High tea consumption diminishes salivary 17β-estradiol concentration in Polish women

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We hypothesized that among reproductive-age women consuming large quantities of tea, the production of estradiol would be suppressed. It has been shown that catechins and theaflavines, the major constituents of tea, inhibit aromatase, an enzyme which catalyses the conversion of androgens to oestrogens. Our study included Polish women living in urban (*n* 61) and rural (*n* 48) areas. Women collected daily saliva samples for one complete menstrual cycle and filled out dietary questionnaires. Saliva samples were analysed by RIA for concentration of 17β-estradiol (E2). Women with high (above the median) average daily consumption of black tea had reduced levels of salivary E2 in comparison with women who drank less black tea (below the median). This effect was observed within the whole study group, as well as separately within urban (*P*=0·0006) and rural (*P*=0·013) groups. High intake of the sum of subclasses of tea catechins and epigallocatechin gallate, assessed using the United States Department of Agriculture database (http://www.nal.usda.gov), was also associated with lower concentrations of E2 within all women (*P*=0·01 and *P*=0·0001, respectively) and within the urban group (*P*=0·0001 and *P*=0·004, respectively). Similar relationships were observed between the sum of subclasses of theaflavines and thearubigines and E2 levels for the whole group (*P*=0·002) and for urban women (*P*=0·02). Women with high consumption of tea had lower levels of E2 concentration throughout the entire menstrual cycle. These results may have implications for reducing hormone-related cancer risk by a relatively easy dietary intervention.

Catechins: Epigallocatechin 3-gallate: 17β-Estradiol: Tea: Theaflavines

Changes in lifestyles and dietary habits in the post-industrial era can dramatically influence metabolism and the level of sex-steroid hormones (Ellison & Lager, 1986; Jasienska *et al.* 2000, 2005, 2006; Wenger, 2004). It has been suggested that prolonged exposure to oestrogens during the life span due to early menarche, late menopause, lower parity and environmental oestrogenic toxins may be responsible for the increased rate of breast cancer (Verkasalo *et al.* 2001; Liu *et al.* 2004; Zografos *et al.* 2004). A diet rich in flavonoids and isoflavones, in particular, can decrease circulating oestrogen levels as well as menstrual cycle length (Lu *et al.* 1996; Kumar *et al.* 2002). Furthermore, epidemiological data mainly from Asian countries indicate that high consumption of soya products containing an abundant amount of these compounds is inversely correlated with colon carcinoma and hormone-related cancers (Gittes, 1991; Lee *et al.* 1991; Seow *et al.* 1998; Wu *et al.* 2000). Soyabean products are consumed much less frequently in European countries than in Asia. The intake of foods containing isoflavonoids is almost negligible in the typical Polish diet, although flavan-3ols, the major constituents in tea, are consumed in large quantity. These flavonoids not only seem to decrease estradiol levels but also exhibit chemopreventive effects against breast cancer (Park & Surh, 2004). The inhibitory impact of tea constituents like catechins and theaflavines on aromatase, an enzyme involved in the final step of oestrogen production, is likely responsible for this preventive effect through modulation of circulating oestrogen levels (Chen *et al.* 2002; Satoh *et al.* 2002; Way *et al.* 2004). Since black tea, and to a lesser extent green tea, is consumed frequently in Poland, the Polish population provides an ideal context for investigating the potential relationship between tea consumption and estradiol levels. We studied healthy young women during one full menstrual cycle to investigate the relationship between salivary 17β-estradiol levels and the daily intake of: (1) black tea, (2) black and green tea together or 'total' tea, (3) the two major

groups of constituents in tea, namely theaflavines and catechins, and (4) epigallocatechin 3-gallate (EGCG), the most biologically active compound.

Materials and methods

Study design and participants

Participants of the study were Polish women living in urban (*n* 61) and rural (*n* 48) areas. During the period from June 2001 to June 2003, urban women were recruited for the study through newspaper and television advertisements, while rural women were recruited through their parish. Women were selected for participation if they met the following criteria: age between 24 and 36 years, regular menstrual cycles, no fertility problems, no gynecological or chronic disorders (i.e. diabetes, hypo/hyperthyroidism), not taking any hormonal medication or using hormonal contraception, and not being pregnant or lactating for 6 months before recruitment. Subjects' body weight, height and body fat (by bioimpedance) were measured by a trained anthropologist. A general questionnaire (self-administered or through an interview) was used to collect information on education, reproductive history and past use of hormonal medication, tobacco and alcohol. Physical activity was assessed based on a physical activity log completed daily by each subject during the menstrual cycle (Jasienska *et al.* 2006).

Ethical consideration

The study protocol was reviewed and approved by the Jagiellonian University Medical College Ethics Committee.

Hormone analysis

Women collected morning saliva samples daily for one entire menstrual cycle. Saliva samples for 20 d of each cycle (reverse cycle days −5 to −24) were analysed for 17β-estradiol (E2) concentration using an ^{125}I-based RIA kit (#39 100; Diagnostic Systems Laboratories, Webster, TX, USA) along with published modifications to the manufacturer's protocol (Jasienska *et al.* 2004). The sensitivity of the estradiol assay was 4 pmol/l. The average intra-assay variability was 9 % and the inter-assay variability ranged from 23 % for lower values (15 pmol/l) to 13 % for higher values (50 pmol/l). Prior to statistical analyses, all cycles were aligned following published methods (Lipson & Ellison, 1996) based on identification of the E2 drop at mid-cycle (day 0), which provides a reasonable estimate of the day of ovulation. The E2 values for 18 consecutive days from each cycle aligned on day 0 were used in data analyses.

Dietary assessment

Subjects were trained to estimate the volume of tea by using a photographic album identifying the size of a cup (Album of Food; Food and Nutrition Institute, Warsaw, Poland), to fill out the food diary questionnaire, and to record as much information about the type of tea and its preparation as possible. The majority of participants in our study used one teabag per cup of hot water with short brewing time. Seven food diary questionnaires were filled out by each participant during the menstrual cycle and the data were entered into a computer. A specially written computer program was used to estimate the daily intake of a particular class of polyphenols for each participant based on merging the average consumption of a particular type of tea with the flavonoid content of the tea, expressed as mg/100 ml tea infusion standardized to 1 % infusion (as consumed) by the United States Department of Agriculture (2003) database. These standardized values were calculated using the weight of the teabag (or loose tea leaves) used to make the infusion. Adjustment for brewing time was not necessary as most tea flavonoids are extracted into the infusion after only short brewing times and do not increase substantially with extended duration of brewing (Hertog *et al.* 1993; Arts *et al.* 2000).

The amount of the two main groups of constituents in tea was calculated. The first group contained the sum of six subclasses of catechins (abbreviated to CAT): (−)-epicatechin, (−)-epicatechin 3-gallate, (−)-epigallocatechin, (−)-EGCG, (+)-catechin and (+)-gallocatechin. The second group contained the sum of four subclasses of theaflavines (abbreviated to ThF): theaflavine 3-gallate, theaflavine 3′-gallate, theaflavine 3,3′-digallate and thearubigins.

Statistical analysis

Women were divided into two groups based on their consumption level of black tea, their 'total' tea consumption (black and green), CAT and ThF intake, and EGCG intake. Women with intake levels above the median value for each of these tea products were classified as the 'high consumption' group and women with intake levels below the median value were classified as the 'low consumption' group. We used two-sided *t* tests, separately for urban and rural women, to test for statistical differences in age, tobacco smoking, alcohol consumption, anthropometric and body composition variables, and mean physical activity between groups with low and high consumption of 'total' tea.

The effects of consumption of 'total' tea on E2 levels were then tested by factorial, three-way ANOVA analysis. The division into low and high consumption groups was used as one factor, the place of residence (urban or rural) as the second factor, and day of the menstrual cycle (with eighteen levels) as the third factor. The ANOVA was followed by contrast analyses; an α level of 0·025 (with Bonferroni correction) was used to indicate statistical significance. Similarly structured ANOVA analyses were also performed for the intake of black tea, CAT and ThF, and EGCG. To control for the potentially confounding effect of nutritional status, a four-way ANOVA analysis was performed with BMI as an additional factor in the model for 'total' tea consumption.

Results

Characteristics of studied women

Urban women characterized by low and high consumption of 'total' tea did not show statistically significant differences in age, age at menarche, first birth, number of children, smoking, drinking, physical activity and anthropometric variables (Table 1). Among rural women, however, those with low

Table 1. Characteristics of the study groups*

	Urban					Rural				
	Low consumption (n 26)		High consumption (n 35)			Low consumption (n 27)		High consumption (n 21)		
	Mean	SD	Mean	SD	P value†	Mean	SD	Mean	SD	P value†
Age (years)	29·5	3·05	30·0	3·39	0·55	29·7	3·59	28·9	3·82	0·51
Self-reported age of menarche (years)	12·8	1·17	13·2	1·6	0·32	13·5	1·19	13·6	1·2	0·7
Age at first childbirth (years)	23·3	2·5	25·3	3·4	0·13	21·4	2·06	22·4	2·95	0·3
Number of children	0·7	0·84	0·5	0·85	0·35	1·8	1·51	1·4	1·63	0·4
Body height (cm)	162·6	5·84	164·9	6·11	0·15	161·6	5·11	157·2	5·49	0·01
Body weight (kg)	61·0	10·7	58·1	8·15	0·34	66·1	12·36	56·9	8·34	0·01
BMI (kg/m^2)	23·0	3·32	21·6	2·75	0·07	25·3	4·42	23·0	3·05	0·08
Body fat (%)	27·0	7·7	24·9	6·32	0·25	30·2	6·53	26·0	7·63	0·06
Length of menstrual cycle (d)	29·5	4·73	28·5	3·45	0·37	27·7	3·0	27·6	2·39	0·93
Physical activity (MET-h/d‡)	7·2	1·4	7·6	2·0	0·33	11·6	3·41	10·7	2·87	0·34
Smoking (cigarettes/d)	1·6	4·55	0·7	2·54	0·35	2·7	5·15	1·3	3·0	0·33
Drinking (alcohol units/week)	2·5	2·17	1·8	1·6	0·21	1·1	0·79	1·3	1·66	0·59

* Division into the low and high consumption groups is based on the median consumption of 'total' tea (sum of black and green).

† P values are derived from two-sided t tests, separately for urban and rural women.

‡ Metabolic equivalent (MET) is the ratio of the work metabolic rate to the resting metabolic rate. One MET is defined as 1 kcal/kg per h (Ainsworth et al. 2000).

'total' tea consumption were significantly taller and heavier than women with high 'total' tea consumption, although differences in other characteristics were not statistically significant (Table 1).

We estimated the amount and type of tea consumed as well as the amount of CAT and ThF, the two main subclasses of tea polyphenols, from 798 questionnaires filled out by the women participating in our study. All the study participants drank black tea; the mean daily intake was 473 ml. In contrast, green tea was consumed by only twenty-one participants. The mean daily intake of both black and green tea combined was 499 ml.

Estradiol and black tea consumption

Within both the urban and rural women, the group with high (above the median) consumption of black tea had a lower mean estradiol level than the group with low (below the median) black tea consumption (F 17·2, P=0·0001). For urban women, mean E2 levels were 21·7 and 18·8 pmol/l for the low and high consumption groups, respectively

(P=0·0006). For rural women, mean E2 levels were 18·4 and 16·1 pmol/l for the low and high consumption groups, respectively (P=0·01; Table 2).

To test whether ThF, the main constituent of black tea, was responsible for this effect, we analysed whether groups of women who differed in ThF intake had different E2 levels. We found that women with high consumption of ThF had lower levels of E2 than women with low consumption of ThF (F 9·24, P=0·002). Contrast analyses showed that the difference in E2 levels between the low and high consumption groups was statistically significant within urban women (P=0·02) but not rural women (P=0·04).

Estradiol and 'total' tea consumption

'Total' tea consumption also showed a statistically significant relationship with E2 levels (F 16·96, P=0·0001). Within urban women, those with high 'total' tea consumption had reduced E2 concentration compared to women with low 'total' tea consumption (F 58·1, P=0·0001; Fig. 1). Such a

Table 2. Mean levels of salivary 17β-estradiol (in pmol/l) for groups of urban and rural women characterized by low and high (below and above median) consumption of 'total' tea (sum of black and green), black tea and constituents*

	Urban					Rural				
	Low consumption		High consumption			Low consumption		High consumption		
	Mean	SD	Mean	SD	P value†	Mean	SD	Mean	SD	P value†
'Total' tea	23·9	16·75	18·0	11·74	0·0001	17·0	10·48	18·0	12·17	0·34
Black tea	21·7	16·29	18·8	12·6	0·0006	18·4	11·26	16·1	10·88	0·013
CAT	21·2	16·75	18·9	11·95	0·004	17·2	11·17	16·7	10·89	0·45
ThF	20·9	16·73	19·1	12·19	0·02	17·86	11·35	15·9	9·7	0·04
EGCG	23·2	18·07	19·0	11·76	0·0001	17·39	11·05	16·2	10·78	0·17

CAT, sum of six subclasses of catechins: (−)-epicatechin, (−)-epicatechin 3-gallate, (−)-epigallocatechin, (−)-EGCG, (+)-catechin and (+)-gallocatechin; EGCG, epigallocatechin 3-gallate; ThF, sum of four subclasses of theaflavines: theaflavine 3-gallate, theaflavine 3′-gallate, theaflavine 3,3′-digallate and thearubigins.

* For details of procedures, see p. 990.

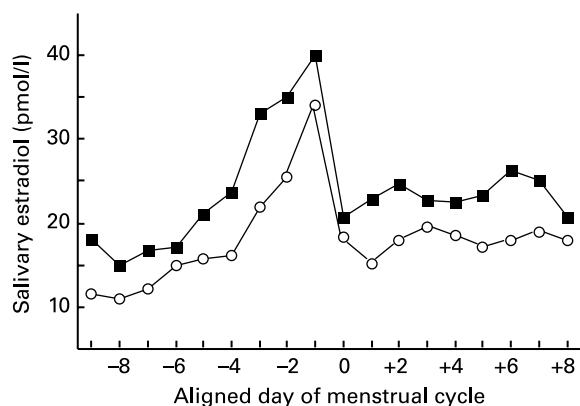† P values are derived from the contrast analyses following the ANOVA analyses.

Fig. 1. Estradiol profiles in groups of urban women with low (■) and high (○) consumption of 'total' (black and green) tea. Division into groups is based on median consumption. For details of procedures, see p. 990. Values are means (CI omitted for clarity).

relationship was not evident for the rural women (F 0·91, $P=0.34$). Urban women drank more 'total' tea than rural women, mainly because more urban women drank green tea compared to the rural women (Table 3). Since higher consumption of 'total' tea significantly enhanced the amount of CAT consumed, we analysed the influence of CAT intake above and below the median value on estradiol profiles. For urban women, daily CAT intake delivered from both kinds of tea showed a statistically significant association with E2 levels (F 8·37, $P=0.004$; Fig. 2). For rural women, daily CAT intake above and below the median did not show a statistically significant association with E2 levels (F 0·57, $P=0.45$).

Estradiol and epigallocatechin 3-gallate consumption

Since EGCG is the dominant and most bioactive compound in tea in terms of its inhibitory effect on aromatase activity, we tested whether groups of women who differed in EGCG intake showed different E2 levels. For urban women, E2 levels were significantly lower (F 25·46, $P=0.0001$) for the group with high EGCG intake (above the median value) compared to the group with low EGCG intake (below the median

value; Fig. 3). No difference was detected for the rural women (F 1·91, $P=0.17$).

BMI and physical activity as additional factors

The relationship between 'total' tea consumption and E2 remained statistically significant after controlling for the potentially confounding effect of BMI (F 31·55, $P=0.0001$). The relationship between BMI and E2 levels was not statistically significant (F 3·1, $P=0.05$). Within the urban women, the low 'total' tea consumption group had higher levels of E2 after controlling for BMI than the high 'total' tea consumption group (F 85·06, $P=0.0001$). Within the rural women, differences in E2 between the low and high 'total' tea consumption groups remained statistically non-significant after including BMI in the model (F 0·009, $P=0.92$).

In order to control for the potentially confounding effect of physical activity on estradiol levels we performed three-way factorial ANOVA analyses, separately for the urban and rural women. Division into low and high 'total' tea consumption groups was used as one factor, day of the menstrual cycle as the second factor and division into tertiles of physical activity (following a published method by Jasienska *et al.* 2006) as the third factor. In urban women, the relationship between 'total' tea consumption and E2 level remained statistically significant (F 49·8, $P=0.0001$). We also found a statistically significant inverse relationship between physical activity and E2 levels (F 4·98, $P=0.007$) confirming our previously published results (Jasienska *et al.* 2006). A similar relationship was observed in rural women as well: while physical activity had an effect on E2 levels (F 12·57, $P=0.0001$), there was also a statistically significant inverse relationship between 'total' tea consumption and E2 levels (F 5·5, $P=0.02$).

Discussion

Endocrine regulation is very susceptible to lifestyle factors, in particular diet and environmental modification. For example, high levels of physical activity (Ellison & Lager, 1986; Jasienska & Ellison, 1998, 2004; Jasienska & Thune, 2001; Willer, 2003; Wenger, 2004; Jasienska *et al.* 2006) or certain dietary habits, such as enhanced consumption of soya products (Cassidy *et al.* 1994; Nagata *et al.* 1998; Wu *et al.* 2000;

Table 3. Consumption of 'total' tea (sum of black and green), black tea and constituents in groups of women characterized by low and high (below and above median) consumption*

	All women				Urban				Rural			
	Low consumption		High consumption		Low consumption		High consumption		Low consumption		High consumption	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
'Total' tea (ml)	252·5	127·03	778·8	348·5	243·6	136·7	846·8	382·92	261·0	118·98	621·5	177·0
Black tea (ml)	233·3	111·09	712·1	328·43	236·9	11·39	781·2	360·15	229·9	113·0	570·04	190·23
CAT (mg)	48·5	24·56	174·5	101·56	48·2	24·85	204·4	114·67	48·9	24·76	126·9	48·54
ThF (mg)	197·1	76·24	561·7	235·72	191·8	79·94	638·4	258·0	202·8	73·35	428·4	97·54
EGCG (mg)	20·9	14·12	120·1	89·99	20·0	14·12	131·2	87·56	21·7	13·88	98·0	93·18

CAT, sum of six subclasses of catechins: (−)-epicatechin, (−)-epicatechin 3-gallate, (−)-epigallocatechin, (−)-EGCG, (+)-catechin and (+)-gallocatechin; EGCG, epigallocatechin 3-gallate; ThF, sum of four subclasses of theaflavines: theaflavine 3-gallate, theaflavine 3′-gallate, theaflavine 3,3′-digallate and thearubigins.
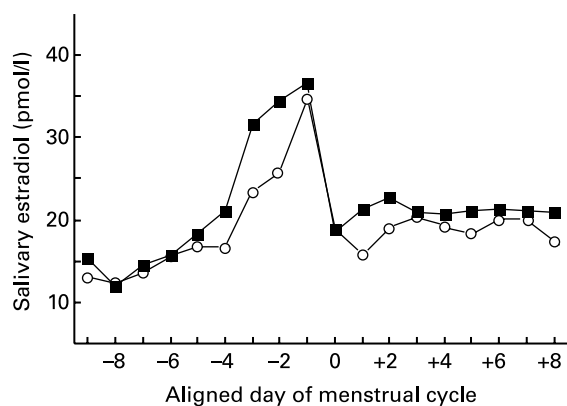* For details of procedures, see p. 990.

Fig. 2. Estradiol profiles in groups of urban women with low (■) and high (○) consumption of black tea. Division into groups is based on median consumption. For details of procedures, see p. 990. Values are means (CI omitted for clarity).

Kumar *et al.* 2002) can lower levels of sex steroid hormones in women. Based on repeated observations of higher oestrogen levels in cancer tissue (for a review see Mueck *et al.* 2002), a reduction in estradiol levels may modify the risk of cancer. Since the incidence of cancer is related to the DNA-damaging activity of carcinogens, the association between E2 concentration in circulation and the extent of DNA damage seems to implicate estradiol as a reliable indicator of the risk of cancer (Key *et al.* 2002; Kapiszewska *et al.* 2005).

Localized oestrogen levels are determined by the enzyme CYP19, the aromatase which catalyses the conversion of androgens, including androstenedione and testosterone, to oestrogens, including estrone and estradiol (Conley & Hinshelwood, 2001). Thus, the selective inhibition of oestrogen biosynthesis can be achieved through the inhibition of aromatase (Chen *et al.* 2002). Recently, a few aromatase inhibitors have been developed for use in cancer therapy (Joensuu *et al.* 2005). The naturally occurring flavonoids were shown to inhibit aromatase and other steroidogenic enzymes as well as modulate oestrogen metabolism through their ability to bind with oestrogen receptors (Rosenberg Zand *et al.* 2002). Therefore, flavonoids, the most abundant compounds in
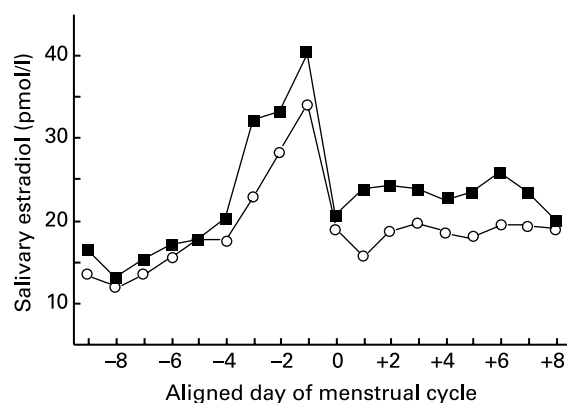
plant foods such as fruits and vegetables, and certain beverages, including tea, wine, beer and juices, seem to be good candidates for shifting overall hormonal levels. Tea is a potent source of flavonoids in the diet, with levels approaching 200 mg/cup for a typical brew of black tea (Lakenbrink *et al.* 2000).

The most extensively studied flavonoids, in terms of their influence on steroid metabolism, have been genistein and active isoflavone, widely present in soya products, and epigallocatechin gallate, derived from tea. Since black tea, as opposed to soya products, remains commonly consumed throughout Poland, the Polish population seems to be an appropriate group for studying the association between tea intake, along with its constituents, and E2 levels in women. In addition, the differences in lifestyle between rural and urban areas in Poland led us to analyse this relationship for women living in both types of region in Poland. Different lifestyles may affect the developmental conditions for women growing up in these regions, as suggested by the relatively later age at menarche and lower final body height for rural women compared to urban women (Laska-Mierzejewska, 1995; Bielicki & Szklarska, 1999; Laska-Mierzejewska & Olszewska, 2004). Lifestyle differences may influence the reproductive physiology, including hormonal metabolism, of adult women as well.

A popular habit in Poland is to drink tea at the workplace a few times a day, especially among urban, white-collar workers. Recently, the consumption of green tea has been promoted by the media and become more common, especially among well-educated people. This trend is similar to that described for the Netherlands (Hertog *et al.* 1993). Nonetheless, our ongoing research may be the last opportunity to study the effects of drinking tea, since transformation of the political system and the development of a free market in Poland are associated with replacing the habit of drinking tea with drinking carbonated soft drinks.

In our study, the average amount of black tea consumed was significantly higher than the average amount of green tea, as expected. When we analysed the association between salivary E2 levels during one menstrual cycle and tea intake, we found a statistically significant lowering effect on E2 for the sum of black and green tea intake for urban but not rural women. This difference may be related to the higher consumption of green tea by urban women. Even a small amount of green tea increased the CAT intake in our study subjects, as green tea contains threefold more catechins than black tea. It has been shown that the plasma level of CAT after the consumption of green tea is higher than after consumption of black tea (Lee *et al.* 2000) and also that the bioavailability of the subclasses of CAT differs (Rietveld & Wiseman, 2003).

We tested the association between estradiol levels and the intake of ThF and CAT by comparing groups of women whose average intake was above and below the median values. Both CAT and ThF possess strong aromatase inhibitory activity (Park & Surh, 2004; Way *et al.* 2004). We found a statistically significant lowering effect of ThF on estradiol levels for the total group of women in our study and, again, for the urban but not rural group of women.

The most biologically active compound among the CAT subclasses, EGCG, makes up more than 50 % of the catechins

Fig. 3. Estradiol profiles in groups of urban women with low (■) and high (○) consumption of epigallocatechin 3-gallate (EGCG). Division into groups is based on median consumption. For details of procedures, see p. 990. Values are means (CI omitted for clarity).

found in green tea. Its biological effect is also the strongest. When administered orally to rats, EGCG induces the reduction of sex steroid concentrations due to the inhibition of aromatase activity (Satoh *et al.* 2002). In our study, the estradiol profiles of urban women suggest that high EGCG intake from tea is associated with reduced E2 levels in the luteal phase of the menstrual cycle but not in the follicular phase (Fig. 3). This lowering effect during the luteal phase may point to the important chemopreventive activity of EGCG, since breast cells are more proliferative during the luteal phase than the follicular phase (Nagamani *et al.* 1979; Lu *et al.* 2000). In light of the present finding, it would be interesting to evaluate the association between estradiol concentrations and tea intake separately for the follicular and luteal phase in the future.

A study of aromatase isolated from the microsomes of rat ovarian and human placental tissue (Way *et al.* 2004) indicates a dose-dependent relationship between EGCG and E2 in that only the presence of EGCG above a certain level exerts an inhibitory effect. In addition, ThF showed a stronger inhibitory effect on aromatase than EGCG (Way *et al.* 2004). Therefore, the significantly lower mean EGCG intake for rural women compared with urban women ($P = 0.03$) may explain the lack of association between EGCG and estradiol levels in rural women.

Although an increase in levels of E2, especially over the long term, may be associated with an increased risk of breast cancer, we are not aware of data which would allow the cancer risk associated with increased E2 levels to be estimated. However, the differences we observed in the mean levels of E2 for women characterized by low and high tea consumption may allow differences in the probability of conception to be estimated. For example, the E2 levels for urban women were almost 33 % higher for the group with low 'total' tea consumption compared to the group with high consumption. Based on estimates from the logistic regression curve developed from conception research (Lipson & Ellison, 1996), such an increase in E2 levels would be associated with an almost three fold increase in the probability of conception (Jasienska *et al.* 2004).

In conclusion, we demonstrated that drinking tea can decrease E2 concentrations. This lowering effect was observed in our total study group but remained pronounced only for urban women, who tended to drink more tea in general and more green tea in particular, than rural women. The main strength of the present study was the precise assessment of E2 concentration across the menstural cycle based on daily collection of saliva samples combined with frequent dietary assessment. Most other studies use a single blood sample to characterize hormone levels.

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