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Postprandial increases in serum antioxidant capacity in older women

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Cao, Guohua, and Ronald L. Prior. Postprandial increases in serum antioxidant capacity in older women. *J Appl Physiol* 89: 877–883, 2000.— Eight women were recruited for studying the effects of a meal on overall antioxidant status. Subjects resided in a metabolic research unit for two 36-h periods. During *period A*, subjects fasted overnight (12 h) and were then given a breakfast, a lunch, a snack, and a dinner. During *period B*, subjects fasted for 23 h and were then given a dinner. These meals were designed to contain negligible antioxidants. Blood samples were collected for analyzing total antioxidant capacity (TAC) and individual antioxidants. The results showed that serum TAC significantly increased by up to 23% after the consumption of the lunch and dinner during *period A*. Serum TAC did not increase until after the consumption of the dinner during *period B*. Among the antioxidants (vitamin C, α-tocopherol, bilirubin, and uric acid) examined, serum uric acid was the only one that showed a significant postprandial increase, which was also parallel to the postprandial response in serum TAC. These results indicate that food intake, even if low in antioxidants, can increase the serum total antioxidant activity.

meal; reactive oxygen species; free radical; oxygen radical absorbance capacity

THE OCCURRENCE OF REACTIVE oxygen (ROS) and nitrogen species (RNS) is an attribute of normal aerobic metabolic processes. Among the most significant biological sources of ROS are those that lead to O_2-derived superoxide radicals from electron transport associated with mitochondrial membranes. It is well documented that, under normal physiological conditions, an estimated 1–3% of respired oxygen is converted to superoxide radicals. Other ROS include hydroxyl, peroxyl radicals, and H_2O_2. RNS include nitric oxide (NO·) and nitrogen dioxide (NO_2·). NO· is formed from the amino acid L-arginine. NO_2· is made when NO· reacts with O_2. There is a considerable body of biological evidence that ROS and RNS can be damaging to cells and thus might contribute to cellular dysfunction and diseases. The existence and development of cells in an oxygen-containing environment would not be possible without the presence of a complicated defense system that includes enzymatic and nonenzymatic antioxidant components. Therefore, a healthy aerobic life is characterized by a steady formation of ROS and RNS balanced by a similar rate of their consumption by an antioxidant system.

The nonenzymatic antioxidants, most of which have low molecular weights and are able to directly and efficiently quench ROS and RNS, constitute an important aspect of the body's antioxidant system. Measurement of total antioxidant capacity (TAC) from all of these nonenzymatic antioxidants becomes necessary and more important in many conditions because of the difficulty in measuring all of them individually. Our oxygen radical absorbance capacity (ORAC) assay (5, 12) is one of the tests recently developed to measure the TAC of biological samples. The main advantage of the ORAC assay over other similar methods is its use of a biologically and pathologically important ROS and its application of an area-under-curve technique in the quantitation process (6, 22). The area-under-curve technique considers both inhibition time and inhibition percentage of free radical action by an analyzed antioxidant sample. The ORAC assay has been used by different laboratories (1, 13, 17, 20, 21, 23, 26) and has provided significant information regarding the antioxidant capacity of various biological samples from pure compounds, such as melatonin, dopamine, and flavonoids, to complex matrices, such as tea, fruits, vegetables, herbs, and animal tissues (1, 4, 5, 9–11, 13, 17, 20, 21, 23, 24, 26). In this human study, we found a significant postprandial increase in serum TAC by using the ORAC assay and two other TAC assays. The results not only have their own physiological implications but also help design a better controlled animal or human study that measures serum or plasma antioxidant status.

SUBJECTS AND METHODS

Subjects and diets. Eight healthy female subjects [age 66.9 ± 0.6 (SE) yr, body mass index 26.1 ± 0.7 kg/m^2] were recruited to participate in this study. All study participants were in good health as determined by a medical history questionnaire, physical examination, and normal results from clinical laboratory tests. All of the subjects fulfilled the following eligibility criteria: *1*) no history of cardiovascular,

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hepatic, gastrointestinal, or renal disease; *2*) no alcoholism; *3*) no antibiotic or supplemental vitamin and/or mineral use for >4 wk before the start of the study; and *4*) no smoking. The study protocol was approved by the Human Investigation Review Committee of Tufts University and the New England Medical Center, and written informed consent was obtained from each study participant.

Subjects were required to reside in the Metabolic Research Unit at the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University for two 36-h periods (*periods A* and *B*). During *period A*, eight subjects were asked to come to the Metabolic Research Unit in the evening and fast overnight. In the morning, an intravenous catheter was inserted into one forearm. A 10-ml fasting blood sample (the baseline zero time sample) was obtained at about 8 AM, after which the subject was given a breakfast drink (1,004 kJ; Table 1). Blood samples (10 ml) were collected again at 0.25, 0.5, 1, 2, and 4 h after consumption of the breakfast drink. A lunch meal was given (2,385 kJ; Table 1) immediately after the blood sampling at 4 h. Additional blood samples were obtained at 7, 9, 11, 13, 15, and 24 h after the initial blood sampling. A snack (1,063 kJ; Table 1) was given immediately after the 7-h blood sampling. A dinner (2,621 kJ; Table 1) was given immediately after the 11-h blood sampling. The breakfast, lunch, snack, and dinner were designed to contain as little as possible of known antioxidants (no significant amounts of vitamin C, α-tocopherol, carotenoids, and flavonoids) but provide the recommended daily allowance for protein and energy (Table 1). Blood samples were collected more frequently after breakfast compared with after lunch or dinner because of the tests needed for other treatments, which have been published (8). During *period B*, we were able to retest five of the eight subjects to confirm that the postprandial change in serum TAC that we observed during *period A* was due to meals and not the endocrinological or any other physiological factors exercised around a meal time. Therefore, during *period B*, subjects were given a dinner, but not a breakfast, lunch, or snack. The blood sampling procedure and schedule were the same as those used in *period A*.

Serum TAC analyses. Three different methods were used for the serum TAC analyses. They were the *1*) ORAC assay, *2*) trolox equivalent antioxidant capacity (TEAC) assay, and *3*) ferric reducing ability/power (FRAP) assay.

Serum ORAC was determined by the automated method of Cao and co-workers (5, 12) using serum deproteinized with 0.5 mol $HClO_4$/l (1:1 vol/vol). Briefly, in the final assay mixture (0.4 ml total volume), *R*-phycoerythrin (16.7 nM) was used as a target of free radical attack, with 2,2′-azobis(2-amidinopropane) dihydrochloride (4 mM) as a peroxyl radical generator. Trolox was used as a control standard. Final results were calculated by using the differences of areas under the R-phycoerythrin decay curves between the blank and a sample and are expressed as micromoles of Trolox equivalents per liter.

Serum TEAC was measured by using the method of Miller and co-workers (19) with commercially available kits (Total Antioxidant Status, lot 21440, Randox Laboratories, Lakewood, CA). This method for measuring antioxidant activity is based on the inhibition by antioxidants of the absorbance of the radical cations of 2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonate) (ABTS) at 600 nm. ABTS radical cations are formed by incubation of ABTS with metmyoglobin and H_2O_2. The final results are expressed as micromoles of Trolox equivalents per liter.

Serum FRAP was determined by the method of Benzie and Strain (3). The FRAP assay measures the ferric-reducing ability of plasma or serum. At low pH, when a ferric (Fe^{3+})-tripyridyltriazine complex is reduced by antioxidants to the ferrous (Fe^{2+}) form, an intense blue color with an absorption maximum at 593 nm develops. In the FRAP assay, Fe^{2+} was used as a standard. The final results were converted to micromoles of Trolox equivalents per liter. The relative activity of Trolox in the FRAP assay was 2.0; i.e., the direct reaction of Fe^{2+} gave a change in absorbance of one-half that of an equivalent molar concentration for Trolox (3).

Determination of protein, uric acid, bilirubin, α-tocopherol, and vitamin C. Protein, uric acid, and bilirubin were measured in serum by using a Cobas Mira spectrophotometric centrifugal analyzer with reagent kits purchased from Roche

Table 1. *Food items and nutrient content of breakfast, lunch, dinner, and snack*

Item	Amount	Energy, kJ	Protein, g	Fat, g	Carbohydrate, g
Breakfast		1,004	10.4	9.7	45.0
Coconut milk	18.9 g				
Pro Mod powder*	13.2 g				
Polycose liquid†	37.7 g				
Coffee creamer	62.9 g				
Sugar, white	18.9 g				
Water	250.2 g				
Lunch		2,385 ± 108	39.7 ± 1.3	10.9 ± 0.9	76.6 ± 3.7
Turkey breast	120 g				
Margarine	1 pat				
Bread, white, commercial	2 slices				
Crackers	4				
Ginger ale, regular	480 g				
Dinner		2,621 ± 124	29.3 ± 0.9	10.0 ± 0.8	103.2 ± 6.0
Sole, cooked	150 g				
Rice, white, regular, cooked	75 g				
Corn oil	5 g				
Angel food cake	34 g				
Ginger ale, regular	240 g				
Snack		1,063	6.0	0.91	55.4
Ginger ale, regular	240 g				
Bagel, white flour, plain	1				

Data for lunch and dinner are presented as means ± SE. All the subjects received the same amounts of breakfast and snack. *Concentrate protein from whey (Ross Laboratories, Columbus, OH). †Glucose polymers, 43% solution (Ross Laboratories).

Diagnostic Systems (Branchburg, NJ). α-Tocopherol in serum was analyzed by reverse-phase HPLC (18) coupled to an ESA coulometric detection system (ESA, Chelmsford, MA). Vitamin C was determined by HPLC analysis of plasma deproteinized immediately after separation with 0.5 mol $HClO_4$/l (2).

Statistical analyses. Postprandial responses in serum TAC, uric acid, α-tocopherol, bilirubin, and plasma vitamin C were evaluated by using a paired *t*-test (Systat). Linear regression analyses of the change in serum TAC vs. the change in serum uric acid were computed by using MGLH in Systat for Windows. A linear fit adequately described the data as assessed by the correlation coefficient.

RESULTS

Postprandial responses in serum TAC. Fasting serum TAC before the consumption of breakfast during *period A* and before breakfast time (no breakfast) during *period B* are shown in Table 2. The fasting ORAC and FRAP were significantly different between *period A* and *period B*, indicating effects of season or daily life (diet, exercise) on the serum TAC. Besides being expressed in micromoles per liter, serum TAC was also expressed in nanomoles per milligram protein to correct for any possible effects resulting from blood dilution. These subjects received normal saline continuously through an intravenous catheter maintained in one forearm for blood sampling. Also, the water intake was not restricted for these subjects during both periods. The responses in serum ORAC, FRAP, and TEAC after the initial blood sampling are shown in Figs. 1 and 2 for both periods.

Apparently there were three peaks in the response of serum ORAC during *period A*, one after breakfast, one after lunch (at 4 h), and one after dinner (at 11 h), although the increase in serum ORAC after the breakfast meal was not statistically significant. The increase in serum ORAC after the consumption of the lunch meal was significant, and the increase lasted for 11 h during the period. When expressed in nanomoles per milligram protein, serum ORAC (Fig. 1) at 7, 9, 11, 13, or 15 h was significantly higher than that at 0 h; the percent increases were 19, 21, 13, 20, and 23%, respectively. When expressed in micromoles per liter (Fig. 2), serum ORAC at 9, 13, or 15 h was also significantly higher than that at 0 h; these percent increases were 17, 16, and 15%, respectively. However, during *period B*, when no breakfast, lunch or snack was given, serum ORAC did not increase significantly until after the consumption of a dinner meal, which was given at 11 h. Serum ORAC, expressed in nanomoles per milligram protein, was significantly higher at 13 or 15 h than at 0 or 11 h during the period. When expressed in micromoles per liter, serum ORAC showed a trend of in-

Table 2. *Serum total antioxidant capacity and individual antioxidant measures before consumption of breakfast*

Item	*Period A*		*Period B*	
	μmol/l	nmol/mg protein	μmol/l	nmol/mg protein
ORAC	552 ± 34*	8.52 ± 0.37	680 ± 27†	10.33 ± 0.57†
FRAP	388 ± 27*	5.96 ± 0.28*	526 ± 36	7.99 ± 0.55
TEAC	1325 ± 48	20.4 ± 0.59	1328 ± 41	20.2 ± 0.67
Uric acid	232 ± 25	3.57 ± 0.25	290 ± 34	4.41 ± 0.52
α-Tocopherol	24.4 ± 1.7	0.38 ± 0.03		
Bilirubin	9.6 ± 1.2	0.15 ± 0.02	11.0 ± 1.5	0.17 ± 0.02
Vitamin C	40.2 ± 7.5	0.62 ± 0.10	60.3 ± 9.6	0.92 ± 0.14
Protein, g/l	65 ± 1.2		66 ± 1.6	

Data are presented as means ± SE of $n = 8$ (*period A*) or 5 (*period B*) subjects, unless otherwise indicated; †$n = 4$. Vitamin C was measured in plasma. ORAC, oxygen radical absorbance capacity; FRAP, ferric reducing ability/power; TEAC, trolox equivalent antioxidant capacity. **Period A* vs. *period B*, $P < 0.05$.

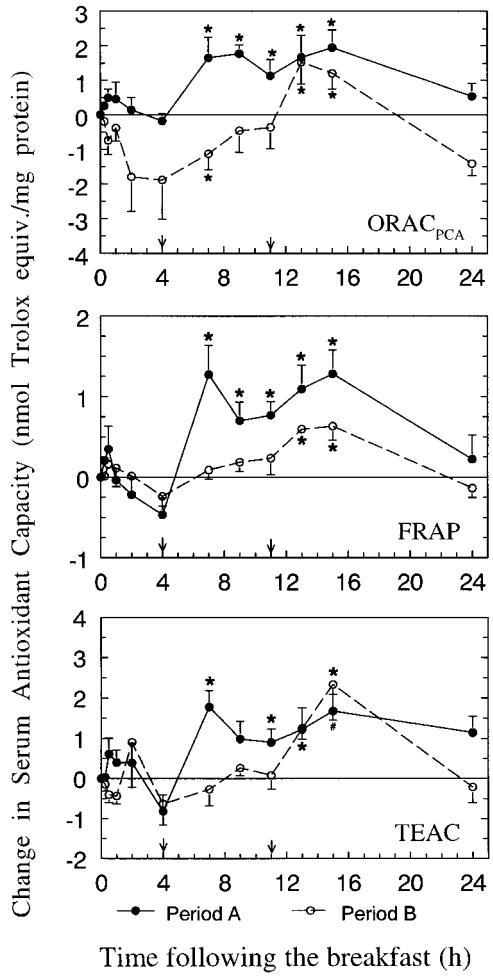

Fig. 1. Postprandial changes (means ± SE) in serum total antioxidant capacity (TAC) measured as oxygen radical absorbance capacity (ORAC), ferric reducing ability/power (FRAP), and Trolox equivalent antioxidant capacity (TEAC) expressed in nanomoles per milligram protein. PCA, serum treated with perchloric acid. Baseline ORAC, FRAP, and TEAC data are presented in Table 2. During *period A*, a breakfast drink was given at *time 0* followed by lunch, snack, and dinner at 4, 7, and 11 h, respectively. During *period B*, only the dinner was given. Data are expressed in nanomoles per milligram protein to correct for any possible effect resulting from blood dilution. These subjects received normal saline continuously through an intravenous catheter maintained in 1 forearm for blood sampling. Also, water intake was not restricted for these subjects during both periods. Significantly different ($P < 0.05$) from *time 0* for **period A* and #*period B*. Down arrows indicate meal time (no breakfast and lunch were given during *period B*).

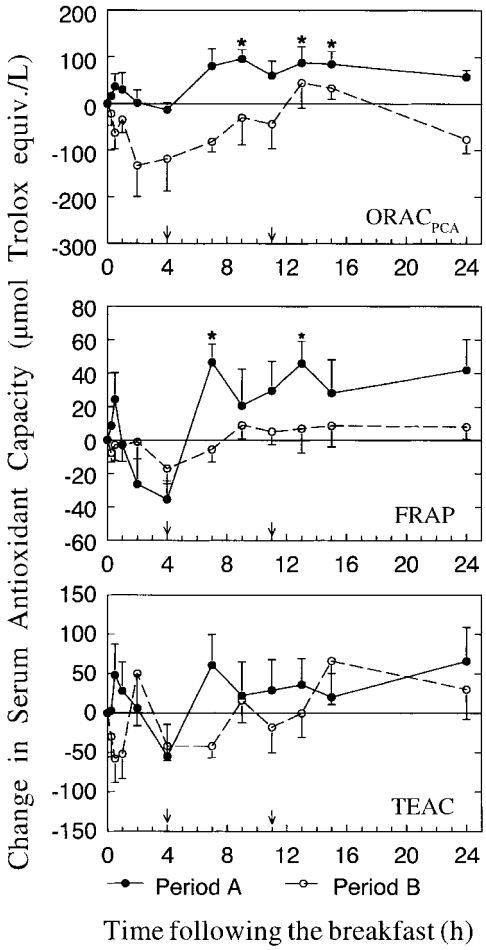

Fig. 2. Postprandial changes in serum TAC measured as ORAC, FRAP, and TEAC and expressed as micromoles per liter. Baseline ORAC, FRAP, and TEAC data are presented in Table 2. *Significantly different ($P < 0.05$) from *time 0*. See Fig. 1 legend for additional details about the experiments.

creasing after the dinner, but the increase was not statistically significant due to the effect of blood dilution, which resulted from normal saline infusion during the fasting period.

As seen with serum ORAC, there were also three peaks in the response of serum FRAP during *period A*: one after breakfast, one after lunch, and one after dinner. Serum FRAP increased significantly during *period A* after the consumption of the lunch meal, and the increase lasted for 11 h. When expressed in nanomoles per milligram protein (Fig. 1), serum FRAP at 7, 9, 11, 13, or 15 h was significantly higher than that at 0 h, with percent increases at 21, 12, 13, 18, and 21%, respectively. When expressed in micromoles per liter (Fig. 2), serum FRAP at 7 and 13 h was significantly higher than at 0 h, with percent increases at 12 and 12%, respectively. During *period B*, in the absence of breakfast and lunch, serum FRAP did not increase significantly until after the consumption of the dinner meal. Serum FRAP, expressed in nanomoles per milligram protein, was significantly higher at 13 or 15 h than at 0 h during the period.

Serum TEAC showed a similar postprandial response pattern as serum ORAC and FRAP during both *period A* and *period B* when it was expressed in nanomoles per milligram protein (Fig. 1). However, when expressed as micromoles per liter, serum TEAC showed no significant postprandial response during either periods (Fig. 2).

Postprandial responses in blood individual antioxidants. Fasting concentrations of serum uric acid, α-tocopherol, bilirubin, and plasma vitamin C before the consumption of the breakfast during *period A* and before the breakfast time (no breakfast) during *period B* are shown in Table 2. There were no significant differences in these parameters between *period A* and *period B* (α-tocopherol was not measured in *period B*).

Responses in serum uric acid, bilirubin, α-tocopherol, and plasma vitamin C levels after the consumption of the breakfast (*period A*) or breakfast time (*period B*) are shown in Figs. 3 and 4. Among these blood antioxidants, only serum uric acid showed a postpran-

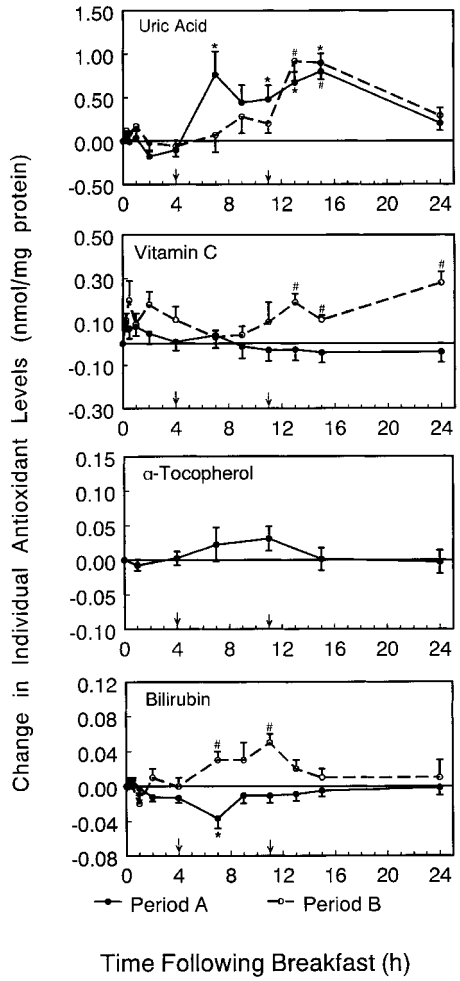

Fig. 3. Postprandial changes in serum uric acid, plasma vitamin C, α-tocopherol, and bilirubin concentrations (nmol/mg protein). Baseline uric acid, α-tocopherol, bilirubin, and plasma vitamin C concentrations are presented in Table 2. Significantly different ($P < 0.05$) from *time 0* for **period A* and *#period B*. See Fig. 1 for additional details about the experiments.

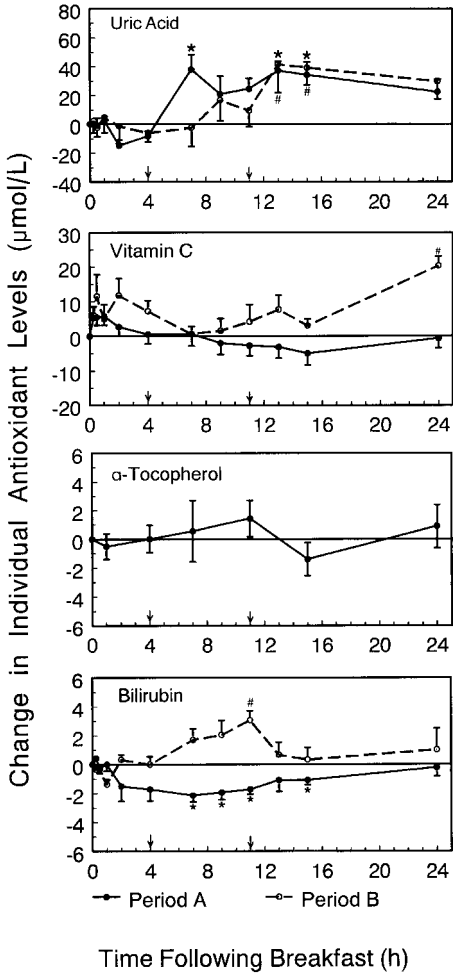

Fig. 4. Postprandial changes in serum uric acid, plasma vitamin C, α-tocopherol, and bilirubin concentrations (μmol/l). Baseline uric acid, α-tocopherol, bilirubin, and plasma vitamin C concentrations are presented in Table 2. Significantly different ($P < 0.05$) from *time 0* for **period A* and #*period B*. See Fig. 1 for additional details about the experiments.

dial response, which was parallel to serum TAC measured as ORAC or FRAP. There were two peaks in the response of serum uric acid during *period A*, one after lunch and another after dinner, whether it was expressed as micromoles per liter or nanomoles per milligram protein. During *period B*, when no breakfast, lunch, or snack was given, serum uric acid did not increase significantly until after the consumption of the dinner meal. The postprandial change in serum uric acid was linearly correlated with the change in serum ORAC or FRAP ($P < 0.001$) (Fig. 5); however, the correlation coefficients were relatively low.

During *period A*, serum bilirubin concentrations, when expressed in nanomoles per milligram protein, decreased significantly after the lunch but not after the breakfast or dinner (Fig. 3). Serum bilirubin also showed a continuous decrease after the initial sampling when it was expressed in micromoles per liter during the period (Fig. 4). However, during *period B*, when no breakfast and lunch were given, serum bilirubin showed a continuous increase until after the consumption of the dinner meal (Figs. 3 and 4).

There were no significant changes in plasma vitamin C and serum α-tocopherol concentrations during *period A*. However, during *period B*, plasma vitamin C showed a significant increase after the dinner was consumed.

DISCUSSION

The present study showed a clear postprandial response in serum TAC in older women. Serum TAC, which was measured by using different analytic techniques (ORAC, FRAP, and TEAC), increased significantly after the consumption of meals that were designed to contain negligible antioxidants. The postprandial responses in serum ORAC, FRAP, and TEAC were parallel to each other and gave two significant peaks during *period A*: one seen 3–4 h after the lunch and one seen 4 h after the dinner. It appeared that there was also a peak after the breakfast, but the change was not statistically significant. Serum TAC during *period A* remained elevated above the basal level between the lunch and dinner. This could be a result of the extended effect of the lunch but is more likely an effect of the snack that was given between the lunch and dinner during *period A*.

The postprandial increase in serum TAC observed in the human subjects in this study was clearly a food-related observation. When the subjects fasted for 23 h before a dinner was given during *period B*, their serum TAC did not increase until after consumption of the dinner. This postprandial increase in serum TAC indicates the importance of controlling diets in clinical studies that involve the measurement of antioxidant status. A measured increase in serum or plasma antioxidant capacity after the consumption of a diet con-

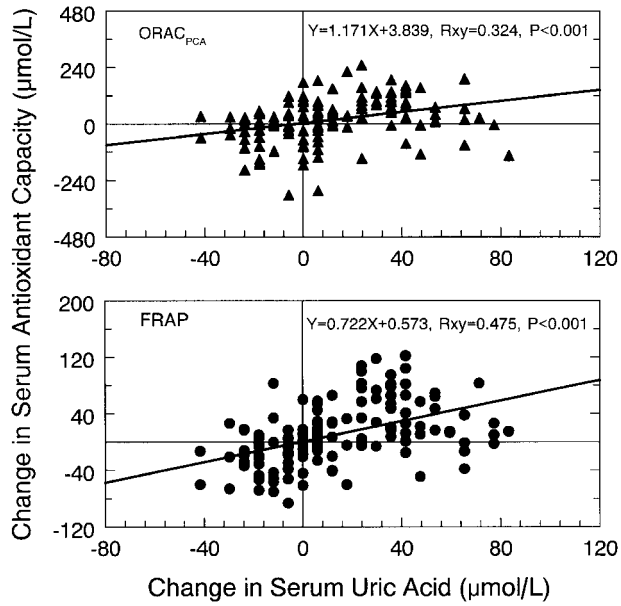

Fig. 5. Postprandial change in serum TAC (*Y*) as measured by ORAC and FRAP as a function of postprandial changes in serum uric acid concentration (*X*). Thick line, line of best fit.

taining antioxidants could be a general effect of food intake, not a specific effect from the antioxidants contained in the diet. The increase in serum antioxidant capacity after a meal could result from an increased production of antioxidant amino acids from proteins contained in the meal and/or from an increased internal antioxidant release into the blood during the process of absorbing, storing, or metabolizing the ingested nutrients. The FRAP assay used in this study does not measure sulfhydryl-containing amino acids or peptides (3), but serum FRAP showed a significant postprandial increase parallel to serum ORAC, indicating that increased amino acid production after a meal was not a primary mechanism underlying the postprandial increase in serum antioxidant capacity.

Interestingly, the time course of the response in serum TAC was similar to that of "the thermic effect of food," a well-known phenomenon referring to the increase in oxygen consumption or energy expenditure after the consumption of a meal. When one meal was fed to four male and four female volunteers, LeBlanc and Soucy (15) observed a gradual increase in oxygen consumption for up to 80 min; the value for the increased oxygen consumption was still higher than 50% of the peak value for increased oxygen consumption by 140 min. The thermic effect of food (kJ/min) in young women after ingestion of meals was energy-content dependent; the total food-induced thermogenesis values were significantly higher in subjects with higher energy intakes (25). The postprandial response in serum TAC appeared to be also energy-content dependent. The response in serum TAC in the older women during *period A* after breakfast (1,004 kJ) was much weaker than that after lunch (2,385 kJ) or dinner (2,621 kJ). For example, the maximum increase in serum ORAC (nmol/mg protein) during *period A* after breakfast (1,004 kJ) was 6%, whereas those after lunch and dinner were 21 and 23%, respectively.

Because oxygen consumption or energy expenditure increases after a meal (the thermic effect of food), it would be reasonable to postulate a food-induced increase in free radical (ROS and/or RNS) production from the increased oxygen metabolism and thus a food-induced decrease in serum antioxidant capacity (TAC). The amount of oxygen radicals derived from oxygen is dependent on the amount of oxygen consumed. The more oxygen consumed, the more oxygen radicals formed. Apparently, the human body is protected against the potential damaging effects of the postprandial increase in oxygen consumption or free radical production. What we observed in this study is actually a postprandial or "food-induced" increase in serum antioxidant capacity. This food-induced increase in serum antioxidant capacity can be viewed as an adaptive response to the food-induced oxidative stress. The adaptive response in the antioxidant defense system to free radical production in vivo has been demonstrated in calorie-restricted animals. Chronic caloric restriction without essential nutrient deficiency is recognized as the most effective manipulation to extend the life span and retard the aging process in laboratory rodents and other short-lived species. Calorie restriction reduces both free radical production (16) and serum antioxidant capacity measured as ORAC (7). The thermic effect of food, basal metabolic rate, and total heat production of calorie-restricted animals were also significantly lower than those for ad libitum-fed controls (14).

In this study, uric acid was the only individual antioxidant found to be responsible for part of the increased serum antioxidant capacity after a meal. This postprandial increase in serum uric acid might have critical physiological implications. Two evolutionary alterations have led to high tissue concentrations of urate in humans: loss of peroxisomal urate oxidase and active reabsorption of urate from the kidneys. However, other physiological processes are also responsible for the postprandial increase in serum TAC, because the contribution of the increased serum uric acid to the increased serum ORAC (0–15 h) was only ~29%.

The plasma vitamin C, when expressed in nanomoles per milligram protein, increased significantly after the consumption of the dinner given during *period B*. We do not have a clear explanation for it, because all the meals were designed to contain negligible antioxidants, which include vitamin C, although the consumption of a vitamin C-containing beverage along with the dinner or after the dinner by these fasted subjects cannot be excluded during the period. However, the increased vitamin C level (after the dinner) could not fully account for the increased serum TAC (after dinner) during *period B*. During *period A*, plasma vitamin C remained unchanged, whereas postprandial increases in serum antioxidant capacity were observed.

In conclusion, we observed in older healthy women a significant postprandial increase in serum antioxidant capacity, which was partly due to the food-related increase in serum uric acid concentration. This postprandial increase in serum antioxidant capacity indicates an adaptive physiological response in antioxidant defense systems.

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