Clinical Chemistry 44:6
1309–1315 (1998)

Automation and Analytical Techniques

Comparison of different analytical methods for assessing total antioxidant capacity of human serum

Guohua Cao[1,2] and Ronald L. Prior[1*]

Three assays were compared for the determination of total antioxidant capacity in human serum: the oxygen radical absorbance capacity (ORAC) assay, the Randox Trolox-equivalent antioxidant capacity (Randox-TEAC) assay, and the ferric reducing ability (FRAP) assay. There was a weak but significant linear correlation between serum ORAC and serum FRAP. There was no correlation either between serum ORAC and serum TEAC or between serum FRAP and serum TEAC. The effect of dilution on the serum TEAC value and the use of inhibition percentage at a fixed time, without considering the length of inhibition time in the quantitation of results, adversely affected the Randox-TEAC assay. The FRAP assay is simple and inexpensive but does not measure the SH-group-containing antioxidants. The ORAC assay has high specificity and responds to numerous antioxidants. By utilizing different extraction techniques in the ORAC assay, one can remove serum proteins and also make some gross differentiation between aqueous and lipid-soluble antioxidants. However, the ORAC assay requires ~60 min more than the FRAP or Randox-TEAC assay to quantitate results.

Production of reactive species, including free radicals, is an integral part of human metabolism. Because of the high potential to damage vital biological systems, reactive species have now been incriminated in aging and in more than 100 disease states *(1, 2)*. Living organisms have developed complex antioxidant systems to counteract reactive species and to reduce their damage. These antioxidant systems include enzymes such as superoxide dismutase, catalase, and glutathione peroxidase; macromolecules such as albumin, ceruloplasmin, and ferritin; and an array of small molecules, including ascorbic acid, α-tocopherol, β-carotene, ubiquinol-10, reduced glutathione (GSH),[3] methionine, uric acid, and bilirubin *(3)*.

Several methods *(4–11)* have been developed to assess the total antioxidant capacity of human serum or plasma because of the difficulty in measuring each antioxidant component separately and the interactions among different antioxidant components in the serum or plasma. The total peroxyl radical trapping parameter assay of Wayner et al. *(4)* was the most widely used assay of antioxidant capacity during the last decade. As Rice-Evans and Miller *(12)* pointed out, the major problem with the original total peroxyl radical trapping parameter assay lies in the oxygen electrode endpoint; an oxygen electrode will not maintain its stability over the period of time required. Therefore, a high degree of imprecision is inherent in this method. The method of Glazer *(5)* assumes that the decrease in fluorescence of B-phycoerythrin (B-PE) or R-phycoerythrin (R-PE) in the presence of 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) is linear with time and that a period of complete protection (the length of the lag phase) of B- or R-PE against AAPH is related to antioxidant concentrations. The method of Ghiselli et al. *(6)* is basically a duplicate of the method of Glazer. However, the decrease in the fluorescence of B- or R-PE is not linear with time *(9, 10)*. More recently, the 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

[1] US Department of Agriculture, Agriculture Research Service, Jean Mayer Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.
[2] Nutritional Science Department, University of Connecticut, Storrs, CT 06269.
*Address correspondence to this author at: USDA, ARS, HNRCA, 711 Washington St., Boston, MA 02111. Fax 617-556-3299; e-mail prior@hnrc.tufts.edu.
Received May 16, 1997; revision accepted March 4, 1998.

[3] Nonstandard abbreviations: GSH, reduced glutathione; PE, phycoerythrin; AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TEAC, Trolox equivalent antioxidant capacity; FRAP, ferric reducing ability of plasma; ORAC, oxygen radical absorbance capacity; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonate); PCA, perchloric acid; and Randox-TEAC, Randox Trolox-equivalent antioxidant capacity.

(Trolox)[4] equivalent antioxidant capacity (TEAC) assay of Miller et al. *(7, 12)*, the ferric reducing ability of plasma (FRAP) assay of Benzie and Strain *(11)*, and our oxygen radical absorbance capacity (ORAC) assay *(9, 10)* were developed. The TEAC assay is based on the inhibition by antioxidants of the absorbance of the radical cation of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), and has been commercialized by Randox Laboratories. This method has produced useful information regarding the antioxidant activities of phytochemicals *(13–16)*. The FRAP assay measures the ferric-to-ferrous iron reduction in the presence of antioxidants and is very simple and convenient in terms of its operation. The ORAC assay is based largely on the work reported by Glazer's laboratory *(5)* and depends on the unique properties of phycoerythrins. It is, to date, the only method that takes free radical action to completion and uses an area-under-curve technique for quantitation; it thus combines both inhibition percentage and the length of inhibition time of the free radical action by antioxidants into a single quantity *(10)*. The ORAC assay has been used by different laboratories *(17–22)* and has provided substantial information regarding the antioxidant capacity of various biological samples from pure compounds such as melatonin *(17)*, dopamine *(19)*, and flavonoids *(20, 23, 24)* to complex matrices such as tea *(25)*, fruits *(26)*, vegetables *(25)*, *student rasayana* (an herbal mixture) *(21)*, and animal tissues *(18, 22, 27, 28)*.

The measured antioxidant capacity of a sample depends on which technology and which free radical generator or oxidant is used in the measurement *(9, 29, 30)*. Therefore, the comparison of different analytical methods constitutes a key factor in helping investigators to choose a method and to understand the result obtained using the method. The objective of the present study was to compare the TEAC, FRAP, and ORAC assays for assessing the total antioxidant capacity in human serum.

Materials and Methods

REAGENTS

R-PE from *Porphyridium cruentum*, 2,4,6-tripyridyl-*s*-triazine, ferric chloride, and ferrous chloride were purchased from Sigma Chemicals. The R-PE that we used in these experiments usually loses >90% of its fluorescence within 30 min in the presence of 4 mmol/L AAPH. The AAPH was obtained from Wako Chemicals USA. Trolox was obtained from Aldrich.

SAMPLES

Serum samples were obtained from 45 healthy subjects (women, 31; men, 14; age, 71.1 ± 1.2 years, mean ± SE) for the correlation analysis between serum ORAC, FRAP, and TEAC values. Serum samples were also taken from eight female subjects (ages, 66.9 ± 1.7 years) over 8 weeks at biweekly intervals to see the effects of sampling time on the antioxidant capacity. During the 8 weeks, these female subjects kept their free-living life-style and consumed a self-selected diet. All serum samples except those collected biweekly over 8 weeks were stored at −80 °C and analyzed within 1 week for ORAC, FRAP, and TEAC on the same day. The samples collected biweekly over 8 weeks were aliquoted, stored at −80 °C, and analyzed within 1 week for ORAC, TEAC, and FRAP, but on different days. 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.

ORAC ASSAY

The automated ORAC assay was carried out on a COBAS FARA II spectrofluorometric analyzer (Roche Diagnostic Systems) at an excitation wavelength of 540 nm and an emission wavelength of 565 nm. The procedure was based on a previous report of Cao et al. *(9)*, modified for the COBAS FARA II *(10)*.

Both serum and serum nonprotein fractions extracted with perchloric acid (PCA) and acetone were used in the ORAC assay. For preparation of a serum nonprotein fraction, the serum was diluted with 0.5 mol/L PCA (1:1, by volume) or acetone (1:8, by volume). The samples were then centrifuged at 100 000*g* for 10 min at 4 °C, and the supernatants were removed as the serum nonprotein fractions and diluted for the ORAC assay.

RANDOX-TEAC ASSAY

The automated TEAC assay was carried out on a COBAS MIRA spectrophotometric analyzer (Roche Diagnostic Systems) with commercially available kits ("Total Antioxidant status"; lot 21440, Randox Laboratories). It was thus referred to as the Randox-TEAC assay in this study.

FRAP ASSAY

The automated FRAP assay was carried out on a COBAS FARA II spectrophotometric analyzer *(11)*. The final results were converted to mmol Trolox equivalents/L. 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 one-half that of an equivalent molar concentration for Trolox *(11)*.

DETERMINATION OF URIC ACID, BILIRUBIN, PROTEIN, α-TOCOPHEROL, AND ASCORBIC ACID

Uric acid, total bilirubin, and total protein were measured in serum, using a COBAS MIRA spectrophotometric analyzer, with reagent kits purchased from Roche Diagnostic Systems. Serum α-tocopherol was analyzed by reversed-phase HPLC *(31)* coupled to an ESA coulometric detection system (ESA Inc.). Ascorbic acid was deter-

[4] Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

mined by HPLC analysis of deproteinized plasma *(32)*. Samples were injected onto a Bio-Sil ODS 5S 150 × 4 mm reversed-phase column (Bio-Rad) and analyzed on a Waters HPLC system with a LC4B Bioanalytical Systems amperometric electrochemical detector.

STATISTICS

The effects of sampling time on the serum total antioxidant capacity measurements of the same subjects over an 8-week period were analyzed by ANOVA using Systat (Systat). Correlation and regression analyses of one total antioxidant capacity measurement vs another and the antioxidant capacity of a serum or antioxidant sample vs its concentration were also computed using Systat. $P < 0.05$ was considered to indicate statistical significance.

Results

The within-run CVs obtained with ORAC, TEAC, and FRAP assays using human serum were 3.5%, 2.5%, and 0.6%, respectively. The between-run CV obtained with ORAC, TEAC, and FRAP assays using human serum were 5.6%, 5.0%, and 4.0%, respectively.

The total antioxidant capacities measured as $ORAC_{total}$, $ORAC_{pca}$, $ORAC_{ac}$, FRAP, and TEAC in the serum of eight elderly women over a period of 8 weeks is shown in Table 1. Results of ANOVA showed that the effects of sampling time (from sampling week 0 to week 8) on the serum $ORAC_{total}$, $ORAC_{pca}$, $ORAC_{ac}$, FRAP, and TEAC were not significant.

A weak but significant linear correlation was found between serum $ORAC_{total}$ and serum FRAP ($r = 0.349$, $P = 0.019$; Fig. 1). There was no correlation either between serum $ORAC_{total}$ and serum TEAC (Fig. 2) or between serum FRAP and serum TEAC (Fig. 3).

The relative antioxidant capacities of individual serum antioxidants and their estimated contributions to serum $ORAC_{total}$, $ORAC_{pca}$, $ORAC_{ac}$, FRAP, and TEAC are shown in Table 2. The individual antioxidants measured in serum in this study were uric acid, α-tocopherol, ascorbic acid, and bilirubin. Serum albumin concentrations were calculated using serum total protein based on the ratio of albumin:total protein in an elderly population (unpublished data). The relative ORAC value of ascorbic acid determined in this study and listed in Table 2 was 1.0 Trolox equivalent, which was higher than the value that we reported previously for sodium ascorbate *(9)*. This difference can be explained by taking into account the possibility that partially oxidized ascorbate was used. The main known contributors to serum $ORAC_{total}$ and TEAC were albumin (27.8% and 28.0%, respectively) and uric acid (7.1% and 19.3%, respectively). The primary known contributors to $ORAC_{pca}$, $ORAC_{ac}$, and FRAP were uric acid (39.2%, 45.4%, and 61.7%, respectively) and ascorbic acid (7.2%, 10.5%, and 10.1%, respectively). However, other substances in addition to the ones included in Table 2 were the biggest contributors to serum $ORAC_{total}$,

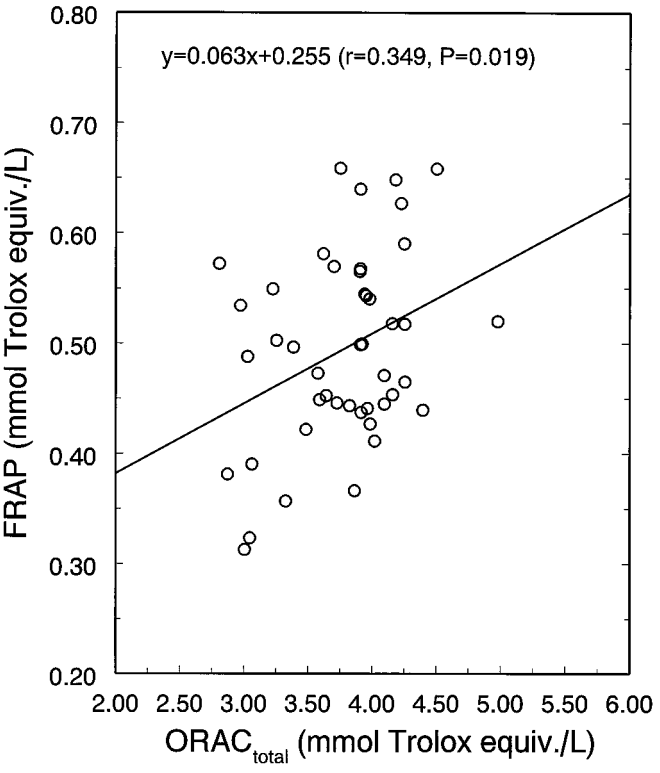

Fig. 1. Scatter plot of serum $ORAC_{total}$ and serum FRAP.
There is a significant linear correlation between serum $ORAC_{total}$ and serum FRAP. Forty-five human subjects were used in the analysis.

Table 1. Total antioxidant capacities of serum measured at biweekly intervals in the same subjects.

	mmol Trolox equivalent/L[a]				
	$ORAC_{total}$[b]	$ORAC_{pca}$[b]	$ORAC_{ac}$[b]	FRAP	TEAC
Week 0	3.10 ± 0.20	0.63 ± 0.09	0.41 ± 0.10	0.40 ± 0.08	1.41 ± 0.12
Week 2	3.12 ± 0.32	0.56 ± 0.11	0.38 ± 0.09	0.42 ± 0.07	1.39 ± 0.13
Week 4	3.05 ± 0.43	0.58 ± 0.10	0.42 ± 0.07	0.40 ± 0.07	1.26 ± 0.08
Week 6	3.40 ± 0.21	0.61 ± 0.07	0.41 ± 0.06	0.45 ± 0.07	1.39 ± 0.11
Week 8	3.60 ± 0.78	0.58 ± 0.08	0.39 ± 0.06	0.42 ± 0.07	1.37 ± 0.14

[a] Data are presented as mean ± SD of seven to eight female subjects (age, 66.9 ± 1.7; body-mass index, 26.1 ± 2.0). All samples were taken at about 0800 on Tuesday after an overnight fast.

[b] $ORAC_{total}$, ORAC analyzed using whole serum; $ORAC_{pca}$, ORAC analyzed using serum treated with perchloric acid; and $ORAC_{ac}$, ORAC analyzed using serum treated with acetone.

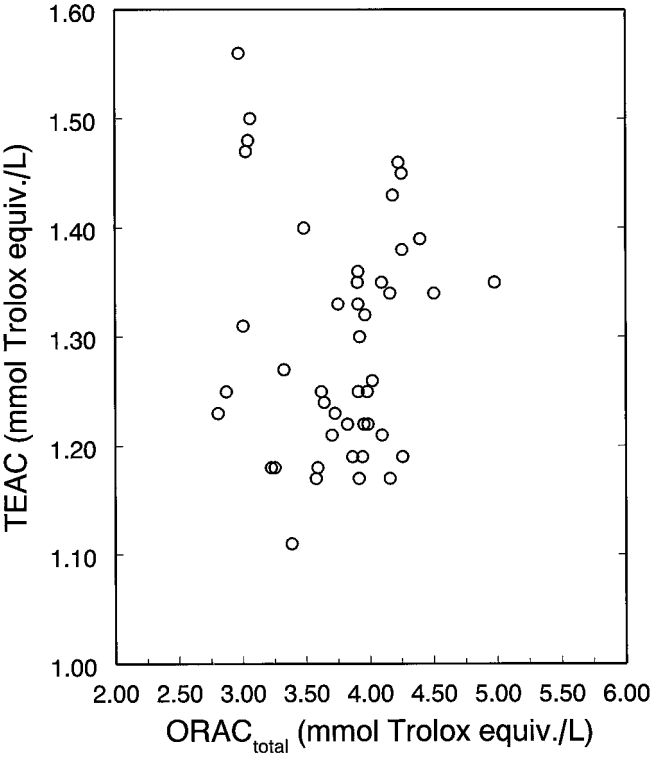

Fig. 2. Scatter plot of serum ORAC$_{total}$ and serum TEAC.
There is no significant correlation between serum ORAC$_{total}$ and serum TEAC.

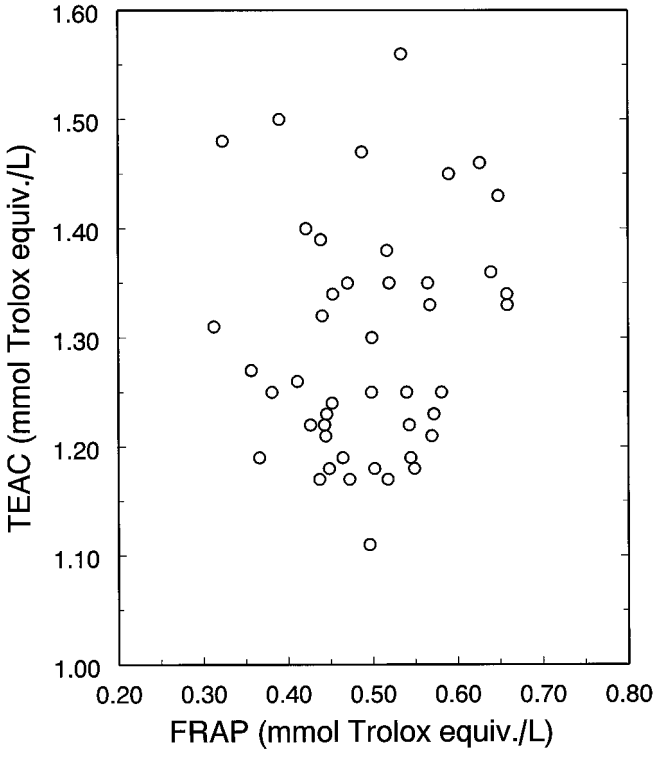

Fig. 3. Scatter plot of serum FRAP and serum TEAC.
There is no significant correlation between serum FRAP and serum TEAC.

ORAC$_{pca}$, and TEAC (62.7%, 52.3%, and 46.9%, respectively).

The dose-response characteristics of serum in the ORAC, FRAP, and Randox-TEAC assays are shown in Fig. 4. Excellent linear correlations between the measured antioxidant capacity and the volume of serum used in the measurement were found for all these antioxidant capacity assays. However, the dilution of serum samples produced up to a 15% increase in the Randox-TEAC values, as indicated by the intercept >0 for TEAC in Fig. 4. These results indicate that dilution of serum would not affect the final ORAC and FRAP values but would affect the final Randox-TEAC values.

The dose-response characteristics for the antioxidant capacity of GSH was examined in the ORAC, FRAP, and Randox-TEAC assays. The results are shown in Fig. 5. GSH is an antioxidant whose concentration is low in human serum. However, the functional part of GSH as an antioxidant is the SH group, which is also present in nonprotein antioxidants such as lipoic acid and some amino acids. Therefore, GSH was used here to represent the SH-group-containing nonprotein compounds. Linear dose-responses were found for GSH within the concentration ranges indicated in Fig. 5 for all of the three different antioxidant capacity assays. However, the antioxidant capacity of GSH determined with the ORAC, FRAP, and Randox-TEAC assays was 0.59, 0.02, and 0.66 mol Trolox equivalents/mol, respectively, indicating the inability of the FRAP assay to detect the antioxidant capacity of GSH.

Discussion

The results of this study demonstrated that the antioxidant capacity of human serum, measured as either ORAC (ORAC$_{total}$, ORAC$_{pca}$, or ORAC$_{ac}$), FRAP, or TEAC, was stable over an 8-week time period. These results suggest that the total antioxidant capacity of serum is part of a tightly regulated homeostatic mechanism. This was expected because an efficient antioxidant defense system is important in the control of oxidative stress caused by free radicals and other reactive species, which are continuously generated in the body.

The weak correlation between the ORAC and FRAP assay and the lack of correlation either between the ORAC and TEAC assay or between the FRAP and TEAC assay is not surprising because they use different technologies. Both ORAC and TEAC assays are inhibition methods: A sample is added to a free radical-generating system; the inhibition of the free radical action is measured; and this inhibition is related to the antioxidant capacity of the sample. The ORAC assay uses AAPH as a free radical-generating system, B- or R-PE as a sensitive target of the free radical attack, and an area-under-curve technique for the quantification *(9, 10)*. AAPH undergoes spontaneous decomposition and produces peroxyl radicals, with a rate primarily determined by temperature *(33)*. The analyzed antioxidant samples are not likely to affect this rate,

Table 2. The relative activity of individual serum antioxidants and their estimated contribution to serum $ORAC_{total}$,[a] $ORAC_{pca}$, $ORAC_{ac}$, FRAP, and TEAC.[b]

	Albumin[c]	Uric acid	α-Tocopherol	Ascorbic acid	Bilirubin	Other
Concentration, μmol/L	605 ± 34	257 ± 71	24.4 ± 4.9	42.3 ± 15.5	9.05 ± 2.84	
Activity[d] in						
ORAC	1.49	0.90	1.00	1.00	0.84	
FRAP	0.05	1.00	1.00	1.00	2.00	
TEAC	0.63	1.02	0.97	0.99	1.50	
Contribution (%) to						
$ORAC_{total}$	27.8	7.14	0.75	1.31[e]	0.28	62.72
$ORAC_{pca}$	0.00	39.2	0.00[f]	7.17	1.29	52.34
$ORAC_{ac}$	0.00	45.4[g]	6.07	10.5	1.89	36.14
FRAP	7.26	61.7	5.84	10.1[e]	4.34	10.76
TEAC	28.0	19.3	1.74	3.08[e]	1.00	46.88

[a] $ORAC_{total}$, ORAC analyzed using whole serum; $ORAC_{pca}$, ORAC measured using serum treated with perchloric acid; and $ORAC_{ac}$, ORAC measured using serum treated with acetone.

[b] All serum antioxidant parameters and individual antioxidants were measured in eight subjects biweekly over 8 weeks.

[c] Albumin was estimated using a ratio of albumin to total protein (610 mg/g).

[d] The activity was expressed as mol Trolox equiv./mol.

[e] The value may be overestimated because part of ascorbic acid may be oxidized during storage at −80 °C.

[f] α-Tocopherol was almost undetectable using an HPLC system in the PCA nonprotein fraction of the serum.

[g] 79% of the uric acid concentration was used in the calculation because acetone treatment decreased the uric acid level by 21%.

particularly when the chemical structure of AAPH and the very high molar ratio (>2000) of AAPH to an antioxidant sample are considered *(23)*. Therefore, the ORAC assay has high specificity; it measures the capacity of an antioxidant to directly quench free radicals. The area-under-curve technique combines both inhibition percentage and the length of inhibition time of free radical action by an antioxidant into a single quantity, which makes it superior to similar methods that use either an inhibition percentage at a fixed time or a length of inhibition time at a fixed inhibition percentage *(10)*.

The Randox-TEAC assay uses ABTS and H_2O_2 to generate ABTS radical cations in the presence of metmyoglobin as a peroxidase. The inhibition percentage of the ABTS radical cation formation by the added antioxidant sample at a fixed time point is quantified as the result *(7)*. Added antioxidants quench ABTS radical cations formed by the interaction of H_2O_2 with metmyoglobin. However, the direct interaction of an added antioxidant sample with the reagents cannot be totally excluded because the molar ratio of H_2O_2:metmyoglobin:ABTS:Trolox standard in the

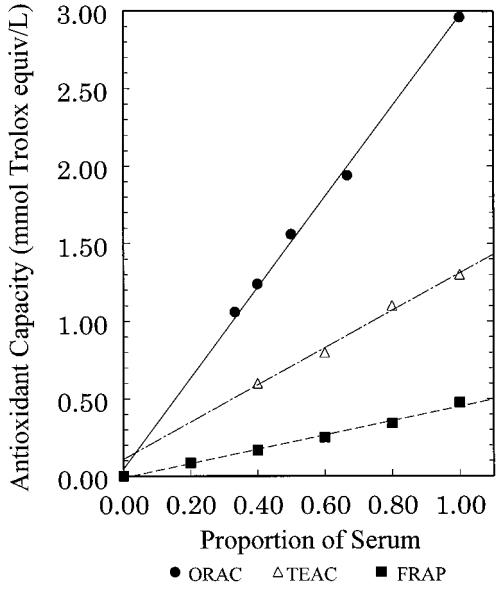

Fig. 4. Dose-response characteristics of serum in the ORAC, FRAP, and TEAC assays.

ORAC: $y = 2.923x + 0.047$, $r = 0.999$, $P < 0.01$; FRAP: $y = 0.462x - 0.011$, $r = 0.996$, $P < 0.01$; TEAC: $y = 1.22x + 0.11$, $r = 0.996$, $P < 0.01$.

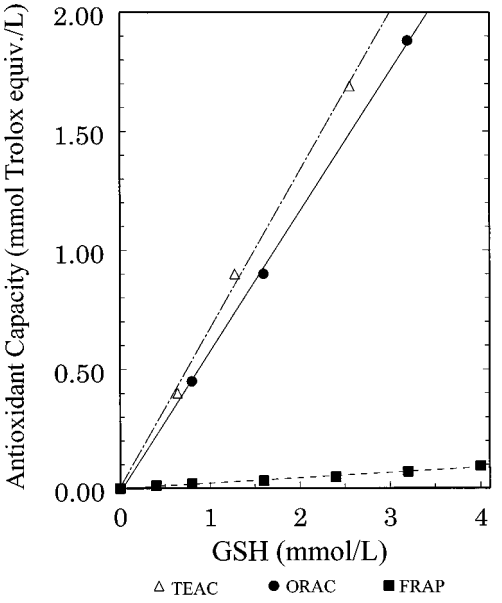

Fig. 5. Response to GSH in the ORAC, FRAP, and TEAC assays.

ORAC: $y = 0.589x - 0.016$, $r = 1.000$, $P < 0.01$; FRAP: $y = 0.022x - 0.00002$, $r = 0.994$, $P < 0.01$; TEAC: $y = 0.664x - 0.005$, $r = 0.998$, $P < 0.01$; the GSH concentrations used in the ORAC and FRAP assays were about 1/80 and 1/4, respectively, of those used in the TEAC assay.

Randox-TEAC assay is only 10.2:0.25:25:1. These interactions may reduce or even increase the production of radical species. This was supported by the increase in the Randox-TEAC values produced by the dilution of a serum sample and the nonlinear dose-response found for quercetin (data not shown) in the Randox-TEAC assay. Therefore, the specificity of the TEAC assay in measuring capacity of a sample to directly quench free radicals is not always guaranteed *(34)*. The difference in the kinetics of albumin and urate from Trolox in inhibiting the production of ABTS radical cations, as reported by Schofield and Braganza *(35)*, actually suggests that using an inhibition percentage at a fixed time without the consideration of the length of inhibition time is not suitable in those cases *(10)*. The inhibition time was fixed at 3 min in the Randox-TEAC assay, but 6 min was used in the original TEAC assay procedure *(7, 12)*.

The FRAP assay measures the ferric reducing ability of a sample. It is totally different from the ORAC and TEAC assays, because there are no free radicals or oxidants applied in the assay. The antioxidant capacity of an antioxidant against a free radical does not necessarily match its ability to reduce Fe^{3+} to Fe^{2+}. This is why the FRAP value for GSH was almost zero. The use of Fe^{2+} as a final indicator in the FRAP assay may cause problems when an analyzed antioxidant, such as ascorbic acid, not only reduces Fe^{3+} to Fe^{2+} but can also react with Fe^{2+} to generate additional free radicals.

The antioxidant capacity of human serum determined by the different methods followed the order of $ORAC_{total}$ > TEAC > FRAP. This can be explained by the overall underestimation of serum antioxidant capacity by the Randox-TEAC assay, a result of the use of the inhibition percentage at a fixed time and the ignorance of the length of inhibition time in the quantitation of the results. Both serum $ORAC_{total}$ and serum TEAC assays measure serum proteins (Table 2), but they were not correlated in this study. In addition, there was no correlation between serum TEAC and serum FRAP. We examined the dose-response several times in the serum TEAC assay and found that the dilution of serum produced up to a 15% increase in the TEAC values (the instructions for Randox-TEAC assay kits indicate that the increase can be up to 20%). The FRAP assay does not measure substantial amounts of serum proteins, including albumin, which was considered an advantage in measuring serum antioxidant capacity *(11)*. We also found that the correlation between $ORAC_{pca}$ and FRAP (data not shown) was better than that between $ORAC_{total}$ and FRAP (Fig. 1) because of the removal of protein in the $ORAC_{pca}$ assay. However, in this study, the FRAP value of GSH was found to be very low, indicating that when the FRAP assay does not measure serum proteins, it also excludes the low molecular weight SH-group-containing antioxidants, such as lipoic acid and some amino acids.

Serum $ORAC_{total}$, $ORAC_{pca}$, and $ORAC_{ac}$ were analyzed using the same technology, i.e., the ORAC assay, but with different sample preparation procedures. PCA and acetone were used in the serum ORAC assay to reflect the antioxidant capacity from the nonprotein antioxidants in the serum, which may be more important than the antioxidant capacity from the whole serum or from the protein fraction of the serum under some conditions. PCA preserves water-soluble antioxidants, including ascorbic acid. The acetone extract contains both lipid- and water-soluble antioxidants, which include α-tocopherol, ascorbic acid, and most of the uric acid. Ammonium sulfate was used previously by us to separate the nonprotein antioxidants in the serum *(9, 10)*. Ammonium sulfate was not used in the present study because we found that it was difficult to completely precipitate the serum proteins from some older subjects.

The commercial kits for the TEAC assay were expensive; the reagent cost per sample in the Randox-TEAC assay was ~9 times that in the ORAC assay. However, the ORAC assay requires a fluorescence detector and takes 70 min to complete. The FRAP assay is both simple and relatively inexpensive.

In summary, there was a weak but significant linear correlation between serum ORAC and serum FRAP. There was no correlation either between serum ORAC and serum TEAC or between serum FRAP and serum TEAC. The effect of dilution on the serum TEAC value and the use of inhibition percentage at a fixed time without considering the length of inhibition time in the quantitation of results makes the Randox-TEAC assay the least desirable. The FRAP assay does not measure the SH-group-containing antioxidants. The ORAC assay has good specificity and responds to numerous antioxidants. By using different extraction techniques in the ORAC assay, one can remove serum proteins and also make a gross differentiation between aqueous and lipid-soluble antioxidants.

We acknowledge the cooperation of Carol Lammi-Keefe of the Nutritional Science Department of the University of Connecticut, Storrs, CT, in facilitating the collaboration in this research project. We acknowledge the technical assistance of N. Lischner, J. McEwen, C. M. O'Brien, and the staffs of the Nutrition Evaluation Laboratory and the Metabolic Research Unit at the HNRCA, who were also critical to the completion of this study.

References

1. Ames BN, Shigensaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci U S A 1993;90:7915–22.
2. Halliwell B, Gutteridge JMC, Cross CE. Free radicals, antioxidants, and human disease: where are we now? J Clin Lab Med 1992; 119:598–620.
3. Yu BP. Cellular defenses against damage from reactive oxygen species. Physiol Rev 1994;74:139–62.
4. Wayner DDM, Burton GW, Ingold KU, Locke S. Quantitative mea-

surement of the total, peroxyl radical-trapping antioxidant capacity of human blood plasma by controlled peroxidation. FEBS Lett 1985;187:33–7.
5. Glazer AN. Phycoerythrin fluorescence-based assay for reactive oxygen species. Methods Enzymol 1990;186:161–8.
6. Ghiselli A, Serafini M, Maiani G, Assini E, Ferro-Luzzi A. A fluorescence-based method for measuring total plasma antioxidant capability. Free Rad Biol Med 1994;18:29–36.
7. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci 1993;84:407–12.
8. Whitehead TP, Thorpe GHG, Maxwell SRJ. Enhanced chemiluminescent assay for antioxidant capacity in biological fluids. Anal Chim Acta 1992;266:265–77.
9. Cao G, Alessio HM, Cutler RG. Oxygen-radical absorbance capacity assay for antioxidants. Free Rad Biol Med 1993;14:303–11.
10. Cao G, Verdon CP, Wu AHB, Wang H, Prior RL. Automated oxygen radical absorbance capacity assay using the COBAS FARA II. Clin Chem 1995;41:1738–44.
11. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996;239:70–6.
12. Rice-Evans C, Miller NJ. Total antioxidant status in plasma and body fluids. Methods Enzymol 1994;234:279–93.
13. Rice-Evans C, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Rad Res 1995;22:375–83.
14. Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, Rice-Evans C. Polyphenolic flavonols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. Arch Biochem Biophys 1995; 322:339–46.
15. Miller NJ, Sampson J, Candeias LP, Bramley PM, Rice-Evans CA. Antioxidant activities of carotenes and xanthophylls. FEBS Lett 1996;384:240–2.
16. Miller NJ, Castelluccio C, Tijburg L, Rice-Evans C. The antioxidant properties of theaflavins and their gallate esters–radical scavengers or metal chelators? FEBS Lett 1996;392:40–4.
17. Pieri C, Maurizio M, Fausto M, Recchioni R, Marcheselli F. Melatonin: a peroxyl radical scavenger more effective than vitamin E. Life Sci 1994;55:PL271–6.
18. Testa R, Testa I, Manfrini S, Bonfigli AR, Piantanelli L, Marra M, Pieri C. Glycosylated hemoglobin and fructosamines: does their determination really reflect the glycemic control in diabetic patients? Life Sci 1996;59:43–9.
19. Miller JW, Selhub J, Joseph JA. Oxidative damage caused by free radicals produced during catecholamine autoxidation: protective effects of O-methylation and melatonin. Free Rad Biol Med 1996;21:241–9.
20. Lin YL, Juan IM, Chen YL, Liang YC, Lin JK. Composition of polyphenols in fresh tea leaves and associations of their oxygen-radical-absorbing capacity with antiproliferative actions in fibroblast cells. J Agric Food Chem 1996;44:1387–94.
21. Sharma HM, Hanna AN, Kauffman EM, Newman HAI. Effect of herbal mixture *student rasayana* on lipoxygenase activity and lipid peroxidation. Free Rad Biol Med 1995;18:687–97.
22. Yu J, Fox JG, Blanco MC, Yan L, Correa P, Russell RM. Long-term supplementation of canthaxanthin does not inhibit gastric epithelial cell proliferation in *Helicobacter mustelae*-infected ferrets. J Nutr 1995;125:2493–500.
23. Cao G, Sofic E, Prior RL. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. Free Rad Biol Med 1997;22:749–60.
24. Wang H, Cao G, Prior RL. The oxygen radical absorbing capacity of anthocyanins. J Agric Food Chem 1997;45:304–9.
25. Cao G, Sofic E, Prior RL. Antioxidant capacity of tea and common vegetables. J Agric Food Chem 1996;44:3426–31.
26. Wang H, Cao G, Prior RL. Total antioxidant capacity of fruits. J Agric Food Chem 1996;44:701–5.
27. Cao G, Giovanoni M, Prior RL. Antioxidant capacity decreases during growth but not aging in rat serum and brain. Arch Gerontol Geriatr 1996;22:27–37.
28. Cao G, Giovanoni M, Prior RL. Antioxidant capacity in different tissues of young and old rats. Proc Soc Exp Biol Med 1996;211: 359–65.
29. Cao G, Cutler RG. High concentrations of antioxidants may not improve defense against oxidative stress. Arch Gerontol Geriatr 1993;17:189–201.
30. Halliwell B, Gutteridge JMC. The definition and measurement of antioxidants in biological systems. Free Rad Biol Med 1995;18: 125–6.
31. Martin A, Foxall T, Blumberg JB, Meydani M. Vitamin E inhibits low-density lipoprotein-induced adhesion of monocytes to human aortic endothelial cells in vitro. Arterioscl Thromb Vasc Biol 1997;17:429–36.
32. Behrens WA, Madere R. A highly sensitive high-performance liquid chromatography method for the estimation of ascorbic and dehydroascorbic acid in tissues, biological fluids and foods. Anal Biochem 1987;165:102–7.
33. Niki E. Free radical initiators as source of water- or lipid-soluble peroxyl radicals. Methods Enzymol 1990;186:100–8.
34. Strube M, Haenen GRMM, Van Den Berg H, Bast A. Pitfalls in a method for assessment of total antioxidant capacity. Free Rad Res 1997;26:515–21.
35. Schofield D, Braganza JM. Shortcomings of an automated assay for total antioxidant status in biological fluids. Clin Chem 1996; 42:1712–4.