Human Nutrition and Metabolism

Catechin Is Present as Metabolites in Human Plasma after Consumption of Red Wine[1,2]

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ABSTRACT   Flavonoids are components of fruits, vegetables and wines. An abundance of flavonoids in the diet is correlated with reduced heart disease mortality, suggesting that they act as protective nutrients. However, little is known about the absorption and metabolism of flavonoids after normal foods are consumed. This study measured the levels of one abundant flavonoid, (+)-catechin, and its metabolites in plasma after five male and four female volunteers consumed 120 mL of red wine (RW) one day and de-alcoholized red wine (DRW) on a separate day. Each wine sample contained 35 ± 1 mg catechin (mean ± SEM). Plasma levels of catechin and its metabolite 3′-O-methylcatechin (3′MC) were determined by gas chromatography-mass spectrometry (GC-MS) of the trimethylsilylated (TMS) derivatives. Glucuronide and sulfate conjugates were determined after enzymatic hydrolysis. Before RW or DRW consumption, plasma levels of catechin, 3′MC and all conjugates were <2 nmol/L. After 1 h, average levels of catechin, 3′MC and all conjugates increased to 91 ± 14 nmol/L (RW) and 81 ± 11 nmol/L (DRW). At 1 h, 21 ± 1% of the metabolites were methylated and <2% of catechin and 3′MC were unconjugated. Catechin was present as both a sulfate conjugate and a conjugate containing both glucuronide and sulfate residues. 3′MC was present primarily as a glucuronide conjugate. At every time point, catechin was present almost exclusively as metabolites, and these levels were independent of ethanol. Therefore, if flavonoids are protective nutrients, the active forms are likely to be metabolites, which are far more abundant in plasma than the forms that exist in foods.   J. Nutr. 129: 1662–1668, 1999.

KEY WORDS: • wine • catechin • flavonoids • metabolites • humans

Epidemiologic studies demonstrate that wine consumption is associated with reduced incidence of heart disease and wine consumption provides an explanation for the "French Paradox" (Criqui and Ringel 1994, Gronbæk et al. 1995, Renaud and de Lorgeril 1992, St. Leger et al. 1979). Red wine is a rich source of flavonoids; many believe that flavonoids are at least partially, if not mainly responsible for the protective effects of wine (Frankel et al. 1993, Renaud and Ruff 1995). Other flavonoid-rich foods such as onions, green tea and fruits are also associated with reduced heart disease mortality (Criqui and Ringel 1994, Hertog et al. 1993 and 1995). Although some still believe that the beneficial effects of wine consumption are due largely to ethanol (Criqui and Ringel 1994, Rimm et al. 1996), the fact that beer and spirits do not offer the same protective effect suggests otherwise (Criqui and Ringel 1994, Gronbæk et al. 1995, Klatsky and Armstrong 1993, Sasaki and Kesteloot 1994, St. Leger et al. 1979).

Animal and in vitro studies have shown that flavonoids can prevent artherogenesis and thrombosis (Demrow et al. 1995, Fitzpatrick et al. 1993, Hayek et al. 1997, Renaud and Ruff 1995, Xu et al. 1998). Flavonoids have been shown to be powerful antioxidants for LDL and can modify eicosinoid synthesis (Schramm et al. 1997, Vinson et al. 1995). Despite numerous investigations, the mechanisms by which flavonoids affect human health and disease are not well understood. Progress in this area has been limited by the lack of quantitative data on the absorption, metabolism and distribution of flavonoids after consumption of normal foods. This investigation focuses on the metabolism of (+)-catechin, one of the most abundant flavonoids in foods and wine (Kühnau 1976).

Many factors affect the fate of orally ingested nutrients. The dose is obviously important, but the matrix in which it is delivered is also important. Ethanol, in particular, can affect absorption or modify metabolism or excretion (Hobbs et al. 1996, Weiner et al. 1988). Other components in foods, including other flavonoids, can also affect the fate of specific substances (Fuhr and Kummert 1995). This study addresses the metabolism of catechin after consumption of a very common but complex source, red wine, and the effect of alcohol on the circulating levels of catechin and its metabolites in adult humans.

The metabolism of catechin has been studied in humans and animals at doses that exceed the amounts found in wine
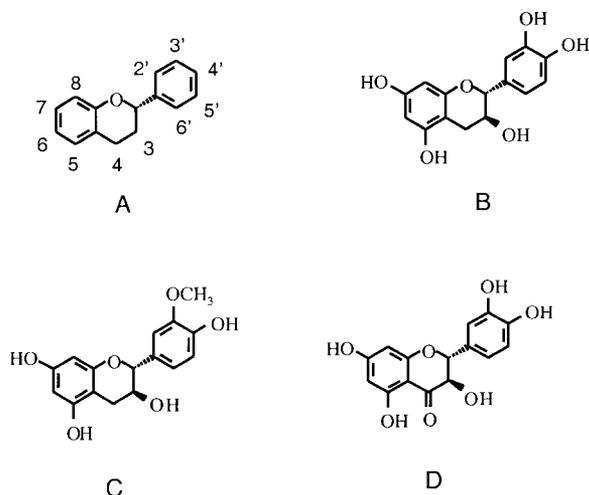
FIGURE 1 Numbering of (*A*) the flavonoid ring system and the structures of (*B*) (+)-catechin, (*C*) 3′-*O*-methyl-(+)-catechin and (*D*) the internal standard, (+)-taxifolin.

and foods by a factor of at least 50 (Das 1971, Das and Sothy 1971, Giles and Gumma 1973, Hackett et al. 1982 and 1983, Macheix et al. 1990, Shaw and Griffiths 1980, Shaw et al. 1982, Wermeille et al. 1983). One such study showed that after ingestion of 2 g of purified [^{14}C] catechin, plasma radioactivity peaked at a level corresponding to 40 μmol/L catechin. At that time, most of the catechin was present as metabolites, whereas 12.5% was in unmetabolized form (Hackett et al. 1983). Analysis of urine showed that 60% of catechin was methylated at the 3′ position and that catechin and 3′-*O*-methylcatechin (3′MC)[4] were present as sulfate and glucuronide conjugates (Wermeille et al. 1983).

In this study, the levels of catechin and its metabolites were determined in human plasma after the consumption of red wine (RW) and de-alcoholized red wine (DRW). Analysis after enzymatic treatment of plasma allowed the determination of individual conjugate forms of both catechin and 3′MC. The plasma levels of the specific classes of metabolites were used to estimate key pharmacokinetic variables, including the half-lives of absorption (A$_{1/2}$) and elimination (E$_{1/2}$), the areas under the curves (AUC), and the maximum concentrations (C$_{max}$) and times (T$_{max}$). This information will provide a foundation to assess the nutritional significance of catechin and other flavonoids in foods and wine.

MATERIALS AND METHODS

Chemicals and reagents. (+)-Catechin was obtained from Fluka Chemicals (Ronkonkoma, NY) and (+)-taxifolin was purchased from Apin Chemicals (Abingdon, UK). The 3′ and 4′ methyl ethers of catechin and its isomer, epicatechin, were synthesized, purified and unambiguously identified as previously described (Donovan et al. 1999). The structures of these compounds are shown in **Figure 1**. The derivatizing reagent, *N*,*O*-bis (trimethylsilyl)-trifluoroacetamide (BSTFA), was purchased from Pierce (Rockford, IL). β-Glucuronidase (G-0251; EC 3. 2. 1. 31), sulfatase (S-9754; EC 3. 1. 6. 1) and D-saccharic acid 1,4-lactone were purchased from Sigma Chemical (St. Louis, MO). All solvents and reagents were Fisher HPLC or

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Optima grade; other reagents were purchased from either Fisher (Pittsburgh, PA) or Aldrich (Milwaukee, WI).

Preparation and analysis of wine. Ethanol was removed from a 1996 *Cabernet Sauvignon* (100%, produced by University of California at Davis) by rotary evaporation (<40°C) to 50% of the original volume. The wine was divided into two samples one of which was normalized to the original volume with water (DRW); the other was normalized with water and ethanol to contain 103 g/L ethanol (RW). The wine was prepared in this manner to ensure that the RW and DRW samples were identical with the exception of ethanol content. The levels of catechin and other low-molecular-weight phenolic compounds (i.e., ≤1 kDa) in both wine samples were determined on eight different occasions throughout the study period with the use of HPLC analysis as previously described (Waterhouse et al. 1998).

Subjects and study design. Nine volunteers (five men, four women; age, 29 ± 3 y; weight, 78 ± 4 kg) consumed a flavonoid-free diet containing no fruits, vegetables, chocolate, coffee or tea for 2 d before the experiment. After a 14-h fast, each subject consumed, in random order, 120 mL of either RW or DRW providing 35 ± 1 mg catechin. A crossover design was used, allowing subjects to serve as their own controls. The subjects returned to consume the alternate wine sample after at least 2 d had passed with diet modification. Blood was drawn into 10-mL EDTA-containing tubes (Becton Dickinson, Franklin Lakes, NJ) before wine consumption and at 0.5, 1, 2, 3, 4 and 8 h after wine consumption. Plasma was prepared and 1.0-mL aliquots were mixed with 25 μL phosphate buffered ascorbic acid (PBA, 200 g/L ascorbic acid, 0.4 mol/L NaH$_2$PO$_4$, pH 3.6), flushed with nitrogen and frozen at −70°C. The clinical protocol was approved by the Human Subjects Committee at the University of California at Davis.

Analysis of plasma. A modified version of a previously described method was used for plasma analysis (Luthria et al. 1997). To prevent oxidation of the analytes, all solvents and reagents were deoxygenated by purging with nitrogen and were kept on ice. The 1.0-mL aliquots of plasma were thawed and an additional 100 μL PBA was added. The plasma was divided into two 500-μL samples for duplicates and 250 μL of a 0.6 mol/L CaCl$_2$ solution was added to each sample. Taxifolin was used as an internal standard, and 17 μL of a 2165 nmol/L solution in PBA was added to all plasma samples to achieve a final concentration of 82 nmol/L. The plasma was incubated at 37°C in a shaking water bath for 45 min in nitrogen-flushed tubes containing 100 U sulfatase and 2500 U β-glucuronidase dissolved in 120 μL water. Individual forms were incubated without enzymes (free), glucuronidase only (glucuronide conjugates), or sulfatase and 0.2 mol/L saccharic acid 1,4-lactone (sulfate conjugates). After incubation, the plasma was extracted with 1 mL methylene chloride and 500 μL water, vortexed for 1 min and centrifuged at 4500 × g for 10 min at 4°C. The aqueous supernatant was removed, and the remaining portion was extracted a second time with 750 μL water. The aqueous extracts were mixed and extracted twice with ethyl acetate (first with 2.0 mL, then with 1.5 mL). The combined ethyl acetate extracts were passed through anhydrous sodium sulfate packed in a Pasteur pipette, dried under nitrogen gas and then redissolved in 20 μL pyridine and derivatized with 30 μL BSTFA at 65–75°C for 2 h. The samples were then analyzed by gas chromatography with mass spectrometry detection (GC/MS) on a Hewlett Packard 6890 GC equipped with a 5973 quadrupole MS using a DB-5 capillary GC column (30 m × 0.25 mm i.d., 0.25-μm film thickness, J & W Scientific, Folsom, CA). Helium was used at 0.7 mL/min; injections of 2 μL were made, and the column temperature was programmed from 100°C (3 min) to 260°C at 30°C/min and held at this temperature for 30 min. Major fragmentation ions and molecular ions for catechin (m/z = 355,650), 3′MC (m/z = 310,592) and taxifolin (m/z = 368,664) were monitored in selective ion monitoring mode using a dwell time of 100 ms/channel.

A partial ion chromatogram of plasma after wine consumption is shown in **Figure 2**. For quantitation, standard curves were prepared in blank plasma (i.e., plasma containing undetectable levels of catechin and 3′MC). Catechin and 3′MC were added to the blank plasma at final concentrations of 0, 7, 17, 35, 69, 172 and 258 nmol/L catechin and 0, 7, 16, 33, 66, 164 and 246 nmol/L 3′MC. Taxifolin was added to all plasma samples at a final concentration of 82 nmol/L. The
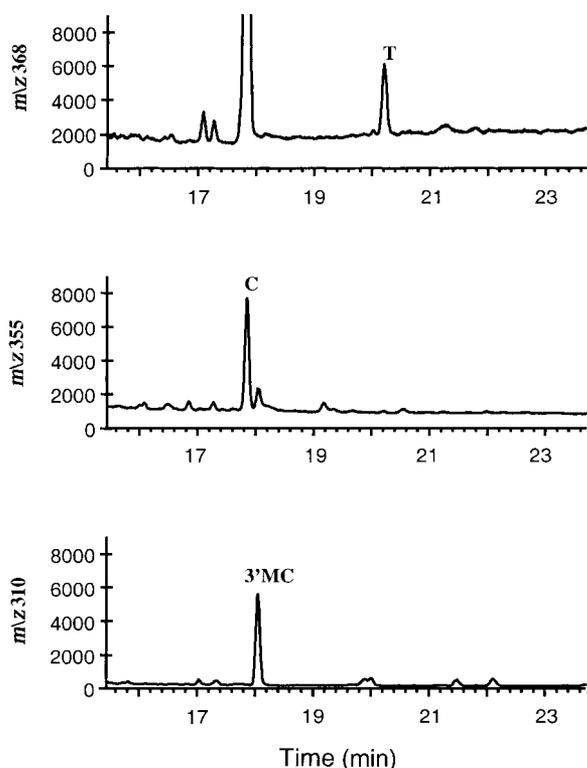
FIGURE 2 A partial ion GC/MS chromatogram of a human plasma sample 1 h after red wine consumption by one male volunteer. (+)-Catechin (C), 3′-O-methyl (+)-catechin (3′MC) and the internal standard (+)-taxifolin (T) are labeled at the fragmentation ions used for quantitation.

plasma was then incubated, extracted and analyzed exactly as described for the samples. Calibration curves were established by plotting the ratio of the peak areas of the analyte (catechin or 3′MC) to the internal standard (taxifolin) vs. the amount of analyte (nmol/L) added to blank plasma. The standard curves for catechin and 3′MC were linear and had average r^2 values of 0.997 and 0.994, respectively. The slopes of four standard curves prepared over 10 d varied by <5% (CV). The limit of detection (signal to noise ratio = 3) was 2.0 nmol/L for catechin and 0.7 nmol/L for 3′MC.

Determination of metabolite classes. Total unmethylated catechin and total 3′MC were determined after the hydrolysis of both the glucuronide and sulfate conjugates in duplicate for all subjects at all time points. The sulfate conjugates of catechin and 3′MC were also determined in duplicate for all subjects at all time points. The free, glucuronide, sulfate, and total amount of unmethylated catechin and 3′MC were determined in duplicate using the pooled plasma of three subjects (2 men, 1 woman; age, 31 ± 3 y; weight, 74 ± 1 μ/kg) at an early time point (1 h) and a late time point (3 or 4 h) after RW and DRW consumption. Conjugates containing both the glucuronide and sulfate residues were estimated in these subjects by subtracting the free, sulfate and glucuronidated forms from the totals.

Pharmacokinetic and statistical analysis. All pharmacokinetic variables were generated as described by Gibaldi (1991). Modeling was performed using a least-squares nonlinear regression pharmacokinetics program (WinNonlin Version 1.1; Scientific Consulting, Cary, NC). Curves were generated for each subject using a one-compartment model without a lag time and uniform weighting of all time points. In all cases, this model provided an adequate fit of the experimental data (r^2 = 0.93 ± 0.2); the half-lives of absorption ($A_{1/2}$) and elimination ($E_{1/2}$) and the maximum concentrations (C_{max}) and times (T_{max}) were generated from the model. The $E_{1/2}$ was also determined by plotting the natural logarithm of the plasma concentrations vs. time at 3, 4 and 8 h and calculated using the formula $E_{1/2} = -\ln(2)/\beta$, where β is slope of the linear regression. The AUC were determined using the linear trapezoidal rule and by integration of the curve generated by the model after extrapolation to infinity. Values are expressed as means ± SEM of the nine subjects unless otherwise noted. Data were analyzed with the use of the paired t test with differences considered significant at $P < 0.05$.

RESULTS

Phenolic composition of the wine. Each subject consumed 34 ± 1 and 35 ± 1 mg of catechin in the 120-mL RW or DRW sample, respectively. The red wine used in this study was chosen for its high catechin content; however, because only 120 mL of wine was administered, the dose of catechin was only 0.46 ± 0.03 mg/kg body weight. Additionally, each subject consumed 69 ± 5 mg of other low-molecular-weight phenolics that were mostly flavonoids. The phenolic composition of the test wine is reported in **Table 1**. There were no significant differences in the phenolic compositions of the RW and DRW. HPLC analysis also confirmed that the test wine was free of taxifolin, allowing the use of taxifolin as an internal standard in the plasma analysis.

Plasma levels of total catechin and 3′-O-methylcatechin. Before consumption of RW or DRW, plasma levels of total catechin (catechin, 3′MC and all conjugates) were <2 nmol/L. Plasma levels of total catechin after RW consumption are shown in **Figure 3**. Maximum levels of total catechin were 91 ± 14 nmol/L (RW) and 81 ± 11 nmol/L (DRW) at 1h. Maximum levels varied from 50 to 176 nmol/L (RW) and 46 to 139 nmol/L (DRW) among individuals. At 8 h, levels of total catechin were <25% of the maximum levels. There were no significant differences between levels after RW and DRW consumption at any time point in any subject.

The methylated metabolites of catechin were <1 nmol/L before wine consumption. Plasma levels of the methylated metabolites after RW consumption are shown in Figure 3. Maximum levels of methylated metabolites were 19 ± 4 and 18 ± 4 nmol/L 1 h after RW and DRW consumption, respectively. At 1 h, methylated metabolites accounted for 20 ± 2% (RW) and 22 ± 3% (DRW) of total catechin. At 4 h, methylated metabolites accounted for only 14 ± 2% (RW) and 12 ± 1% (DRW) of total catechin. Differences in total levels of methylated metabolites were observed between individuals, and maximum levels ranged from 10 to 49 nmol/L.

TABLE 1

Levels of phenolic compounds in each 120 mL red wine (RW) and de-alcoholized red wine (DRW) sample[1]

Compound	RW	DRW
	mg	
Catechin	34 ± 1	35 ± 1
Flavan-3-ols[2]	51 ± 2	53 ± 2
Hydroxycinnamates	4 ± 1	5 ± 1
Flavonols	12 ± 1	11 ± 1
Anthocyanins	34 ± 2	34 ± 2
Benzoic acids	2 ± 1	2 ± 1
Taxifolin[3]	ND	ND
Total[4]	103 ± 1	104 ± 1

[1] Values are means ± SEM, n = 8. In the analysis by HPLC, there were no significant differences between RW and DRW.

[2] Does not include catechin; includes epicatechin, dimers and trimers of flavan-3-ols, and their gallate esters.

[3] ND, not detected, ≤0.1 mg.

[4] Total low-molecular-weight (≤1 kDa) phenolic compounds as measured by HPLC.
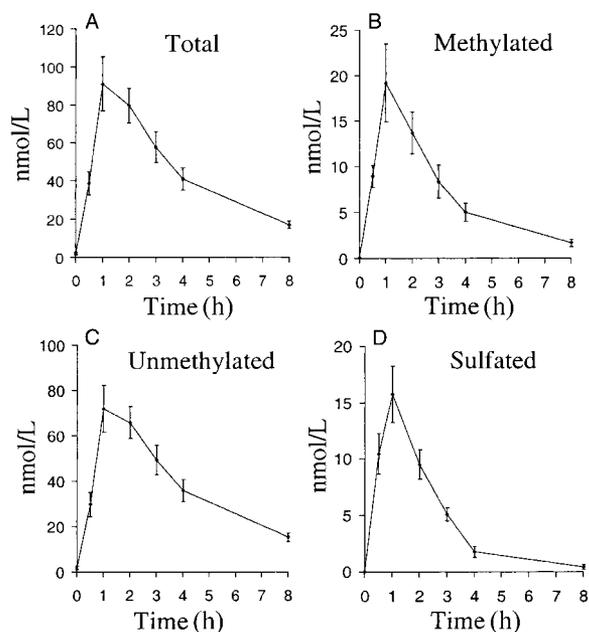
FIGURE 3 Plasma levels of total catechin, methylated metabolites, unmethylated metabolites and sulfated metabolites after consumption of 120 mL red wine (RW) by five male and four female volunteers. (*A*) Total is the sum of catechin, 3′-O-methylcatechin (3′MC) and all conjugates; (*B*) methylated is 3′MC and all conjugates; (C) unmethylated is the sum of catechin and all unmethylated metabolites; (*D*) sulfated is the amount of unmethylated catechin in sulfated form; methylated metabolites were not detected in sulfated form. Values are plotted as means ± SEM, *n* = 9. There were no significant differences in plasma levels of any metabolites at any time point after consumption of RW and de-alcoholized red wine (DRW).

however, no significant differences were observed between the levels of methylated metabolites after RW and DRW consumption.

Glucuronide and sulfate conjugation. Levels of free catechin and free 3′MC in plasma were extremely low (1–2 nmol/L) and were near the limit of detection in this study. Free catechin was detected in plasma at 1 h but accounted for <2% of the unmethylated metabolites. At 3–4 h, no free catechin was detected in plasma. Free 3′MC accounted for an average of 7% of the methylated metabolites at 1 h and 5% of the methylated metabolites at 3–4 h. The sum of free catechin and free 3′MC, however, did not account for >2% of total catechin in plasma. Levels of free catechin or free 3′MC were similar after consumption of RW and DRW.

Sulfate conjugates of the methylated metabolites were not detected at any time point. Conversely, the unmethylated metabolites were present as sulfate conjugates as shown in Figure 3. Maximum levels were present 1 h after wine consumption and ranged from 7 to 28 nmol/L. Thirty minutes after RW and DRW consumption, 40 ± 6% and 45 ± 7%, respectively, of the unmethylated metabolites were sulfated. However, in both cases, only 5% were sulfated at 4 h, and the elimination half-life of the sulfate conjugates was <1 h (**Table 2**). Because sulfated metabolites were eliminated relatively quickly, sulfated metabolites accounted for <16% of the AUC of the unmethylated metabolites and <11% of the AUC of total catechin and metabolites. There were no significant differences in the amounts of sulfate conjugates after RW and DRW.

The proportions of individual conjugate forms of catechin and 3′MC in plasma at an early (1 h) and later (3–4 h) time point after RW and DRW consumption are shown in **Figure 4**. At both time points, a large proportion of the unmethylated metabolites were present as conjugates containing both the glucuronide and sulfate residues, and the proportion of metabolites in this form increased over time. The proportion of unmethylated catechin present purely in glucuronidated form and purely in the sulfated form both decreased. Fewer changes were observed in the proportions of the methylated metabolites. 3′MC was present primarily in the glucuronidated form

TABLE 2

Pharmacokinetic values for catechin metabolites in plasma of five male and four female volunteers after red wine (RW) and de-alcoholized red wine (DRW) consumption[1]

Form of catechin in plasma[2]		$E_{1/2}$ model[3]	$E_{1/2}$ calc.[3]	$A_{1/2}$ model[4]	T_{max} model[4]	C_{max} model[4]	C_{max} calc.[4]	AUC model[5]	AUC calc.[5]
				h			*nmol/L*		*nmol · h · L^{-1}*
Total	RW	1.8 ± 0.3	3.1 ± 0.2[b]	0.8 ± 0.1	1.5 ± 0.1	75 ± 12	91 ± 14	382 ± 41	361 ± 43
	DRW	2.2 ± 0.3	3.8 ± 0.3[a]	0.7 ± 0.2	1.5 ± 0.2	78 ± 9	81 ± 11	371 ± 44	353 ± 34
Methylated	RW	1.0 ± 0.1	2.4 ± 0.3	0.7 ± 0.1	1.2 ± 0.1	16 ± 3	19 ± 4	54 ± 10	57 ± 11
	DRW	1.0 ± 0.1	2.4 ± 0.3	0.7 ± 0.1	1.1 ± 0.2	16 ± 3	18 ± 4	48 ± 7	50 ± 7
Unmethylated	RW	2.0 ± 0.3	3.2 ± 0.3[c]	0.8 ± 0.1	1.6 ± 0.1	67 ± 7	72 ± 10	336 ± 36	303 ± 34
	DRW	2.6 ± 0.3	4.1 ± 0.4[d]	0.8 ± 0.2	1.6 ± 0.3	64 ± 7	63 ± 9	363 ± 39	304 ± 28
Sulfated	RW	0.9 ± 0.1	0.9 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	16 ± 2	16 ± 3	39 ± 4	37 ± 5
	DRW	0.7 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	1.0 ± 0.1	15 ± 2	18 ± 3	42 ± 6	34 ± 6

[1] Values were generated by a one-compartment pharmacokinetic model or calculated from plasma levels and are expressed means ± SEM, *n* = 9.
a,b,c,d Means not sharing a superscript for each form of catechin are significantly different, *P* < 0.05.
[2] Total is sum of catechin, 3′-O-methylcatechin and all conjugates. Methylated is sum of 3′-O-methylcatechin and all conjugates. Unmethylated is sum of catechin and all unmethylated metabolites. Sulfated is unmethylated catechin in sulfated-only form; methylated metabolites were not detected in sulfate-only form.
[3] Half-life of elimination from plasma generated by the model and calculated with plasma levels at 3, 4 and 8 h.
[4] Half-life of absorption ($A_{1/2}$) was generated by the model; maximum concentration (C_{max}) was generated by the model and calculated from plasma levels at 1 h; time at maximum concentration was generated by the model (T_{max}).
[5] Areas under the curves (AUC) were calculated by the trapazoidal rule and by integration of the curve generated by the model after extrapolation to infinity.
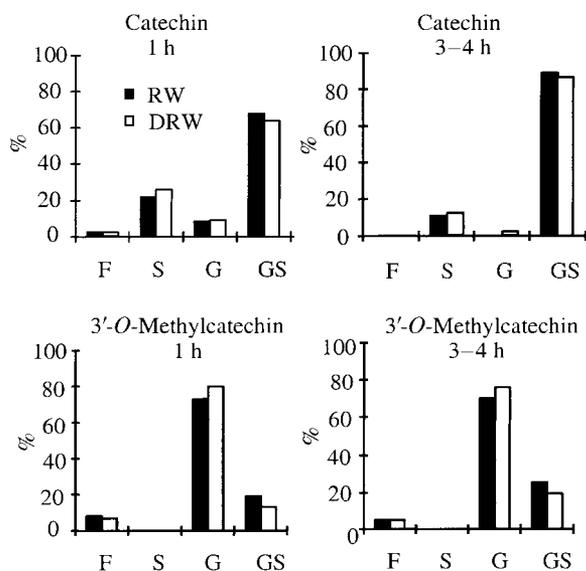
FIGURE 4 Proportions of the conjugate forms of catechin and 3′-O-methylcatechin (3′MC) 1 h and 3–4 h after consumption of 120 mL red wine (RW) or de-alcoholized red wine (DRW). Forms are free (F), sulfated (S), glucuronidated (G) or glucuronidated and sulfated (GS). Data were obtained from pooled plasma of three subjects. Values are means of duplicate analyses in which the difference between determinations was <2% for the free form and <5% for all other forms.

at both time points. Although 3′MC did not exist in a form with only a sulfate, small amounts of a form containing both the glucuronide and sulfate conjugates were detected at both time points.

DISCUSSION

Progress in the evaluation of the role of flavonoids in human health has been hindered by the lack of sensitive methodology to determine plasma levels of flavonoids and their metabolites after consumption of the doses that occur in foods. Previous methods to determine the levels of catechin in plasma have not reported levels of the individual conjugate forms or the levels of the methylated metabolites. Future studies of flavonoid metabolism must use methodology that is capable of separating and detecting all potentially important classes of metabolites. In this study, a sensitive GC/MS method, coupled with selective enzymatic hydrolysis, revealed the levels of specific classes of catechin metabolites in plasma after consumption of red wine.

A selective detection method was crucial in this study because many other flavnoids may be present in plasma after red wine consumption. Mass spectrometry detection of specific ions limited the possibility of interferences by other flavonoids because most other flavonoids have different molecular weights and fragmentation ions. One flavonoid in wine, epicatechin, has the same molecular weight and fragmentation ions as catechin; however, epicatechin and its 3′and 4′ methyl ethers, were chromatographically separated from the analytes and were not interferences in this analysis.

Maximum levels of total catechin varied from 50 to 176 nmol/L 1 h after consumption of a single serving of red wine containing 35 mg catechin. The levels of total flavonoids in plasma are likely to be higher due to the presence of other flavonoids in wine and foods. For example, a recent study of plasma flavan-3-ols from green tea showed that glucuronidated and sulfated metabolites ranged from 648 to 1066 nmol/L 1 h

after consumption of 235 mg flavan-3-ols (Lee et al. 1995). Another study showed that plasma levels of glucuronide and sulfate conjugates of epicatechin reached 700 nmol/L after consumption of 164 mg epicatechin from chocolate (Richelle et al. 1999). Other classes of flavonoids are also reported to be absorbed from foods, including flavonols (de Vries et al. 1998a and 1998b, Hollman et al. 1995, 1996 and 1997), anthocyanins (Lapidot et al. 1998, Paganga and Rice-Evans 1997), flavanones (Fuhr and Kummert 1995) and isoflavones (Watanabe et al. 1998). Furthermore, the subjects in this study were consuming flavonoid-restricted diets before wine consumption, and it is not known how this affects the absorption or metabolism of catechin. If flavonoid-containing foods are consumed before full elimination of flavonoids from the previous meal, progressively higher plasma levels could result.

Catechin from red wine was present almost exclusively as metabolites in plasma. Conversely, in earlier studies in which humans consumed gram quantities of catechin, substantial amounts of catechin were present in free form in plasma (Giles and Gumma 1973, Hackett et al. 1983). This may be because enzymes responsible for catechin metabolism were unable to metabolize the entire dose before circulation when administered at gram levels. Here, plasma 3′MC was also conjugated, and free catechin and 3′MC accounted for <2% of total catechin metabolites. These results indicate that immediately after absorption, whether in epithelial cells or the liver, catechin was efficiently metabolized to polar conjugates before systemic circulation.

The increased extent of conjugation observed here would be expected to accelerate elimination and explains why plasma levels did not accumulate as much as in earlier studies with gram-level doses. In addition, larger, more extensively conjugated metabolites are more likely to be excreted by bile (Rozman and Klaassen 1996). A large proportion of the metabolites observed in this study were both glucuronide and sulfate conjugates, and it is likely that these metabolites were excreted, at least to some extent, by this mechanism. It is even possible that some metabolites were excreted by bile before any systemic circulation. These compounds may be reabsorbed and recirculated if intestinal flora are efficient at removing the sulfate and glucuronide residues.

An unexpected result of this study was that 3′MC was not present in a form containing only a sulfate conjugate. The lack of sulfated 3′MC in plasma was consistent in all samples and independent of the ethanol content of the wine. Several earlier reports identified sulfated 3′MC as a urinary metabolite in humans as well as animals after gram-level doses (Hackett et al. 1982 and 1983, Shaw et al. 1982). Clearly, the composition of metabolites changes quantitatively and qualitatively when the dose is much smaller, and previous research with gram-level doses cannot be used to determine the metabolites or pharmacokinetics after consumption of foods. To be relevant to human health, future studies of flavonoid metabolism must be conducted with amounts that occur in foods.

The different classes of catechin metabolites were eliminated at different rates. There was a notable difference in the half-lives of elimination generated by the two methods (calculated vs. model, Table 2); however, both methods indicated that the sulfated metabolites were the most quickly eliminated, followed by the methylated metabolites. The longest half-life was obtained for the unmethylated metabolites, indicating that certain metabolites in that class were eliminated more slowly.

The half-lives of total catechin as well as the unmethylated metabolites were longer after consumption of DRW, but the results were significant only when calculated using the plasma

levels at 3, 4 and 8 h, and not when generated by the pharmacokinetic model (Table 2). These results indicate that alcohol may affect the elimination of certain metabolites; however, the effect was not large enough to create significant differences in the T_{max}, C_{max} or AUC. In addition, there were no significant differences in the amounts of the individual conjugate forms of catechin after RW and DRW. The effects of consumption of larger quantities of ethanol and chronic consumption of ethanol remain to be established.

The biological significance of catechin metabolites in plasma at these levels requires further research. The vast majority of in vitro studies have been performed with the forms of flavonoids that exist in foods, not plasma. These studies have shown that unmetabolized flavonoids have potent antioxidant activities for isolated LDL in the 200-5000 nmol/L range even in the presence of very high levels of oxidants (Frankel et al. 1995, Paganga et al. 1996, Vinson et al. 1995). Very little is known about the biological activities of flavonoid metabolites. Recently, Morand et al. (1998) reported that glucuronide and sulfate conjugates of quercetin, another flavonoid, were nearly four times more powerful than the water-soluble vitamin E derivative, trolox, but several times less powerful than quercetin itself at inhibiting LDL oxidation in vitro. The concentration of glucuronides and sulfates that inhibited 50% of oxidation was only 170 nmol/L. If flavonoid metabolites have biological activities even close to what has been observed with free flavonoids, their presence in plasma would be highly significant.

In conclusion, catechin was present almost exclusively as metabolites in plasma after consumption of red wine. The position of methylation was established in this study, but the positions of glucuronide and sulfate conjugation still must be addressed. If all flavonoids are extensively conjugated after absorption from foods, it is the metabolites, and not the forms that exist in foods, that require further attention. In addition to identification of their complete structures and occurrence in biological fluids and tissues, flavonoid metabolites must be investigated for chemical and biochemical properties related to the prevention of chronic diseases.

ACKNOWLEDGMENT

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