who found that the addition of milk did not significantly affect the FRAP values of tea. Also, in stark contrast to results by Langley-Evans [16], the greatest decrease (micromol per liter) was observed after the addition of skimmed milk. Moreover, in another study by Langley-Evans [26] analyzing the antioxidant potential of a standard infusion of black tea, a FRAP value of 17 020 ± 1900 μmol/L was observed for a 200-mL infusate (1 minute with boiling water). After the addition of 20 mL of semiskimmed milk to these infusions, the FRAP value decreased by 28% to 12 291 ± 1204 μmol/L. These results are much higher than those observed in the present study; however, the tea used by Langley-Evans is not a brand commercially available in the United Kingdom. A variety of FRAP values have been reported for different teas. It is important to have knowledge on the antioxidant capacity of different brands of commercially available teas, commonly consumed, if we are to suggest tea as a viable means of increasing antioxidant status in humans.

Lorenz et al [27] studied the interactions of milk with tea compounds and found that an addition of 10% milk to tea decreased the concentrations of certain catechins in tea, whereas the others were not affected. It has previously been demonstrated that tea polyphenols interact with proteins [28] and that polyphenols can bind to proteins [29,30]. The interaction between flavonoids and proteins affects their antioxidant capacity in vitro [31]. There has also been some evidence that polyphenols possess a high binding affinity for proline-rich proteins such as caseins [32]. Jobstl et al [33] demonstrated the noncovalent cross-linking of epigallocatechin gallate by caseins, highlighting the interaction of tea catechins with milk caseins.



Data are representative of means ± SEM of three independent experiments (all samples were analysed in triplicate).

a = water addition is significantly different ($P < .05$) from whole milk addition of same volume.

b = water addition is significantly different ($P < .05$) from semi-skimmed milk addition of same volume.

c = water addition is significantly different ($P < .05$) from skimmed milk addition of same volume

Fig. 3. (A-E) The total antioxidant capacity (FRAP; micromol per liter) of tea infusions with 10 mL, 15 mL, and 20 mL of water, whole milk, semiskimmed milk, or skimmed milk added.

Van het Hof et al [34] showed that consumption of black tea was followed by a rapid increase of the total catechin concentrations in blood. However, they noticed that black tea was rapidly absorbed and that milk did not impair the bioavailability of tea catechins. Green et al [35] noted an increase in catechin recovery from teas formulated with 50% bovine milk after an in vitro digestion procedure. Hollman et al [36] found that the increase in plasma concentrations of quercetin after black tea consumption was also not affected by the addition of milk to tea. These results do not support the findings of Serafini et al [37] who reported that the increase in antioxidant activity of plasma after black tea consumption was attenuated by the addition of milk. It is therefore possible that the inhibitory effect of milk on plasma antioxidant activity is due to a reduction of the absorption

of total antioxidants and not related to catechins and quercetin in particular.

During the formation of black tea, tea leaves are rolled and allowed to ferment resulting in the formation of relatively high concentrations of theaflavins and thearubigins [38]. Much recent media attention has promoted green tea over black tea due to the higher concentrations of epigallocatechin gallate in the former. Also, though studies have confirmed higher levels of catechins in human plasma after green tea ingestion, studies have failed to show a difference in antioxidant capacity after ingestion of green tea and black tea [39]. These findings suggest that the antioxidant capabilities of black tea may be attributed to the theaflavin and thearubigin content. In the present study, milk between 5% and 10% of the final volume of tea was added. The study was to analyze the total antioxidant activity and not to look at specific fractions. Although the addition of milk may not inhibit the catechin or quercetin concentrations, it may affect other antioxidant components such as the theaflavins and thearubigins that will affect the total antioxidant capacity of black tea.

A number of biochemical assays have been developed and continuously modified in order that the total antioxidant capacity of foods and beverages can be more accurately measured. The most widely used and accepted of these assays are the ORAC (oxygen radical absorbance capacity), TEAC (Trolox equivalent antioxidant capacity), FRAP, and DPPH (2,2′-diphenylpicrylhydrazyl radical) assays. The FRAP assay was used throughout this study as a measure of total antioxidant capacity. Furthermore, knowledge of the antioxidant capacity of the beverages before ingestion is an initial indicator only. Future research will seek to repeat the present study using a battery of antioxidant assays and to investigate the effects in human subjects.

The present study has highlighted that a number of basic parameters such as infusion time, concentration, different quantities of milk, and milks of varying fat content may affect the total antioxidant capacity of tea. Of particular interest was an attempt to mimic conditions that are representative of the common ways in which teas are prepared for human consumption in the United Kingdom. We accept the hypothesis that different volumes of bovine milk and milk of varying fat content affect the total antioxidant capacity of tea. The degree to which the addition of milk reduces the antioxidant capacity of black tea depends on the amount added and the fat content of the milk.

In conclusion, from a public health perspective, tea is rich in antioxidants and may be an important contributor to an individual's overall antioxidant status. The addition of milk may lower the total antioxidant capacity of tea; however, this effect is much greater with skimmed milk compared to whole milk.

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