TOPICAL REVIEW

# HEALTH EFFECTS OF BLACK TEA AND THEIR MODULATION BY MILK

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## ABBREVIATIONS

FRAP: Ferric reducing ability of plasma; TRAP: Total radical-trapping anti-oxidant parameter; ORAC: Oxygen radical absorbance capacity; EC, (-) –epicatechin; ECG (-)-epicatechin gallate; EGC, (-)-epigallocatechin; EGCG,(-)-epigallocatechin gallate; TR, Thearubigin; TF, Theaflavin

## ABSTRACT

There is a very long history of association of tea with health; it evolved from domain of medicine in ancient China to its current global status of beverage having the largest consumption after water. While there is a strong epidemiological link of tea consumption with the risk of cancer and heart diseases, the causal relationships are just beginning to unravel. Flavonoids are one of the most active ingredients in tea. Both the nature and amount of flavonoids available to human body after the consumption of tea, therefore, become critical in defining the efficacy of tea. Multiple factors which govern bioavailability of flavonoids include quantity and quality of available flavonoids in tea, genetic characteristics of tea consuming population, the manner (brewing vs. boiling) in which tea infusion is prepared, and perhaps also by whether or not milk and the amount of milk is added to tea. The addition of milk may have significant implications in bioavailability of tea flavonoids in south Asia where tea infusion is mostly prepared by boiling (in comparison to brewing) tea leaves in presence of milk for time ranging from few to several minutes. In the absence of any significant epidemiology or clinical study from the region, the supporting evidence for public health contribution of tea relies on the evidence derived mainly from western countries for black tea and Japan and China for green tea. In this article, we review the relevant literature available on influence of milk on the health effects of tea. The available literature indicates that amount of milk in tea, if kept below 25%, does not significantly affect bioavailability of catechins in blood plasma. Though milk may delay the time taken to raise plasma antioxidant potential, the levels still reach significantly higher to that of baseline. There is a need, particularly in South Asia, to initiate further studies to generate data, which at this time seem insufficient for any evidence based nutritional recommendation on addition of milk to tea especially when the amount of added milk is high.

***Keywords:*** Black tea and milk, Tea polyphenols, Tea flavonoids, Tea and Health.

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## 1. INTRODUCTION

As a beverage, tea is second to water in terms of its consumption – its annual per capita intake exceeds 40 liters of which most i.e., 80% is black, ~ 18% is green and less than ~ 2% is oolong (McKay and Blumberg, 2002). Tea is well studied for its health effects, which because of its almost universal and large consumption, are of immense public health importance (Rimm and Stampfer, 2004 and Weisburger, 2006). While there is a substantial amount of data on health benefits of tea in *in vitro* model systems, animals and in human studies, scientific discussions on implications of this information on public health are relatively recent and are dominated by green tea (Jhawar, 2004).

Tea is strongly linked to cardiovascular health (CVH) (Gardner et al, 2006; Kuriyama et al, 2006; Hodgson, 2006; Stangl et al, 2006; Peters et al, 2001; Huxley and Neil, 2003; Vita, 2003 and Steptpe et al, 2006) and weakly to prevention of cancer (Gardner et al, 2006; Higdon and Frei, 2003; Arab and Ilyasova, 2003; Tavani and Vecchia, 2004; Oguni et al, 1992; Yu and Hsieh, 1991 and Kinlen et al, 1988). There are strong indications that these beneficial effects of tea are mediated by polyphenolic flavonoids (Hertog et al, 1993, 1995 and Knekt et al, 1996). Among the many mono and polymeric polyphenolic compounds found in green and black tea, catechins have been used as the most prominent representative flavonoids. The intake of catechins correlates inversely with cardiovascular health risk (Arts et al, 2001 and Van het Hof et al, 1999). Since catechins are present in tea in relatively high amounts and can even be detected at low (nM) concentrations in blood plasma, they have been the markers of choice. Estimates on catechin bioavailability are reported by several authors (Higdon and Frei, 2003; Lorenz et al, 2004 and Huang et al, 1999). The health effects of green tea are perceived to be more than those of black tea (Kuriyama et al, 2006; Zhu et al, 2006, Cheng et al, 2006 and Chung, 2006). A long history of existence of green tea, and its cultural integration in the social fabric of Japan and China attracted much attention to identify its functional roles. In 1950s, with the advent of CTC technology, production of black tea grew rapidly driving its share to presently of more than 80% of total consumed tea.

The processing conditions, used in manufacture of black tea, trigger enzymatic oxidations of polyphenols, and are responsible for its very distinct composition. Black tea is often consumed by adding milk to infusion (as prevalent in UK) or its preparation involves boiling of tea leaves in presence of milk (as in South Asia – mainly India, Pakistan and Bangladesh). Thus the nature of final beverage of which effect on health needs to be assessed varies according to cultural practices involved in its preparation and consumption. Change in the composition of tea by processing condition and addition of milk, are concerns that raise some doubt on the functional benefits of black tea. For example, the processing conditions in black tea are said to decrease levels of health imparting flavonoids like catechins (see Cheng, 2006). The other concern, which is considered to take over the health properties of black tea, is the practice of addition of milk. This was first used as a reason by Hertog et al, (1997) to explain the negative relationship of tea consumption with risk factors for CVH in Welsh population. Subsequent studies, however, showed that addition of up to 25% skimmed milk did not affect bioavailability of catechins in blood plasma or decreased it only marginally (Van het Hof et al, 1998; Reddy et al, 2005 and Kyle et al, 2007). This review addresses these two main concerns – the nature of evidence on health benefits of black tea and whether health benefits of black tea are still available when it is consumed or prepared with added milk.

## 2. TYPES OF TEA – GREEN, BLACK AND OOLONG

Teas prepared from the leaves of *Camellia sinensis* are classified into three main types – black, green and oolong. The classification is based on processing condition used in preparation of these teas, which defines their composition. Freshly plucked tea leaves are rich in flavanol monomers known as catechins; the principal catechins being, (-) -epicatechin (EC), (-) - epicatechin gallate (ECG), (-) - epigallocatchin (EGC) and (-)-epigallocatechin gallate (EGCG) (Robertson, 1992). The maceration of leaves during the manufacture of black tea brings cytoplasmic catechins in contact with vacuolar polyphenol oxidase (PPO). This complex oxidation process generates monomeric, dimeric and polymeric flavanols. The most characterized and important oxidized polyphenols in black tea are dimeric theaflavins (TFs) (~10% of total flavonoids) and polymeric thearubigins (TRs) (~60% of total flavonoids). This is the critical aspect of black tea manufacture - the process is maximized in favour of oxidation. The process of oxidation leaves behind low catechin levels in black tea (~20-30%) (Balentine et al, 1997). In contrast, during green tea manufacture PPO is heat killed to limit enzymatic oxidation leaving the catechins unaffected. The other important flavonoids in tea are flavonols (kaempferol, quercetin and myricitin) which exist as glycosides. These molecules are not much affected by processing and their amounts in green and black tea are similar (Balentine et al, 1997). In case of oolong tea, leaves are mildly bruised to allow the release of only some of the PPO and are allowed to oxidize for less time than used for black teas. The composition of oolong tea with respect to key flavonoids like catechin, theaflavin and thearubigin is in between those of green and black teas.

While having a distinct composition, the total polyphenol content in green tea and black tea are similar (Pan et al, 2003 and Vinson, 1998) and their antioxidant potential do not differ significantly (Balentine et al, 1997 and Wiseman et al, 1997). Under black tea processing conditions, catechins oligomerise mainly to dimeric theaflavins and polymeric thearubigins which are also potent antioxidants (Graham, 1992).

## 3. HEALTH BENEFITS OF TEA:

### 3.1. *IN VITRO* ANTIOXIDANT ACTIVITY OF TEA POLYPHENOLS:

Tea polyphenols are strong antioxidants (Vinson et al, 1995; Leung et al, 2001; Frei and Higdon, 2003; Luczaj and Skrzydlewska, 2005 and Gardner et al, 2007) and this has been established using various techniques (Langley-Evans, 2000a; Miller et al, 1996; Cao et al, 1996; Rice-Evans et al, 1995; Robinson et al, 1997; Prior and Cao, 1999). Cao et al, (1996), using the oxygen radical absorbance capacity (ORAC) assay, showed more antioxidant activity for both green and black tea against peroxyl radicals than vegetables such as garlic, kale, spinach and brussels sprouts. Amongst 24 flavonoid species from plants evaluated in trolox equivalent antioxidant capacity (TEAC) assay, catechin and epicatechin ranked amongst the most potent (Rice-Evans et al, 1995). Electron spin resonance (ESR) also has been used to measure antioxidant potential of green and black tea extracts under both aqueous and organic solvent conditions. In both aqueous and organic solvent conditions, the radical quenching ability of green tea extracts was more (~ 24%) effective than black tea (Gardner et al, 1998). EGCG was the most effective antioxidant and when all the four catechins were reconstituted in an artificial system, their effects were summative indicating neither synergy nor antagonism. The antioxidant capacity, measured in terms of thiobarbituric acid-reactive substances (TBARS) and conjugated dienes produced during LDL oxidation, of individual catechins and theaflavins is in order of theaflavin-3,3'-digallate($TF_3$)>ECG>EGCG$\geq$theaflavin-3'-gallate($TF_2$B)$\geq$theaflavin-3-gallate ($TF_2$A)$\geq$theaflavin($TF_1$)$\geq$EC>EGC (Leung, 2001). This indicates that conversion of catechins to theaflavins during fermentation does not decrease their antioxidant potential.

The studies on antioxidant potential suffer from several limitations. The methods of antioxidant measurement are not specific and the values of antioxidant potential are dependent on the method used for their measurement. Moreover, the data from the *in vitro* measurements may not reflect physiological conditions where bioavailability and metabolism play important role (Frei and Higdon, 2003). Considering these limitations of *in vitro* measurements, there have been several attempts to directly measure the increase in antioxidant potential of blood plasma arising after the tea consumption.

### 3.2 *EX VIVO* ANTIOXIDANT CAPACITY OF TEA POLYPHENOLS:

Several clinical studies show that a single dose of tea increases antioxidant potential of blood plasma as measured by FRAP (Benzie et al, 1999, Leenen et al, 2000 and Langley-Evans, 2000b), 2,2''-azino-di-2-ethyl-benzthiazolne sulphonate ($ABTS^+$) (Sung et al, 2000) and total radical antioxidant parameter (TRAP) (Pietta et al, 1998a,b). In general, increase in plasma catechins levels after green tea consumption is at least three times more than black tea. This is because green tea contains higher amounts of catechins. However, the extent of increase in plasma antioxidant potential by black tea is similar to green tea and may arise because of black tea flavonoids like TFs and TRs (Leenen et al, 2000 and Leung et al, 2001).

The effect of tea consumption has also been observed to reduce oxidative stress markers like lipid peroxidation products (Nakagawa et al, 1999, Klaunig et al, 1999 and Freese et al, 1999), oxidative DNA damage (Klaunig et al, 1999 and Lean et al, 1999) and free radical generation (Klaunig et al, 1999). Studies evaluating the efficacy of catechin in protection of oxidation of low density lipoprotein (LDL) in serum, found that though, the catechins preferentially partitioned into protein rich blood plasma fraction (60%) and HDL (23%), their amounts in the LDL particles were, however, below the required levels to provide protection to Cu induced *ex vivo* oxidation (van Het Hof et al, 1999). Widlansky et al (2005) showed that a modest increase in catechin levels after four weeks of black tea consumption is accompanied by increase in endothelial function. On the other hand, in these studies, tea consumption did not improve plasma antioxidant activity (ORAC and FRAP activity), serum C-reactive protein-rich levels, urinary 8-isoprostane and 8-hydroxy-2'-deoxyguanosine. While evaluating effect of tea consumption on endothelial function and F2-isoprostane, Hodgson et al, (2002) also found similar results. It appears that there is no antioxidant or anti-inflammatory intermediate to which the effect of tea dependent increase in endothelial function or decreased cardiovascular disease can be attributed (Widlansky et al, 2005).

### 3.3 EPIDEMIOLOGICAL AND CLINICAL STUDIES:

Epidemiological studies, designed to determine the effect of consumption of green and black tea on risk of cardiovascular disease (CVD), coronary heart disease (CHD) and myocardial infarctions (MI) have been conducted in several countries (USA, European Countries, UK, Australia, Japan and Middle East). The studies conducted in diverse populations in a range of geographies, to a large extent, assure that lifestyle factors unique to one population do not bias the overall conclusions. A meta-analysis of studies published between 1966 to 2000 by Peters et al, (2001) covering association of tea drinking with incidence of stroke, MI, and CHD show that the incidence rate of MI in group consuming 3 cups of tea per day decrease by 11%. Meta-analysis of Huxley and Neil (2003) conducted with prospective cohort studies published from 1966 to 2001, assessed the association of dietary flavonol intake with the subsequent risk of coronary heart disease (CHD) mortality. There was a 20% reduction in CHD mortality in the top third compartment of dietary flavonol intake. Epidemiological study in CHD patients showed that mortality due to cardiovascular diseases (CVD) decreased in people consuming moderate (<14 cups per week, hazard ratio 0.69) and high (>14 cups per week, hazard ratio 0.61) amounts of tea compared to non-drinkers. In a case-controlled study on diet and risk of ischemic heart diseases (IHD) in India conducted in two major cities (New Delhi and Bangalore), there was no such significant association found for tea (Rastogi et al, 2004).

One of the study (Hertog et al, 1997) conducted in UK, however, reported an increased risk of CVD death with consumption of black tea. The study also considered that this may be a population specific effect observed in UK and may arise because of a strong link between prevalence of tea consumption with low socio-economic status (Hertog et al, 1997). This socioeconomic class also showed higher association with other risk factors like obesity, smoking, low intake of fruits and vegetables and a diet high in saturated fats.

In three out of four case-controlled studies, the CVD relative risk decreased with consumption of three cups of tea - RR for CHD was 0.49 in Saudi Arabia (Hakim et al, 2003), 0.31 for MI in United States (Sesso et al, 1999) and 0.29 for MI in Italy (Tavani and Vecchia, 2004).

Two out of six clinical studies conducted with tea in mildly hypercholesterolemic men and women (Davies et al, 2003 and Maron et al, 2003), showed a significant lowering of total and LDL cholesterol. The randomized parallel double blind placebo controlled study performed by Wiseman et al, (2004) showed total cholesterol lowering of 8% in postmenopausal women consuming 8 cups of green tea for 12 weeks. In the group consuming black tea, the decrease was limited to 5%. In randomized, placebo and diet-controlled study, with mildly hypercholesterolemic subjects, consumption of five servings of black tea reduced cholesterol by 3.8% and LDL by 6.4% (Davies et al, 2003) in three weeks. In a recent large randomized double blind placebo controlled parallel study with 240 volunteers, consumption of theaflavin-enriched tea extract equivalent to six cups of black tea for twelve weeks decreased the total cholesterol and LDL by 11.3% and 16.4% , respectively.

While these three recent studies show efficacy of tea for cholesterol lowering, other less controlled studies demonstrate tea consumption has no impact on total or LDL-cholesterol. The explanations for these inconsistencies are diverse and may arise due to differences in several factors including study design, tea preparation protocol, amounts consumed, kind of tea, dietary restrictions, washout phase, composition of the test population and baseline risk factors.

There is evidence that site of action for cholesterol-lowering effects of tea is in both liver and gut. Tea flavonoids or sub fractions of the tea flavonoids could lower cholesterol concentrations by increasing fecal bile acid excretion and LDL-receptor activity (Bursill et al, 2003; Chisaka et al, 1988; Muramatsu et al, 1986; Koo and Noh, 2007; Bursill et al, 2007 and Hsu et al, 2006), or via their estrogenic activity (Breinholt et al, 2000).

There are eight studies, which show green tea, black tea or EGCG significantly improve vascular health benefits of tea. The evidence, mainly obtained in last decade, involve use of imaging to observe state of vascular flexibility in people after consumption of green tea (Nagaya et al, 2006 and Kim, 2006), black tea (Duffy et al, 2001; Hodgson et al, 2002;2005; Hirata et al, 2004 and Lorenz et al, 2007) and EGCG (Widlansky et al, 2007). This evidence is in line with increasing benefits of flavonoids in vascular health demonstrated independently for cocoa (Heiss et al, 2006; Engler and Engler, 2006 and Schroeter et al, 2006), wine (Caimi et al, 2003 and Corder et al, 2006) and grapes (Folts, 2002 and Delmas et al, 2005). The effect of tea is confirmed both after acute (2 h after tea consumption) (Duffy et al, 2001; Hodgson et al, 2005; Lorenz et al, 2007, Hirata et al, 2004 and Nagaya et al, 2006) and chronic tea consumption for weeks (Duffy et al, 2001; Hodgson et al, 2002 and Kim, 2006) tea consumption. In one of the studies, however, the acute effect of tea consumption on vascular health is shown to be abolished when tea is consumed with 10% milk (Lorenz et al, 2007).

## 4. EFFECT OF MILK ON FLAVONOID BIOAVAILABILITY AND FUNCTIONAL BENEFITS OF TEA

Because of potential interaction of tea polyphenols with milk proteins (Bartolome et al, 2000; Prigent et al, 2003; Luck et al, 1994 and Jobstl et al, 2006), addition of milk to tea is expected to lower bioavailability of tea polyphenols. The question on the effect of milk on antioxidant effect of black tea, however, remains open. In *in vitro* studies, milk is shown to decrease antioxidant capacity of tea (Siebert et al, 1996; Arts et al, 2002; Gardner et al, 1998 and Sharma et al, 2008). In a latest study, Sharma et al, (2007), however, found that when antioxidant activity is measured in the assay of β-carotene-linolenic acid model system, milk (2 gms of black tea leaves boiled in 40% v/v of milk) showed no adverse effect on the antioxidant effect of black tea. Weisburger et al, (1997) observed that, in rats, milk in lower amounts (1.85% v/v whole milk with 4.5 % fat) enhances the protective efficacy of black tea against carcinogen induced colon and breast cancer.

In humans, varied results are reported. To our knowledge, nine studies have explored the effect of adding milk to tea in humans – three studies found no effect on plasma antioxidant potential increase (Leenen et al, 2000; van het Hof et al, 1999; and Reddy et al, 2005), one showed elimination of antioxidant potential increase (Serafini et al, 1996), one showed elimination of flow mediated vasodilatation (FMD), (Lorenz et at, 2006) and four showed a decrease of either bioavailability of key flavonoids or antioxidant potential (Van het Hof et al, 1998, 1999; Hollman et al, 2001 and Langley-Evans, 2000b) (for summary see table).

Van het Hof et al, (1998) studied the bioavailability of catechins after consumption of green and black tea. For both teas, catechins reached to their maximum amounts in blood plasma within 2.3 h. The elimination rates were faster for green tea ($t_{1/2}$ = 4.8) in comparison to black tea ($t_{1/2}$ = 6.9). Addition of milk (17 %) did not influence these parameters. Van het Hof et al, (1999) studied catechin bioavailability under repeated tea consumption interspersed at every two hour. The catechin levels reached a steady sate at 8th hour after consumption of fifth cup of tea. The peak catechin levels obtained for green and black tea were 1 mM and 0.3 mM, respectively. Although partitioned in low-density lipoprotein (LDL), catechins did not raise resistance against LDL oxidation. Both, bioavailability and resistance to oxidation were not affected when tea was taken with 10% milk. Study of Hollman et al, (2001) addressed bioavailability of flavonol glycosides under similar conditions of repeated tea consumption - eight cups of black tea were consumed in a day at every two hours which provided 100 mM quercetin glycosides and 6-7 mM kaempferol glycosides. A 10% addition of milk did not influence uptake of quercetin or kaempferol – under both conditions the plasma concentrations reached were 50 nM quercetin and 30-45 nM kaempferol (Hollman et al, 2001). However, in the study of Serafini et al, (1996), inclusion of 25% milk abolished the increase in TRAP monitored after 80 min of consumption of green or black tea (Serafini et al, 1996). This anomaly is, however, explained by the results of a later study by Reddy et al, (2005) who showed that addition of milk delays increase in TRAP by 2 h. Thus in case of tea with added milk the increase is observed at 3rd h compared to that observed after 1st h for tea without milk. This time point was missed in Serafini's study as the study was concluded within 80 min. In the study of Reddy et al, (2005), tea was consumed with 20% milk and antioxidant potential measured as FRAP, lipid peroxidation (TBARS) and catechin bioavailability were measured after 60, 120 and 180 min. Though milk lowered the maximum levels of catechins in blood by 37%, it did not change maximum antioxidant potential except delaying it by 2 h. In the study of Langley-Evans (2000b), volunteers consumed 6 cups of black tea with (10% milk) or without milk starting from 9 am to 2 pm. Blood was sampled for antioxidant activity (FRAP) at three time intervals (9 am, 12 am and 3 pm). In case of tea without milk, the FRAP increased by 65% in initial 3 h and 76% after 6 h. In case of teas with added milk, FRAP remained unaltered for initial 3 h. Subsequently, FRAP increased by 50% but increase was statistically insignificant. This study was conducted with 9 volunteers. In a bigger study (Leenen et al, 2000) with 21 volunteers where twice the amount of milk (20%) was added to tea, FRAP values remained unaltered. In this study, a, single dose of (2 gm tea solids – equivalent to around three cups) black or green tea increased the plasma catechin levels and antioxidant activity measured as FRAP, to its maximum levels in 60 min. While changes in amounts of catechin for teas with milk were not measured, the antioxidant potential was similar in case of tea consumed with and without milk. In a recent study, Kyle et al, (2007) reported similar results showing the delivery of polyphenols and antioxidant potential increase after consumption of tea were not influenced by addition of upto 25% milk. Thus, taken together, a majority of data in humans shows that milk up to 25% either does not affect catechin bioavailability and associated antioxidant potential increase or decreases these parameters only marginally.

In a recently published article, Lorenz et al, (2006) showed that addition of 10 % skimmed milk to tea abolishes its acute vasodilatory (flow mediated vasodilatation, FMD) effects. It would be interesting to monitor vasodilatory effect of milk in tea at longer time points – milk has been shown to delay and not abolish catechin bioavailability (Reddy et al, 2005).

In most of the studies, green tea delivers over three fold more catechins than black tea because it contains higher amounts of catechins. This fact is often quoted as the underlying reason for supposedly low efficacy of black tea. We summarize here some of the observations which suggest that increase in the amounts of catechins in blood plasma alone does not define the functional efficacy of tea. In case of black tea the decrease in catechins is compensated by increase in flavonoids like theaflavins and thearubigins (Lennen et al, 2000). Catechins available from green tea in study of Leenen et al, (2000) were five fold more than black tea but increase in antioxidant potential increase was restricted to only 1.5 fold implicating additional active species in black tea. The increase observed in antioxidant potential lagged by 30 min to that of peak serum catechin levels suggesting catechin action alone may not be responsible. It is also observed that tea consumption dependent improvement in endothelial function did not correlate with catechin increase in serum (Widlansky et al, 2005). Black tea contains a range of complex polymeric polyphenols and studies indicate that they induce eNOS more potently than their monomers (Karim et al, 2000). Considering black tea is a rich source of flavonoids like quercetin, kaempferol, theaflavins and thearubigins, which are independently shown to confer functional benefits, an effect through any of those or by any one of their synergistic combinations is a potential possibility (Beecher, 2003; Widlansky et al, 2005 and Lee et al, 1995).

## 5. FLAVONOID BIOAVAILABILITY AND MODE OF ACTION – SOME POSSIBILITIES:

The arguments based on the *in vitro* functional benefits of tea ingredients are difficult to extend directly in humans because of their low

| | Study | Methods/ Measurements | Type of tea | Dose/ Preparation protocol | Amount/ type of milk |
|---|---|---|---|---|---|
| 1 | van het Hof et al, 1998 | n=12, Cross Over, Wash out 1 week, Bioavailability of catechins | Green tea, Black tea | Bolus, 3 g lyophilized tea solids (~ 4 cups tea) | 17%, Skimmed |
| 2 | Van Het Hof et al, 1999 | n=18, Cross Over, Water as control, Bioavailability of catechins, Resistance to *ex vivo* LDL oxidizability | Green tea, Black tea | One cup every 2 h, Eight cups per day for 3 days, 0.5 g tea solids/ 150 ml | 10%, 1.5 % fat, 3.5% protein |
| 3 | Hollman et al, 2001 | n=18, Cross Over, Washout 10 days, Bioavailability of flavonols | Green tea, Black tea | 1 cup containing 150 ml tea every two hours between 8.0 and 22.0 every day for a total of 8 cups per day, 0.5 g of tea extract (research blend, Thomas Lipton Company, USA) | 10% skimmed milk |
| 4 | Leenen et al, 2000 | n=21, Cross Over, Washout 2 days, FRAP | Green tea, Black tea | Bolus, 2 gm tea solids in 300 ml water | 20% full fat milk |
| 5 | Reddy et al, 2005 | n=9, Cross Over, 7 days, FRAP | Green tea, Black tea | 7 gm of black tea leaf powder was boiled in 350 ml of tap water for 3 min, Bolus - Equivalent to ~ 3 cups consumed in a span of 3 minutes. | 20% milk containing 4% total solids, 3.5% fat and 1.75% protein |
| 6 | Serafini et al, 1996 | n=15, Parallel, Cross Over for milk effect, TRAP | Green tea, Black tea | Bolus 300 ml, 6 (~3 cups of tea) - Twinings Earl Grey and Birko Chinese green tea, KI | 25% whole milk |
| 7 | Langley-Evans, 2000 | n=9, Crossover, FRAP | Black tea | 3.25 g tea in 200 ml water (infusion 650 mg tea solids/ L). One serving every hour starting at 9 am to 2 pm | 10%, semi-skimmed cow's milk |
| 8 | Lorenz et al, 2006 | n=16, Crossover, | Black tea | 5 gms of tea leaves were brewed in 500 ml of water for 3 min | 10%, skimmed milk |
| 9 | Kyle et al, 2007 | n=9, Crossover, Washout 1 week, FRAP | Black tea | 3 gms of tea leaves infused for 7 min in 300 ml of boiled water | 25%, skimmed milk |

| Findings | Outcome |
|---|---|
| 1. Average maximum concentration of total catechins in blood plasma after green and black tea consumption, 0.55 and 0.17 umol/L, respectively. 2. Black tea with and without milk delivers similar (0.17 and 0.18 umol/L, respectively) amounts of catechins 3. Time to reach maximum concentration of catechins after tea consumption with and without milk, 2.2 and 2 h respectively | Addition of milk to tea does not effect kinetics and amount of bioavailability of catechins |
| 1. Repeated tea consumption rapidly increases plasma total catechin levels 2. Maximum steady state concentrations reached after 5 cups of tea (t=8 h) for green and black tea were 1uM and 0.3 uM 3. Although present in LDL, catechins did not raise resistance capacity against LDL oxidation | Addition of milk does not affect plasma increase in antioxidant potential |
| 1. Area under the plasma concentration-time curves for quercetin and kaempferol for black tea as well as for green tea were similar 2. The peak levels reached for quercetin was 59 nM and kaempferol 30-45 nM 3. Addiion of milk does not affect above parameters | Milk in tea does not influence bioavailability of flavonols |
| 1. Area under the curve of plasma FRAP activity (increase above baseline in min.umol/L), after green tea without and with milk, 3490±582 and 3221± 590, respectively. 2. With black tea corresponding values were 2328±473 and 1782±350 respectively. 3. Average maximum increase in plasma catechin concentrations after 90 min of tea consumption was 1.8 uM for green tea and 0.34 uM for black tea | Milk in black tea does not effect kinetics and amount of bioavailability of catechins |
| 1. Plasma total maximum catechin levels reached to their maximum (0.67 uM) after 120 min of tea consumption 2. After consuming tea with milk the maximum concentration reached were 0.41 umole/L and attained within 90 min. 3. The FRAP value reached its maximum in 1 h but took 3 h in presence of milk. The values at 3 h was similar in teas with and without milk (~58 umol/L). 4. Lipid peroxidation measured after 3 h in terms of TBARS was similar in presence and absence of milk. | 1. Addition of milk to tea does not influence its antioxidant potential *in vivo* 2. Tea with and without milk prevents oxidative damage *in vivo* |
| 1. Both green and black teas are effective against *in vitro* lipid peroxidation - green tea shows six time more activity. 2. Addition of milk to tea did not influence *in vitro* lipid peroxidation protection activity of tea 3. Both teas increased plasma TRAP equally. Addition of milk totally abolished the *in vivo* activity. | Addition of milk to tea abolishes antioxidant benefit of black tea |
| 1. Black tea increased FRAP by 65% between 9.0 am and 12 pm. At 15.0 pm, the increase was 76% higher than at 9.0 am (statistically significant). 2. Tea with milk abolished increase in FRAP observed at 12 pm and the increase between 12 pm to 15 pm was restricted to 50% (statistically insignificant) 3. The FRAP values were highly variable for milk added teas - in 4 out of 9 subjects the increase in milk added tea was more than that in without milk. | The increased antioxidant potential mediated by repeated consumption of black tea is atleast partially abolished when tea was consumed with milk |
| 1. Black tea increased FMD significantly (4.2%) after 2 h of consumption. Milk completely abolished these effects 2. Black tea significantly (>4.5 fold) enhanced endothelial Nitric oxide (eNOS) synthase activity in endothelial cells 3. This increase was abolished in presence of milk and milk proteins ($\alpha$, $\beta$ and $\kappa$) -casein wheras bovine serum albumin (BSA), $\alpha$-lactoglobulin and $\beta$-lactoglobulin were ineffective | The vascular health benefits of black tea are abolished in presence of 10% milk. |
| 1. Black tea consumption significantly increased plasma total phenolic compounds, total catechins, flavonols - quercetin and kaempferol and antioxidant potential 2. Increase of antioxidant potential (FRAP value) of blood plasma correlate to that in total polyphenol and catechins 3. The delivery of polyphenols and antioxidant potential increase after consumption of tea were not influenced by addition of milk | Addition of milk to tea does not alter delivery of polyophenols to blood plasma and consequent increase in antioxidant potential |

bioavailability. There is a significant metabolic transformation during absorption in the small intestine and colon. The prominent conjugations in the small intestine involve glucuronide conjugation and o-methylation. The microflora of the colon further transforms flavonoids to smaller phenolic acids and valerolactones (Spencer, 2003). Recent studies indicate that flavonoids derive their efficacy through functional activities independent of their antioxidant effects – this changes the perspectives in which effect of addition of milk to the tea should be looked at. While there is almost universal evidence that flavonoids are potent antioxidant *in vitro*, there relevance as *in vivo* antioxidants appears to be minor (Frei and Higdon, 2003). This is because even under the conditions of high flavonoid intake their amounts in blood plasma reach 100-1000 fold lower than other antioxidants like ascorbic acid (vitamin C), glutathione and urates. Flavonoids, in their native form, are poorly absorbed in body. They are heavily transformed by gut micro flora and since there are indications that flavonoids exert prebiotic effect (Hara, 1997), this process appears to be self-promoting. The functional properties of these metabolic products (methylated catechins, ring-fission products of catechins – valerolactones and their biotransformed derivatives formed in colon, simple phenolic acids – 4-hydroxybenzoic acid, 3, 4-dihydroxybenzoic acid, 3-methoxy-4-hydroxyhippuric acid and vanillic acid), in addition to the native forms seem to determine the physiological benefits of tea (Pietta et al, 1998a; Meng et al, 2002 and Mulder et al, 2005). Though the amounts of unaltered flavonoids required to affect a significant change in antioxidant potential, determined in reconstituted serum *ex vivo*, are much higher (7.5 mmol/ L) in comparison to those achieved after tea consumption (~1.2 mmol/L) (Henning et al, 2004), there was still an increase in antioxidant potential of blood plasma after tea consumption. This is attributed to increase in antioxidant potential of some altered flavonoids or their metabolites (Henning et al, 2004). Rice-Evans et al, (1996), however, showed that phenolic acid produced from flavonoids have lower antioxidant capacity than the parent compound. Mulder et al, (2005) argue that a lower antioxidant capacity of microbial degradation products of tea flavonoids is compensated at two levels. First, these metabolites are better bioavailable and second, because of the prebiotic effects of flavonids, their amounts may get self promoted. The evidence is also emerging that flavonoid play a critical role in cellular signaling (see Chen et al, 2008). Since cellular signaling cascades are common to several cellular processes, they can be central to a myriad beneficial roles reported for flavonoids. The role of black tea flavonoids in defining the sequence and up regulation of key kinases associated with endothelial nitric oxide synthase (eNOS) may as well be contributing to a strong epidemiological indication between tea consumption and heart health (Anter et al, 2004; 2005; Lorenz et al, 2004; Doucas et al, 2006 and Beltz et al, 2006). Tea polyphenols are also shown to induce apoptosis and cell cycle arrest, suppress expression of transcription factors NF-kβ and transcription of C-jun and protein tyrosine kinase activity. These effects of tea may form the molecular basis of its anti-carcinogenic effects (Ahmad and Mukhtar, 1999 and Swapna et al, 2005). The signaling effects of flavonoids can be conferred at several fold lower concentrations than that present in blood plasma (Anter et al, 2004; 2005). The effects may not require build up of a threshold plasma antioxidant potential. Flavonoid dependent rise in antioxidant potential after tea consumption has been the point of debate because of insufficient build up of flavonoids to achieve the physiological effects and a possible contribution from other antioxidants.

## 6. SOURCE OF VARIATION IN FUNCTIONAL BENEFITS OF TEA:

The functional benefits obtained because of consumption of tea are shown to have variations.

Both the active molecules and the testing procedures used to measure efficacy may account for these variations. Majority of the health benefits of tea are derived from flavonoids. The amount and composition of flavonoids in tea plants are governed by the genetic, agronomic and climatic conditions. Depending on the type of tea manufactured (green, oolong and black) there is a further change based on the corresponding processing. These factors may contribute to variations in the functional benefits of various types of teas across different geographies and genetic make up. However, because flavonoids also determine the sensory (mainly taste and colour) properties of tea, to a extent their amounts inadvertently get regulated at the level of quality control, which involves blending of different types of teas for a particular flavor profile. The variations are further compounded by a wide variability in the mode of consumption of tea (brewing vs. boiling, addition of milk vs. no milk and amount of added milk). Taken together, these variabilities may explain the ambiguity or contradictory outcomes reported in many studies. To overcome this, it is recommended that future studies take into consideration standardized tea extracts, which are either ingested as capsules, or their preparation involves controlled conditions to ensure a uniform intake of efficacious molecules. To overcome any possible interference from the dietary and life style factors, a host of measures including controlled and low polyphenol diets, abstinence from other relevant positive (e.g nutraceuticals) and negative (exposure to smoking and excessive intake of alcohol) factors needs to be assured. Even if these factors are considered and taken care of, human physiology is also known to define the response. There are inter-individual differences in flavonoid metabolism. One of the prominent transformation steps of flavonoids involves O-methylation which is mediated by catechol-O-methyltransferase (COMT) (Lu et al, 2003). There is as much as a 3-fold difference in the activity of this enzyme amongst individuals (Dawling et al, 2001) arising out of a low and a high activity gene form. The green tea was effective against breast cancer risk only in women who express at least one low activity form. This suggests that O-methylated transformed forms of catechins are ineffective and women who had lesser activity for transformation benefit the most (Wu et al, 2003). The difference in the activity of untransformed and O-methylated metabolites can change the human response (Lee et al, 2002b).

## 7. CONCLUSIONS:

There is a strong epidemiological link between tea consumption and cardiovascular health. Similar, though weaker relationship exists between tea and prevention of cancer. The data supporting these relationships exists more for green tea. However, there are enough studies, conducted in last few decades, which demonstrate equivalence between green and black tea. One of the most efficacious class of compounds in tea are flavonoids. Though black tea manufacturing process alters nature of flavonoids in black tea, it does not change amount and their antioxidant potential. Also, black tea extracts, TFs, epicatechin and EGCG are shown to regulate cellular signaling cascade by activating specific kinases associated with regulation of NO biosynthesis and apoptosis, and this can influence cardiovascular health and risk of cancer. A concern on the public health impact of tea in different populations and cultures may arise because of a possible interaction with lifestyle, nutrition and genetic factors. Addition of milk in particular has been implicated to interfere with bioavailability of tea flavonoids. Most of these assertions though are indirect and based on *in vitro* studies which show that milk protein bind flavonoids and, therefore, lower their bioavailability. The *in vivo* studies targeting this question are limited. Considered together, these studies suggest that milk at lower amounts (< 25%) does not interfere with bioavailability and associated increase in antioxidant status of plasma, significantly. More studies are, however, required to settle this important question pertaining to majority of tea consuming populations especially when the amount of added milk is high (>25%) and involve boiling of tea leaves with milk. To minimize the contributions of variability to the outcome, it is suggested that studies be conducted with standardized tea extracts in a nutritionally controlled design in defined population.

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