Modulation of Oxidative Damage by Green and Black Tea: Role of Smoking and Gender in a Randomized Trial

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Abstract

Background: Oxidative stress has been implicated as an important modulator of human health and can play a role in both disease prevention and disease development. Objectives: The overall goal of this study was to determine the effects of high tea consumption on biological markers of oxidative stress that mediate lung cancer risk, including, 8-hydroxydeoxy-guanosine (8-OHdG) and F2-isoprostanes (8-iso-PGF2α). Design: We completed a 6-month randomized, controlled, double-blinded trial in a group of former and current smokers who were randomized to receive green or black tea preparations or a matching placebo.

Results: A total of 146 participants (80 females and 66 males) completed the study. At the end of the 6-month intervention, female smokers in the green tea group showed a 35% decrease (p=0.04) in DNA damage while female former smokers in the black tea group showed a 26% decrease (p=0.015) in lipid damage. No significant changes in markers of oxidative stress were observed in men.

Conclusion: This data confirm our previous findings related to the beneficial effect of green tea on oxidative DNA damage among female smokers. The significant beneficial effect of black tea on oxidative lipid damage as well as the gender difference merit further studies.

Keywords: Tea; Gender; Oxidative stress; Clinical trial; Smokers

Abbreviations: 8OHdG: 8-hydroxy-2'-deoxyguanosine; 8-iso-PGF2α: 8-isoprostaglandin F2α; GPx: Glutathione Peroxidase; SOD: Superoxide Dismutase; ROS: Reactive Oxygen Species.

Introduction

Cigarette smoking is the main risk factor for lung cancers in the United States (USA) despite the recent decline in smoking rates in USA, the age-adjusted mortality of lung cancer has not significantly changed especially among former smokers [1].

Moreover, there are now sufficient data to include female sex as an independent risk factor for lung cancer. In addition to cigarette smoking, epidemiologic evidence suggests increased susceptibility to lung cancer in women [2-5]. Cigarette smoke generates high levels of reactive oxygen species (ROS) [6]. Increased ROS production is involved in a diversity of biological phenomena such as inflammation, carcinogenesis, aging, and atherosclerosis as well as oxidative damage to DNA, lipids and proteins.

The oxidized nucleoside 8-hydroxydeoxyguanosine (8OHdG) is an oxidative adduct form of deoxyguanosine [7]. Urinary 8-OHdG level has been validated as a biomarker of the extent of oxidative DNA modification [8-10] and a large number of studies showed that a high level of urinary 8-OHdG may be a risk factor for chronic diseases such cancer, cardiovascular diseases and diabetes [7,11,12].

A wide range of biomarkers of lipid peroxidation is available for use in human samples, however, the prostaglandin-like F2-isoprostanes such as 8-isoprostaglandin F2α (8-iso- PGF2α) are formed *in vivo* through free radical-induced peroxidation of arachidonic acid and are considered by many to be the best indicators of lipid peroxidation as they are not confounded by diet and their levels are increased in many human diseases [13-18].

Tea is one of the most ancient and, next to water, the most widely consumed liquid in the world. In experimental studies, tea preparations exhibited significant inhibitory activity against tumorigenesis [19,20]. Several epidemiological studies suggest that tea drinking may reduce the risk of cancers and cardiovascular diseases [21-23]. However, the differential effects of regular green and black tea intake on urinary markers of oxidative damage by gender and smoking status have not been fully examined in clinical trials. We, therefore, conducted a randomized, controlled, doubled blinded trial to test the efficacy of regular green and black tea drinking in reducing oxidative DNA and lipid damage as measured by urinary 8OHdG and 8-iso-PGF2α, respectively, among current and former smokers.

Subjects and Methods Study population

We recruited current and former smokers between 40 and 80 years of age with 25 or more pack-years of smoking history between September 2003 and December 2007 in Tucson, Arizona. Subjects were screened to exclude pregnant women, regular tea drinkers, persons with a forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC) ratio >85%, history of schizophrenia or cancer, current drug or alcohol abusers, individuals with an abnormal liver function blood test or those

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currently being treated with antidepressants. The study was approved by the Institutional Review Board of the University of Arizona, and all of the subjects provided informed consent before enrollment.

Study Protocol

The study was a 3-arm double-blinded randomized placebo-controlled tea intervention trial. Each subject was randomly assigned to drink 4 cups (12 oz cup) of green tea, black tea, or placebo tea daily for 6 months.

Tea products

All the tea used in the intervention was obtained from the same supplier (Unilever Best foods, NJ, USA). Due to the variability in polyphenol content from lot to lot, a single lot was used to produce the green tea and black tea used in this study. In order to mask the color and taste of the study beverages, a citrus flavor was added to all teas (green, black and control) and all teas were packaged in white tea bags with no labels. Tea analysis was performed in our laboratory and the tea catechin composition of the products used in the study is described in Table 1.

Study procedures

Once informed consent has been obtained, participants underwent eligibility evaluation. Each participant completed an Arizona Smoking Assessment Questionnaire, Arizona Food Frequency Questionnaire (AFFQ), Arizona Tea Questionnaire, and Health Assessment Questionnaire and was evaluated for health and respiratory history. A blood sample was collected for CBC with differentials and comprehensive metabolic panel. Spirometry was performed to assess FEV1 and FVC using Nellcor Puritan Bennett Renaissance PB100 spirometer. Those who met all eligibility criteria underwent a 4-week placebo run-in period. All participants received standardized study cups (12 oz cup). Participants were instructed to prepare their tea by brewing 2 tea bags per cup and to drink 4 cups of tea daily (8 placebo tea bags/48 oz). Participants returned unused bags to the study staff during their scheduled monthly visit to the study clinic. Participants who consumed at least 80% of the placebo tea bags underwent the baseline evaluation and were randomized to one of the intervention groups. Randomization occurred using a random permuted block design (block size=6). Randomization lists stratified by gender and smoking status were prepared by the data manager prior to beginning the study and transferred to file-cards in sealed envelopes. The data manager had no contact with study subjects. All study products were assigned a bar-coded specific inventory number that was incorporated into a three-part label. The inventory number allowed the specific products to be tracked from assignment to dispensing to participants.

The inventory numbers were linked to specific tea products by a code. None of the staff interacting with subjects knew the link between inventory number and code.

Study participants were asked to maintain the beverage consumption pattern (4 cups/ day) for 6 months, returning to the clinic at monthly intervals. They were telephoned during the week before each follow-up visit to confirm the date and time of the next appointment and to identify any problems or side effects associated with study participation. Blood and urine samples were collected at baseline, mid study (month 3) and end of the study (month 6). Induced sputum was collected at baseline and end of the study. Fasting morning blood samples were collected and serum, plasma, buffy coat, and erythrocyte isolated and stored at -80°C prior to the sample analysis. First morning urine was collected on three consecutive mornings including the morning of the visit. The three urine samples were combined in equal parts and aliquots stored at - 80°C prior to the sample analysis.

Adherence

Several techniques were used to maximize participant adherence and retention. Primary adherence to the study intervention was evaluated through self- reporting via monthly intake calendars (tea diaries). For each day, participants recorded the time and number of cups consumed. The monthly tea diaries generated continuous data that allowed identification of problems with the adherence pattern. In addition, participants were instructed to return the unused tea bags to the clinic. A monthly short smoking questionnaire allowed us to identify changes in smoking habits during the period of the intervention.

Data Collection

Demographic, diet, and life-style questionnaires

An in-person screening visit was conducted to ascertain eligibility and to obtain baseline data using a standardized, self- administered, lifestyle questionnaire regarding present and past medical history, job history, years of education, marital status, current use of vitamins and medications. Females were asked about the use of oral contraceptives and estrogen replacement therapy. The questionnaire also sought detailed information on lifestyle habits such as smoking, physical activity, and alcohol consumption. The smoking questionnaire included the following variables: number of cigarettes smoked per day (CPD), total years of smoking, age at onset of smoking, and pack-year (number of packs smoked per day times years of smoking).

As part of the study requirements, all subjects at baseline completed the AFFQ that consists of a semi-quantitative 159-item questionnaire as has been previously validated. Respondents reported how often they usually consume each particular food over the prior 12-month period (as a number of times per day, week, or month) and whether the usual portion size was small, medium, or large. Age–sex-specific portion size assumptions are used in the subsequent nutrient calculations. Vitamin supplement use information is also collected in the AFFQ, and nutrient estimates, both including and excluding vitamin supplements, are estimated. AFFQ output includes the estimated specific nutrients per day including dietary carotenoids, retinol, vitamin A, and vitamin E.

Analysis of Urinary 8-Hydroxy-2'-deoxyguanosine (8OHdG)

Urinary 8OHdG was analyzed using a published HPLC-tandem mass spectrometry method with minor modifications (6). Linear calibration curves have been established from 0.3 to 30 ng/mL. Urinary 8OHdG levels were normalized by urinary creatinine concentrations.

Catechins	Average Catechin Mass (mg)		
	Green Tea	Black Tea	Control Tea
	3 min	3 min	3 min
EGC	42.67	39.31	0
EC	10.82	1.24	0.66
EGCG	72.33	18.26	1.32
GCG	2.71	3.9	1.78
ECG	8.98	3.96	1.74
CG*	0	0	0
Total per1 beverage	137.51	66.68	5.51
Total for 1 Tea Bag	68.76	33.34	2.75

Table 1: Catechin analysis of the consumed study beverages.

Analysis of Urinary 8-isoprostaglandin F2 (8-iso-PGF2α)

Urinary 8-iso-PGF2α was analyzed using a published HPLC-tandem mass spectrometry method with minor modifications (6). Linear calibration curves have been established from 0.04 to 4 ng/mL. The urinary 8-iso-PGF2α levels were normalized by urinary creatinine concentrations.

Determination of Antioxidant Enzymes in Erythrocytes

Glutathione peroxidase (GPx) activity in diluted erythrocyte lysates was determined using a Cayman Chemical Glutathione Peroxidase Assay kit. Superoxide dismutase (SOD) activity in diluted erythrocyte lysates was determined using a Cayman Chemical Superoxide Dismutase Assay kit. The erythrocyte antioxidant enzyme activity was normalized by hemoglobin concentrations.

Determination of Serum Fat Soluble Antioxidant Vitamins

The serum levels of retinols, carotenoids and tocopherols were determined by HPLC (6). Standard reference material 968c (fat-soluble vitamins in human serum) supplied by the National Institute of Standards and Technology (NIST, Gaithersberg, MD) was used for assigning values to in-house control materials.

Statistical Methods

Primary end points were changes in the level of creatinine-adjusted urinary 8-OHdG and urinary 8-iso-PGF2α from the baseline to 6 months after commencement of intervention. Descriptive analyses were conducted by gender and smoking status of the study population. Associations between baseline characteristics, urinary 8-OHdG, urinary 8-iso-PGF2α, and intervention group were assessed, using a t test or chi square test. Tests for significance of the change (pre-intervention versus post-intervention values) in urinary 8-OHdG and urinary 8-iso-PGF2α were performed by gender and smoking status. Results were expressed as mean ± standard deviation (SD). Multiple linear regression models were used to estimate the main effects of green and black tea intake on creatinine-adjusted urinary 8-OHdG and urinary 8-iso-PGF2α, with or without adjustment for potential confounders. Potential confounders considered were age, BMI, caloric intake, FEV1/FVC (%), pack years, years of smoking, and dietary total carotenoids. Statistical tests were two-sided with a significant level of 0.05. All statistical analyses were conducted using Stata Statistical Software (Stata 12).

Results

The study CONSORT diagram is shown in Figure 1. Of the 319 persons consented, 59 individuals were not eligible, 154 subjects were randomized and 146 current and former smokers completed the 6-month intervention and provided baseline and 6-month samples. The main reason for non-enrollment was loss of interest (n=98), while the reasons for dropout during run in (n=8) and after randomization (n=8) were moving out of Tucson and not having enough time. There were no statistically significant differences by gender, smoking variables, or treatment group between those who completed the study and those who did not. Among those who completed the study, compliance to the tea intervention was >95%.

The mean age of trial participants was 60 years (range, 40–80); 55% were women, and 58% were current smokers. Baseline characteristics of the study population by gender are shown in Table 2. Our data show that although women have relatively lower pack/year of smoking history (p=0.062) compared to men, they have significantly higher levels of urinary 8-OHdG (p=0.035) and urinary 8-iso-PGF2α (p=0.008).
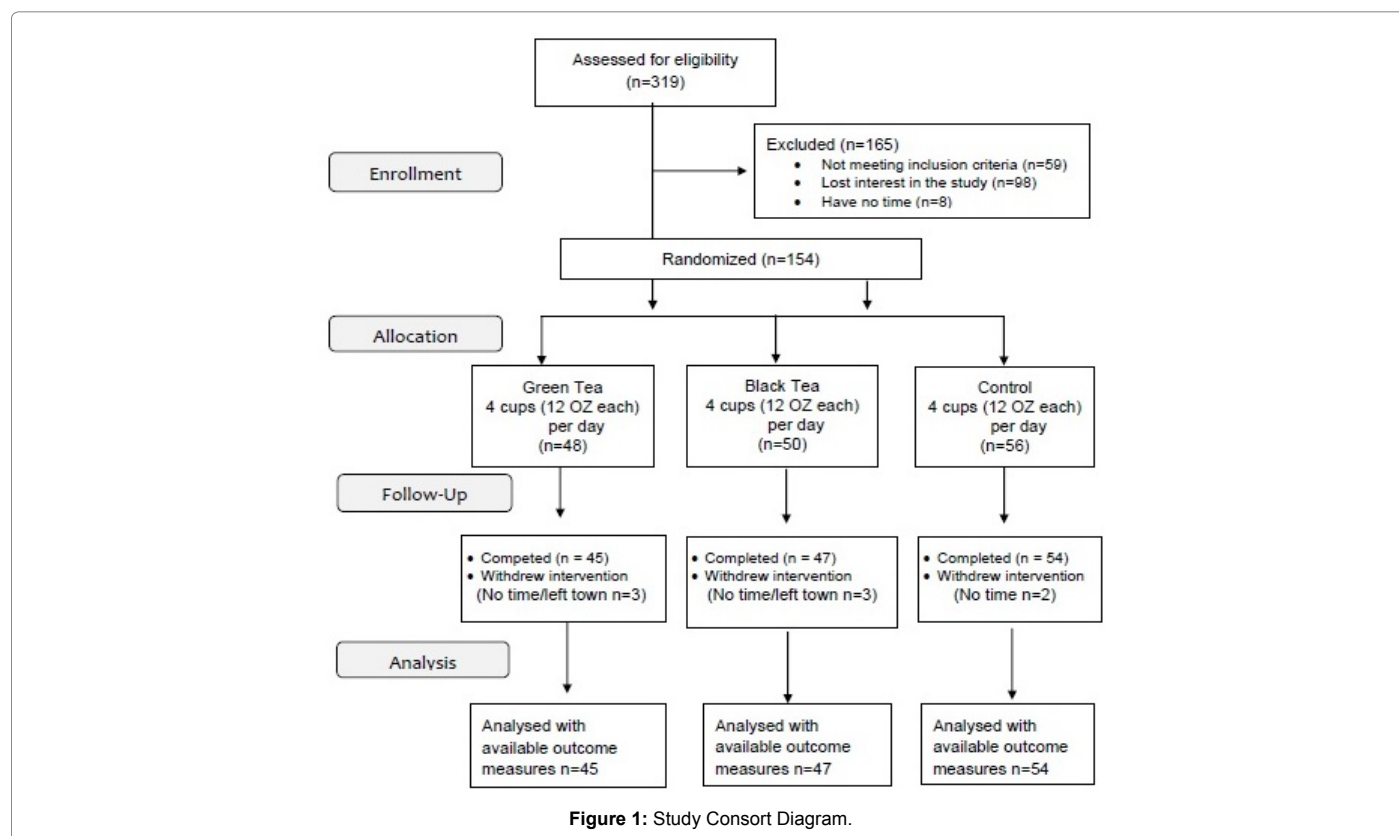

Figure 1: Study Consort Diagram.

Baseline characteristics were similar across the three study groups (Table 3). There were no significant differences in dietary intake or plasma levels of antioxidants between the three groups (data not shown).

The mean changes in urinary excretion of 8-OHdG by tea group compared to placebo are shown in Table 4. Our data showed a significant decrease in urinary 8-OHdG (-35%) among female smokers after 6 months of drinking green tea (p=0.04). Green tea consumption had no effect on urinary 8-OHdG levels in men or female former smokers. Black tea consumption was associated with a non-significant decrease (p=0.08) in urinary 8-OHdG among male former smokers.

The mean changes in urinary excretion of 8-iso-PGF2α by tea group compared to placebo are shown in Table 5. Our data showed a significant decrease in urinary 8-iso- PGF2α (-26%) among female former smokers after 6 months of drinking black tea (p=0.015). Black tea consumption had no effect on urinary 8-iso-PGF2α levels in men or female smokers. Green tea consumption was not associated with levels of urinary 8-iso- PGF2α.

Discussion

Oxidative stress is a key underlying factor in the pathogenesis of many common diseases and is thought to be involved in the initiation, promotion and progression phases of cancer, and its role in each of these phases is complex [24]. It is a major source of chemical-induced cellular injury and is caused by the increased production of ROS and/or decreased efficiency of antioxidant defense mechanisms [25]. Therefore, an oxidant/antioxidant imbalance may play a significant role in lung carcinogenesis among smokers and former smokers.

A number of epidemiologic studies have documented the independent association of gender on lung cancer risk [26-29]. Our data show that although women have relatively lower pack/year of smoking history compared to men, they have significantly higher levels of oxidative damage as measured by urinary 8-OHdG and 8-iso-PGF2α.

Consistent with our findings, several studies [26-28,30,31] reported elevated markers of oxidative DNA damage among female smokers as compared to male smokers, suggesting that women may be more susceptible to oxidative DNA damage induced by cigarette smoking.

	Total (n=146)	Males (n=66)	Females (n=80)	*p* value
Age (year)	60.2 ± 8.8	60.9 ± 8.4	59.6 ± 9.1	0.34
BMI (kg/m2)	28.6 ± 6.8	29.1 ± 6.9	28.3 ± 6.8	0.481
Total carotenoids (µg)	12153 ± 9096	10797 ± 6528	13328 ± 10748	0.101
Multivitamin supplement use (No/Yes)	138/8	64/2	74/6	0.238
Smoking Status				
Former/current smoker	61/85	27/39	34/46	0.846
Pack-years	42.8 ± 19.4	46.1 ± 21.6	40.1 ± 17.0	0.062
Years of smoking	33.7 ± 10.2	35.2 ± 9.1	32.6 ± 11.0	0.116
Systemic oxidative stress				
Urinary 8OHdG (ng/mg creatinine)	4.2 ± 7.1	2.8 ± 2.4	5.4 ± 9.6	0.035
Urinary 8-iso-PGF2α (ng/mg creatinine)	423.3 ± 297.3	344 ± 250	477± 304	0.008
Erythrocyte GPx (nmol/min/g Hb)	24097.1± 8371	24,558 ± 9,488	23,591 ± 7,350	0.5
Erythrocyte SOD (U/g Hb)	5332.5 ± 1894	5,464 ± 1,985	5,182 ± 1,821	0.348

Table 2: Overall characteristics of the study population by gender (means ± SD).

	Green Tea (n=45)	Black Tea (n= 47)	Control (n=54)	*p* value
Female (%)	53%	55%	56%	0.97
Age (year)	59.7 ± 8.5	61.2 ± 8.5	59.8 ± 9.3	0.63
BMI (kg/m2)	29.4 ± 6.5	27.1 ± 5.6	29.2 ± 7.8	0.2
Caloric intake (Kcal/day)	1794 ± 877	1808 ± 720	2007 ± 990	0.41
Total carotenoids (µg)	11354 ± 6674	11486 ± 7851	13394 ± 11552	0.47
Smoking Status				
Smokers (%)	60%	62%	54%	0.69
Pack-years	41.3 ± 16.6	46.7 ± 21.7	40.7 ± 19.2	0.25
Years of smoking	32.1 ± 9.2	36.6 ± 10.7	32.5 ± 10.3	0.06
Systemic oxidative stress				
Urinary 8OHdG (ng/mg creatinine)	3.3± 2.4	4.4 ± 5.3	4.5 ± 10.2	0.66
Urinary 8-iso-PGF2α (ng/mg creatinine)	451.9 ± 391.7	459.0 ± 317.3	369.0± 255.2	0.26
Erythrocyte GPx (nmol/min/g Hb)	22982 ± 8121	23506 ± 8403	25541 ± 8496	0.27
Erythrocyte SOD (U/g Hb)	5589 ± 1965	5196 ± 1803	5238 ± 1926	0.55

Table 3: Overall characteristics of the study population by intervention group (means ± SD).

Urinary 8 OHdG				
	Green Tea Mean change (95% CI)	*p*	**Black Tea Mean change (95% CI)**	*p*
Males				
Total	0.50 (-0.32; 1.42)	0.21	-0.33 (-1.10; 0.60)	0.54
Smokers	0.75 (-0.41; 1.98)	0.19	0.05 (-1.06; 1.23)	0.88
X-smokers	0.18 (-1.10; 1.40)	0.79	-1.32 (-2.60; 0.17)	0.08
Females				
Total	-0.83 (-2.70; 1.40)	0.53	1.6 (-0.09; 3.90)	0.06
Smokers	-1.82 (-4.1; -0.06)	0.04	1.46 (-0.76; 3.22)	0.22
X-smokers	0.72 (-2.7; 5.8)	0.46	1.79 (-1.30; 6.70)	0.18

Table 4: Mean change (95% CI) in urinary excretion of 8-OHdG (ng/mg creatinine) by tea group compared with placebo.

Urinary 8 8-iso-PGF2α				
	Green Tea Mean change (95% CI)	*p*	**Black Tea Mean change (95% CI)**	*p*
Males				
Total	113.2 (-13.8; 257.1)	0.08	90.5 (-39.5; 237.4)	0.16
Smokers	132.3 (-90.7; 256.2)	0.34	102.3 (-126.6; 232.0)	0.55
X-smokers	78.7 (-73.3; 407.1)	0.16	71.6 (-80.4; 400.1)	0.18
Females				
Total	-90.7 (-285.3; 140.5)	0.50	31.9 (-154.9; 255.4)	0.63
Smokers	-137.3 (-448.6; 265.9)	0.61	143.2 (-149.8; 528.0)	0.27
X-smokers	-20.9 (-176.9; 116.5)	0.68	-176.6 (-332.6; -39.2)	0.15

Table 5: Mean change (95% CI) in urinary excretion of 8-iso-PGF2α (ng/mg creatinine) by tea group compared with placebo.

The possible beneficial effects of tea in the prevention of cancer as well as other diseases have been studied extensively. Tea polyphenols appear to inhibit the induction of a variety of cancers, including lung cancer [19,20]. Among current smokers or recent quitters, who had smoked at least 20 pack-years, tea consumption was significantly associated with reduced lung cancer risk [32]. Specifically, a recent meta-analysis of observational studies reported a trend toward reduced risk of lung cancer recurrence associated with higher intake of green tea [21]. Oxidative stress has been implicated as a driving force behind smoking-related diseases, including lung cancer [33,34] and, therefore, biomarkers of oxidative stress could be used as early indicators of a response to smoke exposure. Hatsukami et al. [35] highlighted the biomarkers 8-OHdG and the F2-isoprostanes as being of importance in assessing the extent of smoking-related oxidative stress in the human body.

Our data showed a significant decrease in urinary 8-OHdG (-35%) after 6 months of drinking green tea among female smokers only. Green tea consumption had no effect on men or female former smokers. Several studies have reported that supplementation with green tea or green tea preparations can significantly reduce biomarkers of oxidative DNA damage among smokers [36,37]. However, the beneficial effects of regular green tea consumption seem to be more likely in participants exposed to oxidative challenge [38]. A recent meta-analysis of case-control and cohort studies conducted from 1966 to January 2014 showed that both green and black tea consumption were associated with reduced risk for lung cancer in women. However, no significant association was found in men [39]. Moreover, green tea consumption was not associated with urinary 8-OHdG concentrations in healthy women [40].

Our study used urinary 8-iso-PGF2α as a biomarker for oxidative lipid damage. 8-Iso- PGF2α is biologically active and may play a role in pulmonary pathophysiology [41] and increased lung cancer risk [24,42]. Consistent with our data, previous studies have also shown that female smokers have higher urinary 8-iso-PGF2α [43,44]. Our data showed a significant decrease in urinary 8-iso-PGF2α (- 26%)

among female former smokers after 6 months of drinking black tea. Consistent with our findings, Kubik et al. [45] studied the impact of diet and physical exercise on lung cancer risk in female nonsmokers *vs.* smokers. Their data showed that the intake of black tea was associated with a significant protective effect among female nonsmokers only. Wolfram et al. [46] showed that regular daily black tea consumption for 1 month significantly decreased plasma 8-iso-PGF2α in healthy volunteers. The effect was somewhat more pronounced for females as compared with males. In a recent prospective data analysis of the Singapore Chinese Health Study, the authors reported an inverse dose-response association between higher amounts of black tea intake and all-cause, respiratory- related, and CVD mortality among never-smokers. However, there was no association between black tea and the outcomes among ever-smokers [47].

Conclusion

Our study as well other published data showed that the cellular effects of tobacco smoke may be more pronounced in women than in men. Women may be more susceptible to tobacco smoke and potentially more vulnerable to lung cancer development [48] and therefore are more likely to benefit from a tea intervention. The beneficial effects of regular green tea consumption seem to be more likely in participants exposed to oxidative challenge while the beneficial effects of regular black tea consumption seem to be more likely in former smokers and/or non-smokers.

This data confirm our previous findings related to the beneficial effect of green tea on oxidative DNA damage among female smokers. The significant beneficial effect of black tea on oxidative lipid damage as well as the gender difference merit further studies.

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Clinical Trial Registry number and website

Study ID is NCT02719860 / The ClinicalTrials.gov.

Authors' Contributions

Conception and design: I. A. Hakim, R. B. Harris, L. L. Garland, H.-H. Sherry Chow Development of methodology: I. A. Hakim, R. B. Harris, H.-H. Sherry Chow Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): I. A. Hakim, C. Cordova, D. M. Mikhael, H.-H. Sherry Chow.

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): I. A. Hakim, R. B. Harris, C. Cordova, D. M. Mikhael, H.-H. Sherry Chow Writing, review, and/or revision of the manuscript: I. A. Hakim, R. B. Harris, L. L. Garland, H.-H. Sherry Chow.

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Cordova

Study supervision: I. A. Hakim, L. L. Garland, H.-H. Sherry Chow.

References

1. Thun MJ, Henley SJ, Burns D, Jemal A, Shanks TG, et al. (2006) Lung cancer death rates in lifelong nonsmokers. J Natl Cancer Inst 98: 691-699.

2. Stabile LP, Siegfried JM (2003) Sex and gender differences in lung cancer. J Gend Specif Med 6: 37-48.

3. Rivera MP, Stover DE (2004) Gender and lung cancer. Clin Chest Med 25: 391-400.

4. Thomas L, Doyle LA, Edelman MJ (2005) Lung cancer in women: emerging differences in epidemiology, biology, and therapy. Chest 128: 370-381.

5. Patel JD (2005) Lung cancer in women. J Clin Oncol 23: 3212-3218.

6. Hakim IA, Harris RB, Garland L, Cordova CA, Mikhael DM (2012) Gender Difference in Systemic Oxidative Stress and Antioxidant Capacity in Current and Former Heavy Smokers. Cancer Epidemiol Biomarkers Prev 21: 2193-2200.

7. Wu LL, Chiou CC, Chang PY, Wu JT (2004) Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. Clinica Chimica Acta 339: 1-9.

8. Loft S, Poulsen HE (1996) Cancer risk and oxidative DNA damage in man. J Mol Med 74: 297-312.

9. Loft S, Vistisen K, Ewertz M, Tjonneland A, Overvad K, et al. (1992) Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. Carcinogenesis 13: 2241-2247.

10. Loft S, Fischer-Nielsen A, Jeding IB, Vistisen K, Poulsen HE (1993) 8-Hydroxydeoxyguanosine as a urinary biomarker of oxidative DNA damage. J Toxicol Environ Health 40: 391-404.

11. Valavanidis A, Vlachogianni T, Fiotakis C (2009) 8-Hydroxy-2'-deoxyguanosine (8- OHdG): A critical biomarker of oxidative stress and carcinogenesis. Journal of Environmental Science and Health. Part C 27: 120-139.

12. Sajous L, Botta A, Sari-Minodier I (2008) Urinary 8-hydroxy-2'-deoxyguanosine: a biomarker of environmental oxidative stress? Annales de biologie clinique 66: 19-29.

13. Aviram M (2000) Review of human studies on oxidative damage and antioxidant protection related to cardiovascular diseases. Free Radic Res 33: S85-S97.

14. Bachi A, Zuccato E, Baraldi M, Fanelli R, Chiabrando C (1996) Measurement of urinary 8-Epi-prostaglandin F2alpha, a novel index of lipid peroxidation in vivo, by immunoaffinity extraction/gas chromatographymass spectrometry. Basal levels in smokers and nonsmokers. Free Radic Biol Med 20: 619-624.

15. Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, et al. (1995) Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. N Engl J Med 332: 1198-2203.

16. Mezzetti A, Cipollone F, Cuccurullo F (2000) Oxidative stress and cardiovascular complications in diabetes: isoprostanes as new markers on an old paradigm. Cardiovasc Res 47: 475-488.

17. Lim KHC, Lee CYJ, Earnest A, Seet RCS, Halliwell B (2010) Does radiotherapy increase oxidative stress? A study with nasopharyngeal cancer patients revealing anomalies in isoprostanes measurement. Free Radic Res 44: 1064-1071.

18. Lee CYJ, Seet RCS, Huang SH, Long LH, Halliwell B (2009) Different patterns of oxidized lipid products in plasma and urine of dengue fever, stroke and Parkinson's disease patients: cautions in the use of biomarkers of oxidative stress. Antioxid Redox Signaling 11: 407-420.

19. Yang CS, Wang ZY (1993) Tea and cancer. J Natl Cancer Inst 85: 1038-1049.

20. Shankar S, Ganapathy S, Srivastava RK (2007) Green tea polyphenols: biology and therapeutic implications in cancer. Front Biosci 12: 4881-4899.

21. Tang N, Wu Y, Zhou B, Wang B, Yu R (2009) Green tea, black tea consumption and risk of lung cancer: a meta-analysis. Lung Cancer 65: 274-283.

22. Hakim IA, Alsaif, MA, Alduwaihy M, Al-Rubeaan K, Al-Nuaim A, et al. (2003) Tea Consumption and the Prevalence of Coronary Heart Disease in Saudi Adults: A Saudi National Study. Preventive Medicine 36: 64-70.

23. Apranta D, Joseph Vita (2011) Tea and cardiovascular disease. Pharmacol Res 64: 136-145.

24. Lowe FJ, Luettich K, Gregg EO (2013) Lung cancer biomarkers for the assessment of modified risk tobacco products: an oxidative stress perspective (Review). Biomarkers 18: 183-195.

25. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, et al. (2007) Free radicals and antioxidants in normal physiological functions and human disease. International Journal of Biochemistry and Cell Biology 39: 44-84.

26. Mollerup S, Ryberg D, Hewer A, Phillips DH, Haugen A (1999) Sex differences in lung CYP1A1 expression and DNA adduct levels among lung cancer patients. Cancer Res 59: 3317-3320.

27. Ryberg D, Hewer A, Phillips DH, Haugen A (1994) Different susceptibility to smoking- induced DNA damage among male and female lung cancer patients. Cancer Res 54: 5801-5803.

28. Kure EH, Ryberg D, Hewer A, Phillips DH, Skaug V, et al. (1996) P53 mutations in lung tumours: relationship to gender and lung DNA adduct levels. Carcinogenesis 17: 2201-2205.

29. Gasperino J, Rom WN (2004) Gender and lung cancer. Clinical Lung Cancer 5: 353-359.

30. Mooney LA, Perera FP, Van Bennekum AM, Blaner WS, Karkoszka J, et al. (2001) Gender differences in autoantibodies to oxidative DNA base damage in cigarette smokers. Cancer Epidemiol Biomarkers Prev 10: 641-648.

31. Guo H, Huang K, Zhang X, Zhang W, Guan L, et al. (2014) Women are more susceptible than men to oxidative stress and chromosome damage caused by polycyclic aromatic hydrocarbons exposure. Environ Mol Mutagen 55: 472-481.

32. Gnagnarella P, Maisonneuve P, Bellomi M, Rampinelli C, Bertolotti R, et al. (2013) Red meat, Mediterranean diet and lung cancer risk among heavy smokers in the COSMOS screening study. Annals of Oncology 24: 2606-2611.

33. Allavena P, Garlanda C, Borrello MG, Sica A, Mantovani A (2008) Pathways connecting inflammation and cancer. Curr Opin Genet Dev 18: 3-10.

34. Smith CJ, Perfetti TA, King JA (2006) Perspectives on pulmonary inflammation and lung cancer risk in cigarette smokers. Inhal Toxicol 18: 667-677.

35. Hatsukami DK, Benowitz NL, Rennard SI, Oncken C, Hecht SS (2006) Biomarkers to assess the utility of potential reduced exposure tobacco products. Nicotine Tob Res 8: 169-191.

36. Hakim IA, Harris RB, Brown S, Chow HH, Wiseman S, et al. (2003) Effect of increased tea consumption on oxidative DNA damage among smokers: a randomized controlled study. J Nutr 133: 3303S-3309S.

37. Schwartz JL, Baker V, Larios E, Chung FL (2005) Molecular and cellular effects of green tea on oral cells of smokers: a pilot study. Mol Nutr Food Res 49: 43-51.

38. Ellinger S, Muller N, Stehle P, Ulrich-Merzenich G (2011) Consumption of green tea or green tea products: is there an evidence for antioxidant effects from controlled interventional studies?. Phytomedicine 18: 903-915.

39. Wang L, Zhang X, Liu J, Shen L, Li Z (2014) Tea consumption and lung cancer risk: a meta-analysis of case-control and cohort studies. Nutrition 30: 1122-1127.

40. Hori A, Kasai H, Kawai K, Nanri A, Sato M, et al. (2014) Coffee Intake is Associated With Lower Levels of Oxidative DNA Damage and Decreasing Body Iron Storage in Healthy Women. Nutr Cancer 66: 964-969.

41. Janssen LJ (2001) Isoprostanes: an overview and putative roles in pulmonary pathophysiology. Am J Physiol Lung Cell Mol Physiol 280: L1067-L1082.

42. Epplein M, Franke AA, Cooney RV, Morris JS, Wilkens LR, et al. (2009) Association of plasma micronutrient levels and urinary isoprostane with risk of lung cancer: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev 18: 1962-1970.

43. Yan W, Byrd GD, Ogden MW (2007) Quantitation of isoprostane isomers in human urine from smokers and nonsmokers by LC-MS/MS. J Lipid Res 48: 1607-1617.

44. Taylor AW, Bruno RS, Traber MG (2008) Women and smokers have elevated urinary F(2)-isoprostane metabolites: a novel extraction and LC-MS methodology. Lipids 43: 925-936.

45. Kubik A, Zatloukal P, Tomasek L, Pauk N, Havel L, et al. (2007) Interactions between smoking and other exposures associated with lung cancer risk in women: diet and physical activity. Neoplasma 54: 83-88.

46. Wolfram RM, Oguogho A, Efthimiou Y, Budinsky AC, Sinzinger H (2002) Effect of black tea on (iso-)prostaglandins and platelet aggregation in healthy volunteers. Prostaglandins Leukotrienes & Essential Fatty Acids 66: 529-533.

47. Odegaard AO, Koh WP, Yuan JM, Pereira MA (2015) Beverage habits and mortality in chinese adults. J Nutr 145: 595-604.

48. Kiyohara C, Ohno Y (2010) Sex Differences in Lung Cancer Susceptibility: A Review Gend Med 7: 381-401.