

## Buckwheat Honey Increases Serum Antioxidant Capacity in Humans

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Honey has been known to exert significant in vitro antioxidant activity, in part due to its phenolic content. However, conclusions that the antioxidants in honey are or are not efficacious in the human body cannot be reached if its antioxidant action is not assessed as part of a human study. In the present study, the acute effect of consumption of 500 mL of water, water with buckwheat honey, black tea, black tea with sugar, or black tea with buckwheat honey on serum oxidative reactions was examined in 25 healthy men. Antioxidant capacity of human serum samples was measured using different methods: the oxygen radical absorbance capacity (ORAC) assay, ex vivo susceptibility of serum lipoprotein to Cu<sup>2+</sup>-induced oxidation, and the thiobarbituric acid reactive substances (TBARS) assay. The results showed that the serum antioxidant capacity determined by ORAC increased significantly ( $p < 0.05$ ) by 7% following consumption of buckwheat honey in water. No significant changes in serum antioxidant capacity could be established after the consumption of any of the other beverages. Ex vivo serum lipoprotein oxidation and TBARS values were not significantly altered after consumption of any of the five beverages. This study provides primary evidence of the in vivo antioxidant activity of buckwheat honey. However, long-term studies and epidemiological data are necessary to investigate whether honey consumption can exert overall antioxidant-related health benefits.

**KEYWORDS:** Honey; tea; antioxidant capacity; humans

### INTRODUCTION

Generation of excess free radicals is implicated in an ever-growing number of disease conditions, including cancer, cardiovascular diseases, and neurodegenerative diseases (1–4). Over the past decades, much research has aimed to counterbalance the adverse effects of free radicals by complementing the endogenous antioxidant defense system with dietary antioxidants. Epidemiological studies suggest that consumption of fruits and vegetables may reduce the risk of certain forms of cancer and cardiovascular disease (5–7), likely because of their antioxidant content. These studies have mainly focused on antioxidant vitamins or provitamins and polyphenols present in fruits and vegetables and beverages such as red wine and tea.

Honey may also constitute a dietary source of natural antioxidants. For example, honey has an in vitro antioxidant capacity similar to those of many fruits and vegetables on a fresh weight basis, as measured by the oxygen radical absorbance capacity (ORAC) assay (8). Honey was also more effective than a sugar analogue in protecting serum lipoproteins from oxidation in vitro (8). In addition, honey protects food products against deteriorative oxidation reactions, such as lipid

oxidation in meat (9, 10) and enzymatic browning of fruits and vegetables (11, 12). However, the antioxidant activity of honey varies depending on the honey floral source and positively correlates with the color of the honey (13). Buckwheat honey, a dark honey, had the highest antioxidant activity of honeys from 14 different floral sources tested. The variable antioxidant activity of honeys from different floral sources is due to the variable content of a wide range of antioxidant components such as phenolics, peptides, organic acids, enzymes, and Maillard reaction products (14). A linear correlation between antioxidant activity and both phenolic content ( $p < 0.0001$ ) and protein content ( $p < 0.05$ ) of honeys from various floral sources has been demonstrated previously (14).

One of the common routes for the consumption of honey is as an additive to tea. Black tea is a rich dietary source of polyphenols and inhibits lipoprotein oxidation in human serum samples in vitro (15, 16). Human intervention studies investigating the in vivo and ex vivo antioxidant effects of tea consumption are, however, not consistent (15, 17–25). Although in vitro antioxidant screening tests provide a useful initial guide to the potential of a food product as a dietary antioxidant, these tests do not incorporate factors such as bioavailability and metabolism of its antioxidant components. Thus, ex vivo antioxidant tests are necessary to better represent the physiological impact of food constituents on the human body. The present human

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intervention study is the first to examine the effect of buckwheat honey consumption on serum antioxidant status and ex vivo lipoprotein oxidation. By adding buckwheat honey to black tea, we also sought to enhance the ex vivo antioxidant activity of tea alone. Ex vivo antioxidant effects of the consumption of tea versus that of tea with sugar or tea with buckwheat honey were compared to determine the antioxidant effect of sugars or honey.

## MATERIALS AND METHODS

**Materials.** All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated. 2,2'-Azobis(2-amidinopropane dihydrochloride) (AAPH) was purchased from Wako Chemicals, Inc. (Richmond, VA) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) from Fisher Scientific (Pittsburgh, PA). Buckwheat (*Fagopyrum esculentum*) honey was purchased from Wixson Honey Co. (Dundee, NY). This honey is vended as "monofloral", meaning that the honey must derive at least 51% of the constituent nectar or 45% of contaminant pollen from a single floral source (26). Thus, the honey collected may contain nectars from more than one source, but the nominate floral type predominates.

**Study Design.** Twenty-five male subjects were recruited [age = 29.2 ± 2.3 and body mass index (kg/m<sup>2</sup>) = 24.3 ± 0.6; mean ± SEM]. Results of a medical history questionnaire indicated all subjects were in good health. All of the subjects fulfilled the following eligibility criteria: (1) no history of cardiovascular, hepatic, gastrointestinal, or renal disease; (2) no diabetes mellitus; (3) light or no alcoholic drinking (fewer than one alcoholic drink per day); (4) no current smoking; and (5) no consumption of dietary antioxidant supplements. They were also asked to report any medication intake during the course of the five-week study. The study protocol was approved by the Institutional Review Board of the University of Illinois, and written informed consent was obtained from each study participant.

Five beverages were tested using a blind Latin-rectangle design: (1) water (control); (2) water with 160 g/L buckwheat honey; (3) black tea; (4) black tea with 160 g/L buckwheat honey; (5) black tea with 160 g/L sugar analogue (45% fructose, 35% glucose, and 20% water). Black tea infusions were prepared by extracting commercially available Lipton tea bags (Englewood Cliffs, NJ) for 5 min in boiled tap water (one tea bag, containing ~2.3 g of tea leaves, per 250 mL of water) as suggested by the manufacturer's instructions. All beverages, except water, were consumed hot. A total of five visits were conducted at least 1 week apart, as close as possible to the same day of the week and at the same time of the day. The subjects fasted overnight (for at least 12 h) before each visit. Blood samples were taken at 0, 90, and 120 min. After their first fasting blood sample was taken, each volunteer was given two plain, low-fat bagels and 500 mL of one of the five test beverages, one at each visit in random order, to consume within 30 min.

Seven milliliters of venous blood was drawn into Vacutainer tubes (Fischer Scientific) for serum collection. Serum was left to clot in the dark at room temperature for 30 min, then centrifuged at 1000g for 10 min at 4 °C, and stored at -80 °C until analysis. Another 7 mL of venous blood was drawn into Vacutainer tubes containing EDTA for plasma collection. Plasma was kept on ice and separated by centrifugation (1500g, 4 °C, 10 min) within 30 min after blood drawing and stored at -80 °C until analysis.

**Assessment of Antioxidant Capacity of Beverages and Serum.** The five tested beverages were assessed for in vitro antioxidant activity by the ORAC assay as described by Cao et al. (27) and as previously done in our laboratory (8). Ex vivo serum antioxidant capacity was measured as ORAC<sub>PCA</sub> as described by Cao and Prior (28). The serum nonprotein fraction was prepared by diluting serum with 0.5 mol/L perchloric acid (PCA) (1:1, v/v). The samples were centrifuged at 10000g for 10 min, and the supernatants (serum nonprotein fractions) were removed and appropriately diluted for the ORAC assay. All serum samples were assessed within 1 week after blood drawing. ORAC values were expressed as Trolox equivalents (TE) (mmol/L). All comparisons were made on within-run assessments to limit between-run variability.

**Table 1.** Total Antioxidant and Phenolic Intake by Consumption of 500 mL of Each of the Five Beverages<sup>a</sup>

beverage	ORAC (mmol of Trolox equiv)	total phenolics (mg of gallic acid equiv)
black tea and honey	5.18 ± 0.43a	321.4 ± 15.4a
black tea and sugar analogue	4.32 ± 0.32b	304.3 ± 12.3a
black tea	4.30 ± 0.21b	312.9 ± 14.1a
water and honey	0.94 ± 0.11c	9.1 ± 1.8b
water	0 ± 0d	0 ± 0c
ANOVA <i>p</i>	< 0.0001	< 0.0001

<sup>a</sup> Data expressed as means ± SEM (*n* ≥ 5). Means within a column sharing the same letter are not significantly different by Tukey (*p* < 0.05).

The within-run coefficient of variance (CV) for the assessment of ORAC value was 6.6%.

**Assessment of ex Vivo Serum Lipoprotein Oxidation.** Copper-induced serum oxidation (0.67% serum diluted in PBS buffer) was carried out as described by Regnström et al. (29) and as previously done in our laboratory (8) using 12 μmol/L Cu<sup>2+</sup>. Oxidation kinetics were determined by measuring the absorbance at 234 nm every 10 min at 37 °C until there was no further increase in the formation of conjugated dienes. The serum oxidation lag time (expressed in min) was used as an indicator of the differences in oxidation curves and was defined as the intercept between the baseline and the tangent of the absorbance curve during the propagation phase. All comparisons were made on within-run assessments to limit between-run variability. The within-run CV for the assessment of lag time was ≤ 8.8%.

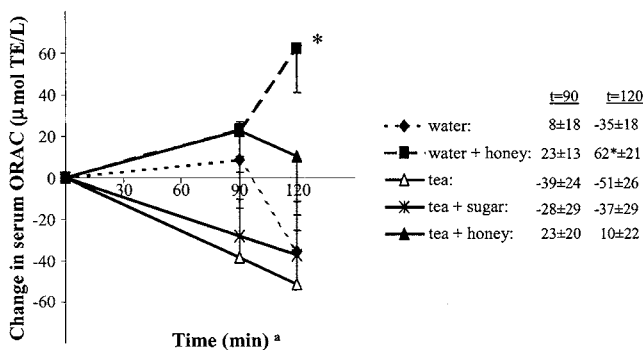
**Assessment of Plasma Thiobarbituric Acid Reactive Substances (TBARS).** For plasma TBARS determinations, 4% (w/v) butylated hydroxytoluene/ethanol was added to aliquots of plasma (for a final ratio of 4:1) before freezing. Plasma TBARS were determined within 2 months of blood sampling using a spectrofluorometric method described by Fraga et al. (30) with some modifications for using a plate reader (31). TBARS were expressed as malondialdehyde (MDA) equivalents (μmol/L).

**Assessment of Total Phenolics of Beverages and Plasma.** Total phenolic content in the beverages was quantified according to a modified version of the Folin-Ciocalteu method as described previously (8). Total phenolics in plasma samples were estimated using a colorimetric assay with Folin-Ciocalteu reagent according to the official AOAC method (32). Total phenolics were expressed as gallic acid equivalents (GAE) (mg/L).

**Statistical Analysis.** All statistical analyses were performed using SAS Software (SAS Institute, Cary, NC, version 8, 1999). Results are expressed as means ± SEM; statistical significance was set at *p* < 0.05. Analysis of variance with post-hoc comparisons according to Tukey was used to examine differences between antioxidant content and total phenolic content of the test beverages. Because of the possible dependence of change in ex vivo oxidation-related variables on initial predrink values, postdrink values were compared after adjustment for predrink or baseline values. Changes between the baseline (*t* = 0) and the 90- and 120-min time points among treatment groups were examined using a repeated-measures 2 × 5 factorial design.

## RESULTS

Total in vitro antioxidant intake (as measured by the ORAC assay) and total phenolic intake by consumption of 500 mL of each of the five beverages are summarized in **Table 1**. The antioxidant capacity of the tea drink was 4.3 mmol of TE/ 500 mL, which is consistent with the previously reported ORAC capacity of black tea, ranging from 5.2 to 16.7 μmol of TE/mL (33). Tea supplemented with buckwheat honey had a significantly higher in vitro antioxidant activity than tea alone or tea with sugar, leading to a 20% increase in in vitro antioxidant activity of tea with honey versus tea without honey. Even though the total in vitro antioxidant activity of tea with honey was



**Figure 1.** Changes in serum antioxidant capacity as measured by ORAC<sub>PCA</sub> at baseline ( $t = 0$ ) and 60 and 90 min after consumption of each of the five beverages. Data are represented as means  $\pm$  SEM. The asterisk indicates significant difference from the control group,  $p = 0.0037$ . Baseline ( $t = 0$ ) serum ORAC<sub>PCA</sub> for the five treatments: water ( $n = 24$ ),  $819 \pm 34$ ; water with honey ( $n = 24$ ),  $888 \pm 38$ ; tea ( $n = 24$ ),  $909 \pm 32$ ; tea with sugar ( $n = 25$ ),  $934 \pm 44$ ; tea with honey ( $n = 25$ ),  $886 \pm 33$ . <sup>a</sup>At  $t = 30$ , all subjects finished the beverage; therefore, 60 min after consumption of the beverage corresponds to  $t = 90$  and 90 min after consumption of the beverage corresponds to  $t = 120$ .

slightly higher than the two other tea drinks, the total phenolic content did not significantly vary among the three tea drinks. Antioxidant and phenolic intake by consumption of buckwheat honey diluted in water was much lower than that of the tea drinks.

In the human intervention study, beverages were consumed over 30 min and blood samples were taken 60 and 90 min after the subjects finished the beverage (90 and 120 min, respectively, after the fasting blood draw). These time points were selected on the basis of preliminary assays in our laboratory in which blood samples were taken from volunteers before and every 30 min after consumption of tea and tea with honey over a time span of 150 min, because maximum rise in plasma antioxidant capacity is most often reached 1–2 h after tea ingestion (34). Changes in serum antioxidant capacity (ORAC<sub>PCA</sub>) of all subjects at baseline ( $t = 0$ ) and 60 and 90 min after consumption of each of the five beverages are depicted in **Figure 1**. The baseline ( $t = 0$ ) serum ORAC<sub>PCA</sub> values for the five treatments were (means  $\pm$  SEM) as follows:  $819 \pm 34$ , water ( $n = 24$ );  $888 \pm 38$ , water with honey ( $n = 24$ );  $909 \pm 32$ , tea ( $n = 24$ );  $934 \pm 44$ , tea with sugar ( $n = 25$ );  $886 \pm 33$ , tea with honey ( $n = 25$ ). All variables were normally distributed, and statistical analyses showed that there was a significant effect of treatment on serum ORAC<sub>PCA</sub>. The change in serum ORAC<sub>PCA</sub> at 90 min after consumption of water with buckwheat honey was significantly higher than the change at 90 min after ingestion of water. ORAC<sub>PCA</sub> concentration increased by  $62.3 \mu\text{mol of TE/L}$  90 min after consumption of buckwheat honey in water, corresponding to a 7% increase in deproteinized serum antioxidant capacity. The increase in serum ORAC<sub>PCA</sub> at 60 min after consumption of tea with buckwheat honey was significantly different from the change in serum ORAC<sub>PCA</sub> at 60 min after consumption of tea alone and tea with sugar. This increase was, however, not statistically different from the change in serum ORAC<sub>PCA</sub> after ingestion of water. The decrease in serum ORAC<sub>PCA</sub> after consumption of tea and tea with sugar was not statistically different from the change in serum ORAC<sub>PCA</sub> after water consumption. Serum lipoprotein oxidation-derived lag times and TBARS values before and after consumption of the beverages are shown in **Table 2**. No significant differences in lag time could be established after consumption of any of the beverages. Similarly, plasma TBARS values were not signifi-

**Table 2.** Ex Vivo Cu<sup>2+</sup>-Induced Serum Lipoprotein Oxidation-Derived Lag Times and Plasma TBARS at Baseline ( $t = 0$ ) and 60 and 90 min after Consumption of Each of the Five Beverages<sup>a</sup>

	lag time (min)	TBARS <sup>b</sup> ( $\mu\text{mol/L}$ )
water ( $n = 24$ )		
$t = 0$ min	$52.8 \pm 4.0$	$1.61 \pm 0.07$
$t = 90$ min	$50.3 \pm 3.6$	$1.52 \pm 0.07$
$t = 120$ min	$55.2 \pm 4.9$	$1.47 \pm 0.06$
water and honey ( $n = 24$ )		
$t = 0$ min	$55.6 \pm 3.5$	$1.56 \pm 0.07$
$t = 90$ min	$53.7 \pm 3.6$	$1.51 \pm 0.06$
$t = 120$ min	$53.2 \pm 4.1$	$1.57 \pm 0.07$
tea ( $n = 24$ )		
$t = 0$ min	$54.9 \pm 2.1$	$1.59 \pm 0.07$
$t = 90$ min	$52.3 \pm 2.3$	$1.51 \pm 0.06$
$t = 120$ min	$55.5 \pm 2.5$	$1.48 \pm 0.06$
tea and sugar ( $n = 25$ )		
$t = 0$ min	$50.8 \pm 2.4$	$1.61 \pm 0.06$
$t = 90$ min	$52.3 \pm 3.2$	$1.57 \pm 0.05$
$t = 120$ min	$51.1 \pm 2.3$	$1.53 \pm 0.08$
tea and honey ( $n = 25$ )		
$t = 0$ min	$54.1 \pm 2.1$	$1.56 \pm 0.05$
$t = 90$ min	$54.1 \pm 3.2$	$1.52 \pm 0.05$
$t = 120$ min	$57.3 \pm 4.5$	$1.54 \pm 0.04$

<sup>a</sup>Data expressed as means  $\pm$  SEM. <sup>b</sup>TBARS expressed as malondialdehyde equivalents.

cantly altered after consumption of any of the beverages. Plasma total phenols, measured in relation to a gallic acid standard curve, ranged from 1.86 to 2.42 mg/mL, but were unaffected by beverage consumption (data not shown).

## DISCUSSION

The results of this study indicate that consumption of buckwheat honey increases the antioxidant capacity of human serum as measured by ORAC<sub>PCA</sub>. This increase of protein-free serum antioxidant capacity could, however, not be supported by a decreased ex vivo lipoprotein susceptibility to oxidation, nor by a decrease in TBARS. The present investigation also illustrates the lack of consistency between the in vitro antioxidant capacity of the phenolic-rich beverages (tea with or without sugar or honey) and their capacity to induce an ex vivo increase in serum antioxidant capacity. In the in vitro antioxidant test, the three tea beverages displayed  $\sim 4.5$ – $5$  times higher antioxidant activity than the buckwheat honey in water. By adding 80 g of buckwheat honey to 500 mL of tea, a 20% increase in in vitro antioxidant activity of the tea was achieved; thus, an additive effect of the antioxidant activity of tea and honey was observed. Despite the low antioxidant content of the buckwheat honey in water, consumption of only this beverage significantly increased serum antioxidant activity by 7%. In a study by Cao et al. (35), serum antioxidant capacity as measured by ORAC<sub>PCA</sub> increased by 9% 120 min after consumption of spinach (294 g), by 14% 60 min after consumption of strawberries (240 g), and by 17% 60 min after red wine consumption (300 mL).

Honey contains a wide range of components, of which the phenolic acids and flavonoids play a significant role in the antioxidant capacity of honey (14). Previously, we characterized the phenolic profile of buckwheat honey (14). The main phenolic compounds identified were the phenolic acids *p*-hydroxybenzoic acid, *p*-coumaric acid, and abscisic acid and the flavonoids pinobanksin, pinocembrin, and chrysin. In the present study, an amount of 4 tablespoons (80 g) of buckwheat honey was consumed per person, providing  $\sim 10$  mg of phenolics. Plasma total phenols, as measured by the Folin–Ciocalteu assay, did not alter after consumption of any of the five test beverages.

However, even though the Folin–Ciocalteu method is still the most commonly used assay for phenols, it is considered to be unreliable when applied to plasma because of nonspecificity, giving phenol values that are unrealistically high (36). Indeed, in our study, plasma total phenol concentrations of  $\sim 2.1$  mg/mL were found. Small changes in phenol concentrations are therefore masked in the assay technique because of interfering compounds, but the *in vivo* antioxidant effect of small increments in plasma polyphenol levels may still be significant. Data on the bioavailability of phenolics are emerging; however, these data are still scarce and contradictory. Large variations in bioavailability have been observed depending on the chemical structure and form of the flavonoid (37). Little is known about the absorption of the above-mentioned phenolic acids and flavonoids that are specific to honey and other bee products. In contrast to many other phenolics in foods or beverages such as tea, honey phenolics are present in the aglyconic rather than glycosidic form due to hydrolysis by the glycosidases present in the bee salivary glands (38, 39). Phenolic aglycons are more readily absorbed through the gut barrier than their corresponding glycosides by passive diffusion (37, 40) and, therefore, the phenolics present in honey may be more readily bioavailable than the tea phenolics. However, a study comparing the pharmacokinetics of quercetin glycoside and its aglycon demonstrated that even though quercetin aglycon was absorbed more quickly, the total areas under the plasma concentration curve over a 32 h period were similar for quercetin aglycon and quercetin glycoside (41). It must also be noted that black tea contains more polymeric polyphenols, such as theaflavins and thearubigins (42) than honey. It is possible that some of these high molecular weight tea phenolics bind more efficiently with proteins present in the serum and are therefore not measured in the ORAC<sub>PCA</sub> assay. Due to the complexity of the black tea polymeric polyphenols, data on the bioavailability of these compounds are lacking. More studies are necessary to investigate whether honey phenolics are more efficiently absorbed than tea phenolics in order to properly evaluate whether the difference in bioavailability may explain the increased serum antioxidant capacity after consumption of honey and not of tea.

Even though black tea has a strong *in vitro* antioxidant activity (15, 16), data in the literature on the *ex vivo* antioxidant effects of tea are equivocal. Several clinical trials have demonstrated that a single dose of black tea improves plasma antioxidant capacity within 1–2 h after tea ingestion (20, 23, 25). Our study, however, failed to demonstrate any significant increase in serum antioxidant capacity or *ex vivo* protection against lipoprotein oxidation after consumption of tea or tea supplemented with sugar or buckwheat honey. Additionally, most studies investigating the effect of tea consumption on lipoprotein oxidation also failed to show a significant *ex vivo* protection of tea consumption on lipoprotein oxidation (15, 17, 18, 24). Similarly, results from epidemiological and clinical studies of the relationship between tea and cardiovascular disease are conflicting. Some epidemiological studies, but not all, support the view that tea or tea flavonoids reduce the risk of cardiovascular disease (reviewed by ref 22, 43–46).

It must be noted that the composition and quantity of tea antioxidants can vary significantly depending on the type of tea and method of preparation (33, 47). In the present study, total phenolic intake from black tea was 0.57 mg/mL, similar to that reported by Lee et al. (48) (0.62 mg/mL) and Lakenbrink et al. (47) (0.53 mg/mL) but lower than that reported in other studies, where the phenol contents of black tea infusions were 3.6 mg/mL (19) and 1.37 mg/mL (23). Many of the tea studies

used either freeze-dried tea powder or research blends that are not commercially available and contained higher concentrations of tea leaves. The lower phenol content of the tea beverages prepared in this study compared to other studies may explain, at least in part, the lack of a change in serum antioxidant capacity by consumption of the tea beverages. However, the underlying reasons for the discrepancy between the effects of buckwheat honey in water compared to buckwheat honey in tea on serum antioxidant capacity remain unknown. Even though both buckwheat honey in water and buckwheat honey in tea show a trend toward an increased serum antioxidant capacity, a significantly increased serum antioxidant capacity was observed only 90 min after consumption of the buckwheat honey in water. Possibly, interactions between the different honey and tea phenolics may occur and negatively influence the bioavailability of the honey phenolics. This hypothesis requires further investigation.

The inconsistency between the effects of the tea beverages *in vitro* and *ex vivo* on oxidative reactions may be attributed to a number of additional factors. First, the present study was designed to determine the acute effects of consumption of only a single dose of tea with or without honey. Concentrations of individual phenolics and their biologically active conjugates may not be high enough after occasional intake to achieve concentrations *in vivo* as great as those obtained with the *in vitro* methods (18, 24). However, longer term trials with repeated consumption of tea and honey may result in accumulation of the antioxidant components, which may in turn result in sufficiently active phenolic concentrations to influence the blood antioxidant status (37). Second, interindividual variations in the serum antioxidant capacity and other oxidation-related variables were substantial; fasting ORAC<sub>PCA</sub> levels of the different subjects, for example, ranged from 500 to 1240  $\mu\text{mol}$  of TE/L. Furthermore, the bioavailability of tea and honey polyphenols may vary considerably due, in part, to differences in genetic polymorphisms among the enzymes involved in polyphenol metabolism (37). Third, the methods used to estimate the antioxidant capacity of plasma or serum are indirect, because they do not allow measurement of *in vivo* oxidative damage. The measurement of specific oxidation products such as F<sub>2</sub>-isoprostanes in plasma and urine may provide better markers of *in vivo* oxidative damage (49). However, in such a short time period, it was unlikely that plasma concentrations of isoprostanes would be significantly altered. Results from a recent study by Hodgson et al. (21) showed that regular ingestion of tea (1 L of tea per day for 7 days) did not change urinary F<sub>2</sub>-isoprostane excretion. Methods measuring TBARS and diene conjugation involving spectrophotometry are limited by the fact that they are nonspecific and subject to interferences (50). ORAC<sub>PCA</sub> is, however, the preferred method for evaluating changes in water-soluble antioxidants in human serum samples based on a detailed comparison of different methods for determining total antioxidant capacity of human serum (28). In the present study, only data obtained from the ORAC<sub>PCA</sub> assay showed any significant change in serum antioxidant capacity, whereas both the TBARS assay and the spectrophotometric method were not sensitive enough to detect significant changes in *ex vivo* susceptibility of plasma/serum against oxidation. In addition, the methods used in the present study are conducted in an aqueous system. Several studies suggest that water-soluble antioxidants are effective in inhibiting serum oxidative processes, in part by recycling lipophilic antioxidants (51, 52). Therefore, the antioxidant effect of tea and honey consumption might be underestimated by measuring only the aqueous serum compartment oxidizability.

In conclusion, our results suggest that ingestion of buckwheat honey may modulate serum antioxidant status. More information on the bioavailability of honey phenolic compounds and longer term studies are, however, necessary to provide more evidence on the in vivo inhibitory effects of honey on lipid peroxidation.

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