

Original Research

Dried Fruits: Excellent *in Vitro* and *in Vivo* Antioxidants

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Key words: fruits, figs, polyphenols, antioxidants

Objective: The goal of this work is to determine the amount and quality of phenol antioxidants in dried fruits and compare them with the corresponding fresh fruits; to compare the nutrients in fresh and dried fruits; to determine if figs are a source of *in vivo* antioxidants when eaten.

Methods: Commercial samples of dried fruits and fresh fruits were compared in the *in vitro* studies using a colorimetric method to measure phenolic antioxidants. The quality of the antioxidants was measured by inhibition of lower density lipoprotein oxidation. Ten normal free-living subjects were tested in the human study. Fasting subjects were given 40 g of figs with or without a carbonated beverage and the plasma antioxidant capacity was measured for six hours using the trolox equivalent antioxidant capacity assay.

Results: Dates have the highest concentration of polyphenols among the dried fruits. Figs and dried plums have the best nutrient score among the dried fruits, and dates among the fresh fruits. Processing to produce the dried fruit significantly decreases the phenols in the fruits on a dry weight basis. Compared with vitamins C and E, dried fruits have superior quality antioxidants with figs and dried plums being the best. Fig antioxidants can enrich lipoproteins in plasma and protect them from subsequent oxidation. Figs produced a significant increase in plasma antioxidant capacity for 4 hours after consumption, and overcame the oxidative stress of consuming high fructose corn syrup in a carbonated soft drink.

Conclusion: Dried fruits and especially figs, are a convenient and superior source of some nutrients, but in the American diet amount to less than 1% of total fruit consumed. Figs are *in vivo* antioxidants after human consumption. The findings suggest that dried fruits should be a greater part of the diet as they are dense in phenol antioxidants and nutrients, most notably fiber.

INTRODUCTION

Fruit and vegetable consumption have been shown by multiple epidemiology studies to reduce the risk of chronic diseases such as cancer [1], heart disease [2], and stroke [3]. A U.S. study showed that diabetes in women was inversely associated with fruit and vegetable consumption [4]. Additionally, there is an inverse relationship between fruit and vegetable intake and blood pressure [5]. The National Cancer Institute and the National Research Council recommend at least five servings of fruits and vegetables. Yet only 17% of 15,000 Americans surveyed in schools, work sites, churches, or nutrition clinics in 1999, ate the recommended number of servings [6]. Initially it was assumed that vitamins C, E, and the provitamin beta carotene were responsible for the health benefits of fruits and vegetables. Recent supplementation studies with pure vitamin

E and beta carotene have cast doubt on this hypothesis. A 4-year study of vitamin E supplementation to more than 9,000 patients at high risk for cardiovascular disease produced no significant benefit [7]. An 8-year prospective study of more than 44,000 healthy men in the Health Professionals Follow-up Study, found no benefits of vitamin C or E for decreasing the risk of stroke [8].

Abundant evidence exists for a beneficial effect of phenol antioxidants (this includes monophenols and polyphenols) on heart disease and cancer. It is hypothesized that phenol antioxidants are the agents in fruits that are at least partly responsible for these protective effects. An early step in atherosclerotic lesion formation is oxidation of low (LDL) and very low (VLDL) density lipoproteins [9]. Oxidation of DNA is an important event in carcinogenesis. The consumption of a diet high in fruits has been shown to decrease oxidative damage of

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Financial Information: There is no current financial interest from this work. The work was funded in part by a grant from the California Fig Advisory Board.

Journal of the American College of Nutrition, Vol. 24, No. 1, 44–50 (2005)

Published by the American College of Nutrition

DNA bases in humans [10]. Fruit and vegetable components, polyphenolic antioxidants such as flavonoids, are protective for heart disease [11] and for stroke in the Netherlands' Zutphen Study [12]. A Finnish study of 10,000 men and women over a 20-year period showed a decreased risk of lung cancer which was attributed to flavonoid intake [13]. The intake of apples, the major dietary source of flavonols in this population, was inversely associated with lung cancer incidence. This benefit was not due to the intake of the antioxidant vitamins. A diet rich in fruits and vegetables has recently been found favorably to affect serum antioxidant capacity and protect against lipid peroxidation [14]. Human consumption of 200 g of strawberries has been shown to increase the plasma antioxidant capacity [15]. This could not be explained solely by the vitamin C from the strawberries.

More than 4000 phenol compounds (flavonoids, monophenols and polyphenols) are found in vascular plants [16]. Phenols found in plants have been considered anti nutrients due to their potential to "tie up" nutrients such as copper and iron and especially proteins, and prevent them from being absorbed. Their function in the plant is to prevent free radical damage to proteins, carbohydrates, lipids and DNA caused by UV light from the sun. We have shown that phenols, especially those with multiple phenolic groups, are better antioxidants than the well-known antioxidant vitamins [17]. Fruits also possess these high quality antioxidants as measured by the oxidation of LDL + VLDL, an *in vitro* model of the initiation of atherosclerosis [18]. In fact, fruits have significantly higher quality phenol antioxidants than vegetables [18].

We hypothesize that the higher quantity and quality of antioxidants in fruits, are responsible for the generally greater benefit of fruits compared with vegetables in epidemiology studies. Since dried fruits are a more concentrated form of fruit, we have investigated the quantity and quality of the antioxidants in these fruits.

METHODS

Measurement of Quantity and Quality of Antioxidants

Three to five samples of each of the fresh and dried fruits (apricots, cranberries, dates, figs, raisins, and pitted prunes aka dried plums) were obtained from local supermarkets. Fresh figs and dates (not usually consumed by the public) were obtained direct from the grower. Fresh Mission and Calimyrna figs were analyzed on the day of receipt from DeBenedetto Farms (Fresno, CA). Fresh Deglit Noor and Zahidi dates were kindly supplied by California Redi-Date (Thermal, CA) and Leja Farms (Coachella, CA). Dried figs of the same variety were provided by the California Fig Advisory Board (Fresno, CA). We used our sample preparation and analysis method already described for fruits [18]. This involved cleaning, weighing,

cutting and freeze-drying. This was followed by weighing and then dissolving the lyophilizate in methanol/water to extract the free phenols. Another sample was dissolved in 50% methanol/50% water/1.2M hydrochloric acid, in order to extract the free and glycosylated phenols and hydrolyze the glycosylated phenols, to give an extract for total phenol analysis. Extracts from sulfite-containing fruits were treated with acetaldehyde before analysis. The quantity of free and total phenols in the fruit extracts was then analyzed by an oxidation-reduction colorimetric assay using the Folin-Ciocalteu reagent [Sigma Chemical Co., St. Louis, MO], with catechin as the standard, and measurement at 750 nm after reaction for 10 minutes.

The quality of the phenol antioxidants in the extracts was measured by determining the IC_{50} (the concentration to inhibit the oxidation by 50%) of the pooled free phenol extracts for each fruit. Fruit extracts were added at phenol concentrations ranging from 0.2 to 2 μ M (as measured by the Folin assay) to a fixed protein concentration of human LDL+VLDL that was isolated by heparin-agarose affinity column [19]. This was followed by oxidation with cupric ions at physiological pH and temperature under standardized conditions. The oxidation mixture was reacted with thiobarbituric acid and the products measured by fluorometry in butanol. A native sample without cupric ions and a blank sample without an antioxidant were also analyzed. All samples were analyzed in duplicate. IC_{50} was determined graphically from the sigmoidal-shaped curve of antioxidant concentration (μ M) vs. % inhibition. For comparison purposes the reciprocal $1/IC_{50}$ was used so that the higher number corresponds to better quality antioxidants. This method has recently been described in detail [19].

To illustrate the ability of phenols from fruits to incorporate in LDL+VLDL in plasma and protect these lipoproteins from subsequent oxidation was measured in Mission figs. Plasma was spiked with the water extract of Mission figs (25–100 μ M phenols) along with a control, and equilibrated for one hour at 37°C. The LDL+VLDL was isolated by affinity column and oxidized with cupric ions under standard conditions as described previously. The kinetics of conjugated diene formation was determined at 234 nm and the lag time (where the initial slow oxidation line converges with the rapid oxidation line) was measured [19]. The longer the lag time compared to the control, the greater the amount of lipoprotein-bound antioxidants present from the fig.

Human Study

Ten normal subjects (5 males and 5 females, aged 25 to 58) participated in the study with informed consent. They were non-smoking individuals not taking any prescription medicine or vitamin supplements. After an overnight fast, they consumed either 240 ml of Sprite™ alone (control), or with 40 g (one serving size) of dried Mission and Calimyrna figs in a random crossover design. Venipuncture was conducted at 0, 1, 2, 4, and 6 hours after consumption. Lunch was eaten after the 4-hour

draw and consisted of a plain low-fat bagel and 240 ml of Sprite™. Plasma was stored at -70°C until assay for antioxidant activity using the trolox equivalent antioxidant capacity (TEAC) kit from Randox Laboratories (San Diego, CA) with trolox as the standard [20]. Statistical comparisons used a paired t-test with $p < 0.05$ considered significant.

RESULTS

Quantity and Quality of Antioxidants

We compared 6 fresh fruits with their corresponding dried versions with respect to nutrients for a serving size of the fruit. Data is taken from the U.S. Department of Agriculture [21] and displayed in Table 1. The lower the nutrient score, the better the source of nutrients. The best nutrient score of the fresh fruits belonged to dates, which in fact had the best nutrient score of all the fruits. The nutrient score was significantly better for the dried fruits compared to the fresh varieties ($p < 0.001$ by a chi-square test). Apricots and figs had the best nutrient scores among the dried fruits.

In Table 2 the data for phenol antioxidants in fresh and dry fruits are shown. The total phenols are always greater than the free phenol due to the liberation of phenolic groups following hydrolysis. Dates had highest concentration of both free and total phenols in both the fresh and dried version. On a fresh weight basis the average free polyphenols for fresh fruits was 205 ± 129 mg/100 g and 390 ± 163 mg/100 g for dried fruits. Total polyphenols averaged 731 ± 907 mg/100 g for fresh and 815 ± 600 mg/100 g for dried fruits. We next wanted to determine how processing affected the phenol content which is illustrated in Fig. 1 for total phenols in fruits on a dry weight basis. The average was 3730 ± 4489 mg/100 g for the fresh fruits and only 910 ± 646 mg/100 g for the dried varieties.

Table 2. Quantity of Free and Total Phenol Antioxidants in Fresh Fruits and Dried Fruits Based on Fresh Weight (Mean \pm Standard Deviation), Rankings Are in Parentheses

Fruit	Free phenols (mg catechin per 100 g) fresh weight	Total phenols (mg catechin per 100 g) fresh weight
Fresh		
Apricots	128 \pm 12 (5)	266 \pm 23 (4)
Cranberries	613 \pm 44 (2)	663 \pm 15 (2)
Dates*	257 \pm 4 (1)	2546 \pm 29 (1)
Figs*	360 \pm 123 (3)	486 \pm 218 (3)
Grapes (green)	14 \pm 10 (6)	195 \pm 116 (6)
Plums	157 \pm 15 (4)	228 \pm 20 (5)
Dried		
Apricots	333 \pm 13 (3)	402 \pm 28 (6)
Cranberries	607 \pm 223 (1)	870 \pm 277 (2)
Dates	400 \pm 86 (5)	1959 \pm 244 (1)
Figs	256 \pm 24 (4)	320 \pm 37 (5)
Raisins	194 \pm 13 (6)	551 \pm 46 (4)
Dried Plums	551 \pm 64 (2)	788 \pm 75 (3)

* Two varieties were used.

Quality of the dried fruits and vitamin antioxidants are shown in Fig. 2. The higher the value of $1/IC_{50}$, the better the quality of antioxidants present. Figs and prunes have the highest quality among the dried fruits. Dried fruits are significantly better antioxidants than the vitamins as a group, $p < 0.03$. We can also compare the quality of 3 fruits (fresh and dried) cranberry, grape, and plum. Fresh fruit data has been published [22]. Respectively for fresh vs dried, the quality is 1.16 and 2.38 for cranberries, 1.33 and 3.45 for grapes, and 1.43 and 4.38 for plums. The difference is almost significant, $p = 0.053$ using a paired t test.

Another way to measure both the quality of the antioxidants and their ability to incorporate into lipoproteins was to measure the lipoprotein-bound antioxidant activity [19]. A water extract

Table 1. Water and Selected Nutrient Values and Total Nutrient Scores for 6 Fresh and Corresponding Dried Fruits (Mean \pm Standard Deviation), Rankings Are in Parentheses

Fruit (serving size)	Water % our assay	Energy (kcal)	Total Fiber (g)	Potassium (mg)	Calcium (mg)	Iron (mg)	Nutrient Score*
Fresh							Fresh
Apricots (165 g)	82 \pm 4	79	3.3 (5) [†]	427 (3)	21.4 (4)	0.6 (6)	18 (2)
Cranberries (95 g)	74 \pm 1	44	4.4 (2)	81 (11)	7.6 (10)	0.2 (11)	34 (5)
Dates# (138 g)	80 \pm 1	390	5.5 (1)	932 (1)	67.0 (1)	1.4 (2)	5 (1)
Figs# (100 g)	83 \pm 1	74	2.9 (7)	232 (8)	35.0 (3)	0.4 (9)	27 (3)
Grapes (green) (154 g)	84 \pm 2	109	1.5 (12)	285 (5)	16.9 (8)	0.4 (8)	33 (4)
Plums (66 g)	87 \pm 3	36	2.0 (10)	114 (10)	3.0 (11)	0.1 (12)	43 (6)
Dried							Dried
Apricots (40 g)	16 \pm 3	95	3.6 (4)	551 (2)	18.0 (7)	1.9 (1)	14 (1)
Cranberries (40 g)	2 \pm 1	123	2.3 (9)	16 (12)	4.0 (12)	0.2 (10)	43 (6)
Dates (40 g)	8 \pm 3	110	3.0 (6)	261 (7)	12.8 (9)	0.5 (7)	29 (5)
Figs (40 g)	11 \pm 5	102	4.9 (2)	285 (6)	57.6 (2)	0.9 (4)	14 (1)
Raisins (40 g)	7 \pm 1	120	1.6 (11)	300 (3)	19.6 (6)	0.8 (5)	25 (4)
Dried Plums (40 g)	22 \pm 1	96	2.8 (8)	298 (4)	20.4 (5)	1.0 (3)	20 (3)

[†] Ranking of fruit for individual nutrients and for nutrient score.

* Sum of ranking scores for individual nutrients. A lower number indicates a higher (better) ranking.

Average of two varieties obtained direct from growers.

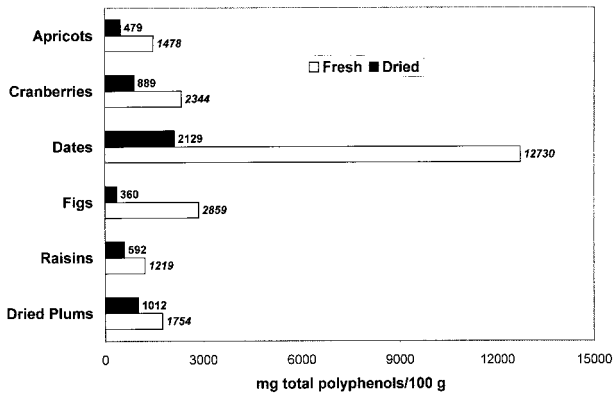


Fig. 1. Comparison of quantity of total polyphenols in fresh and the corresponding dried fruit on a dry weight basis.

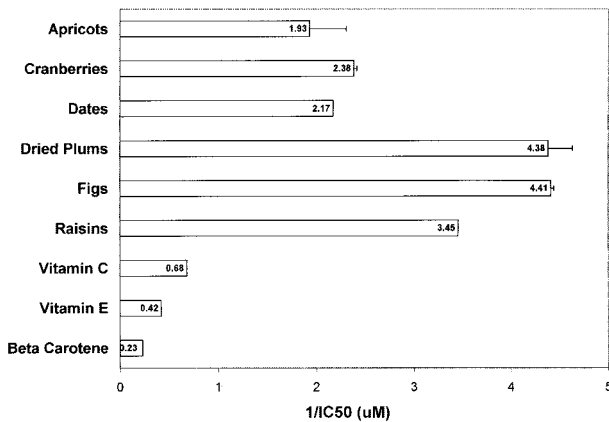


Fig. 2. Comparison of quality of antioxidants of vitamins and dried fruits (mean \pm standard deviation). Dried fruit extracts from several sources of dates and raisins were pooled prior to assay.

of unhydrolyzed Mission figs was spiked in plasma at different concentrations and the LDL+VLDL isolated after equilibration and oxidized with cupric ion under standard conditions. The lag time of oxidation was measured graphically and the results shown in Fig. 3. As can be seen, the extract of fig phenols increased the lag time in a dose-response manner. The changes in lag time were significantly different from the control at 50 and 100 μ M fig polyphenols ($p < 0.05$).

Human Study

The results of the human single serving size consumption of figs on plasma antioxidant capacity is shown in Fig. 4. The initial fasting values were 1.21 ± 0.03 mM for the control and 1.32 ± 0.18 mM for the fig group, and were not significantly different. The consumption of the Sprite™ in the control group produced a significant decrease in plasma antioxidant capacity that reached its minimum level after one hour. The figs produced a net plasma antioxidant capacity increase for 4 hours after consumption with the Sprite™. There was a significant difference between the figs with Sprite™ and the Sprite™

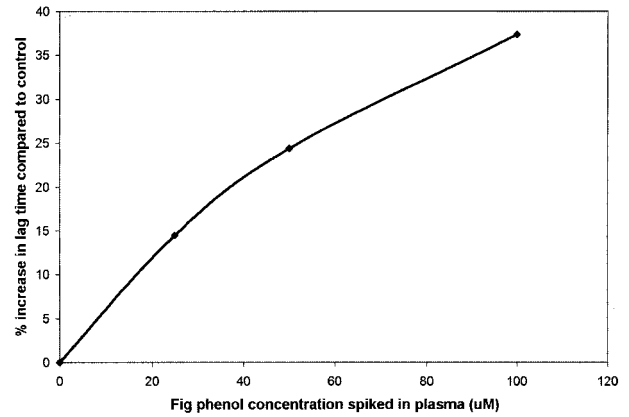


Fig. 3. Lipoprotein-bound antioxidant activity of fig phenols after spiking in plasma at different concentrations and determination of the lag time of LDL+VLDL oxidation.

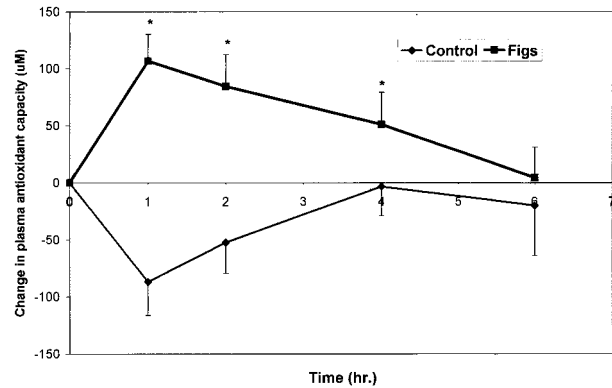


Fig. 4. The average change in plasma antioxidant capacity after consumption of either control (Sprite™) or figs and Sprite™ (mean \pm standard error of the mean) for 10 subjects, * $p < 0.01$ by a paired t-test.

alone at 1, 2 and 4 hours after consumption ($p < 0.01$). This cannot be attributed to vitamin C or E in the fig since the levels are 2 mg or less for the amount of figs ingested. The figs provided *in vivo* antioxidants which overcame the pro-oxidant effect of sugar consumption.

DISCUSSION

We used the Folin-Ciocalteu reagent to measure phenolic antioxidants in the fruit extracts. This colorimetric method has interference from sulfites in preserved dried fruits and acetaldehyde was added to remove sulfites. In addition we determined that fructose (the major monosaccharide in fruits) did not interfere with the assay. Amadori products are formed from amino acids reacting with sugars during the drying process to produce dried fruits [22]. Hydroxymethyl furfuraldehyde (HMF) is formed from Maillard reactions of the Amadori products during the fruit processing. For instance in dried

plums it is present at levels averaging 22 mg/100 g fresh weight [23]. HMF did not react with the Folin reagent. Figs had by far the greatest amount of fiber among the dried fruits. Dried Apricots were ranked second in potassium and first iron, providing 16 and 11%, respectively. Dried figs were ranked number 2 in two nutrients, dietary fiber and calcium and had the best nutrient score among the dried fruits (tied with apricots). Figs provide 25% of the recommended daily value for fiber (25 g), and 6% of the calcium (1000 mg).

Dates have the highest concentration of total phenols among the dried fruits, and cranberries have the highest based on free phenols. The extremely high levels may be due to the extreme temperatures and greater exposure to sunlight for dates compared to the other fruits, i.e. an adaptive response of the plant. The % free phenols increased after drying, averaging 49.6% for fresh and 59.7% for dried but the difference was not significant. It is interesting to compare the dried plum and raisin data using our Folin assay, to that previously published using another method for antioxidant activity of foods, namely the oxygen radical absorbance capacity (ORAC) method. They found that dried plums had twice the amount of free antioxidants compared to raisins [24]. Our method found that dried plums had 2.5 times more free phenol antioxidants than raisins. We found fresh dates at 2546 mg/100 g are also much higher in total phenols than cranberries which have the highest total phenols among the fresh fruits, 678 mg/100 g [18]. Dates are also higher than kidney beans which have the highest phenols of the vegetables, 923 mg/100 g [25]. Dried fruits were significantly higher in total phenols than 20 fresh fruits, 815 vs. 173 mg/100 g, respectively, $p < 0.005$ [18].

Processing was examined with the data in Fig. 1. Figs are the only fruit for which we had samples of both fresh and dried fruit from the same orchard. On a dry weight basis and compared to the fresh fruit, the dried fig lost 87% of total phenols. All 6 fruits averaged an 84% decline in total phenols as a result of drying, which was a significant decrease, $p < 0.02$ by the student's *t* test. Based on dry weight, prunes [23] and raisins [26] also showed a decrease in some individual phenols during the processing of the fresh fruit. It appears that some of the polyphenols in the fresh fruit are destroyed or converted to non-antioxidant forms during the drying process [23, 27]. Compared on a fresh weight basis processing produced a 90% increase in free polyphenols ($p < 0.05$) as a result of the loss of water. There was an 11% increase in total polyphenols as a result of drying.

Antioxidant quality is a measure of the effectiveness of the antioxidant(s) present as a pure compound or a mixture. A comparison of antioxidant quality is shown in Fig. 2 and is measured by $1/IC_{50}$. The higher the value, the better the quality. Phenol antioxidants' quality in the dried fruits was compared to the antioxidant vitamins in Fig. 2 and found to be significantly higher, $p < 0.02$. Also quality seems to improve during the drying process. The IC_{50} for fresh cranberries, green grapes and

plums are 1.16, 1.32, and 1.42, respectively. For dried cranberries, raisins and dried plums, the values are 2.38, 3.45, and 4.38, respectively. This is a significant difference, $p < 0.05$. Quality of antioxidants is an important factor since following consumption of fruits polyphenols are usually present in plasma at concentrations not exceeding 10 μM . The average IC_{50} was $\sim 0.3 \mu\text{M}$ for the dried fruits in Fig. 2. Thus the polyphenols from dried fruits can be potent antioxidants at physiological concentrations.

In the plasma spiking study the fig phenols caused an increase in the lag-time in an almost linear manner as shown in Fig. 3. This was also seen with pure polyphenols and beverages. [28]. From data in Fig. 3, the concentration of fig phenols to increase the lag time 50% compared to the control was calculated to be 143 μM . This value is similar to that found for dried plum juice, 111 μM [28]. The ability to bind lipoproteins is one of the mechanisms by which supplementation of vitamin E is hypothesized to decrease the risk of heart disease [29], and by which consuming polyphenols may be beneficial with respect to reducing the risk of heart disease.

In the human study, plasma antioxidant capacity was measured by TEAC. As seen in Fig. 4, the Sprite™ caused a temporal decrease in antioxidant capacity which reached its lowest level at 1 hour after consumption of the soft drink. Figs produced a significant increase in plasma antioxidant capacity for 4 hours after consumption, and overcame the oxidative stress of consuming high fructose corn syrup in a carbonated soft drink. This increase cannot be attributed to vitamin C since less than 2 mg of the vitamin are found in the dried figs [21]. Although we did not measure glucose, this time corresponds to the maximum plasma glucose as seen for a glucose tolerance test [30]. Post-prandial hyperglycemia has been shown to produce oxidative stress *in vivo* [30, 31]. Thus the high fructose corn syrup in Sprite™ (38 g of carbohydrates/12 oz), or by analogy any soft drink containing sugar, is a pro-oxidant, causing a temporary oxidative stress that lasts for 4 hours after drinking. In fact, up to 4 hours after consumption, there is a significant negative correlation between the control and fig antioxidant capacity (Pearson correlation coefficient -0.9999 , $p < 0.01$), suggesting the same kinetics for both the oxidative stress (prooxidant) from the soft drink and the antioxidant effect from consuming the figs. The antioxidant effect of the figs overcame the oxidative stress of the sugars in the soft drink and the net effect was an increase in antioxidant capacity.

In a previous study, the largest increase in plasma antioxidant capacity (TEAC) after drinking one serving of green tea by normal subjects compared to the baseline was 4% [32]. In our study after eating figs the TEAC increase was 9% (Fig. 4). A fairer calculation would be the difference between the TEAC for figs plus Sprite™ vs. Sprite™ alone at 1 hour. This calculates as an 18% increase. Thus figs produce a much greater increase in plasma antioxidant capacity than green tea. In a pilot study with a single subject, 8 fig bars (Fig Newtons™) were consumed, and produced a maximal increase in plasma

TEAC of 5%. Besides figs, the only other dried fruit to demonstrate human *in vivo* antioxidant activity was raisins from white grapes [33]. In this [33]. In this study subjects consumed 3 g/kg (about 5 servings) of raisins for a week and the fasting plasma antioxidant capacity increased 8%.

Dried fruits are not commonly eaten in the US diet. In 2000 only 2.3 lb/year (corresponding to a mere 3 g/day) of the 6 dried fruits combined, were consumed. This compares with 126 lb/year of fresh fruits and 152 lb/year of processed fruit [34]. Currently we Americans are not following the 5 A Day plan recommended by the National Cancer Institute. In 1999, the mean intake, calculated from 7 study centers and 15,060 subjects were 3.6 servings of fruits and vegetables, with only 1.5 servings/day of fruit [6]. Dried fruits are an excellent source of fiber, nutrients, and complex carbohydrates and thus a good choice for a snack. For example, eating dried fruits produced a significantly lower insulin response than consuming processed snacks containing sugar such as chocolate candy, or cola along with potato chips [35].

There have been a large number of beneficial studies indicating that increased consumption of fruits and vegetables decreases the risk of many cancers. Most of the decreased risk has been attributed to the vegetables rather than the fruits. However an inverse association with lung cancer has been shown for fruit consumption but not vegetables [36]. The benefits of consuming dried fruit are difficult to determine by observational studies due to the low consumption of dried fruit. Two recent case-controlled epidemiology studies in the US found no association of prostate cancer risk and fruit consumption [37, 38]. This was confirmed in two additional studies; a Dutch study and an American study, which in contrast found an inverse association of this cancer with dried fruit consumption [39, 40]. Potent *in vitro* suppressors of cancer cell proliferation have been found in a fraction isolated from figs [41].

CONCLUSION

Dried fruits have a greater nutrient density, greater fiber content, increased shelf life, and significantly greater phenol antioxidant content compared to fresh fruits. The quality of the antioxidants in the processed dried fruit is the same as in the corresponding fresh fruit. Phenols in dried fruit may be important antioxidants as demonstrated by the *in vivo* antioxidant effect from eating a serving size of figs. Therefore, more dried fruits should be recommended to be added to the diet by dietitians and nutritionists.

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Received June 23, 2003; revision accepted March 3, 2004