Naringenin from Cooked Tomato Paste Is Bioavailable in Men\textsuperscript{1}

**ABSTRACT**
Naringenin has been shown to exert antioxidant, cholesterol-lowering and antiinflammatory activities, as well as an indirect modulation on the metabolism of many xenobiotics. It is one of the most abundant polyphenols in tomato. Given the widespread consumption of tomato (Lycopersicum esculentum) and tomato-based products, this study was designed to determine whether plasma levels of naringenin were detectable in five men after consumption of a test meal containing 150 mg of cooked tomato paste. Naringenin intake with the test meal was 3.8 mg. Blood was drawn from fasting subjects and 2, 4, 6, 8 and 24 h after the meal. To compare the results with a control, without tomato paste, a control meal was administrated to the same subjects 2 wk later. Analyses were performed using high-performance liquid chromatography coupled with a CoulArray electrochemical detector. The peak plasma concentration was 0.12 ± 0.03 \( \mu \text{mol/L} \) \( 2 \) h after the meal. Unconjugated naringenin was not detected. Naringenin was not detected in plasma at any time after consumption of the control meal. In addition to naringenin, we detected rutin and chlorogenic acid in tomato paste, but these polyphenols and their derivatives (quercetin and caffeic acid) were not detected in plasma at any time. To the best of our knowledge, this is the first study demonstrating naringenin bioavailability in humans after consumption of a meal containing cooked tomato paste. J. Nutr. 132: 3349–3352, 2002.

**KEY WORDS:** naringenin • tomato • bioavailability • men

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Naringenin (4',5,7-trihydroxyflavanone, molecular weight (MW)\(^3\) 272.3) is a plant bioflavonoid classified as a flavanone. It exerts multiple biological effects that could contribute to the human health protection ascribed to vegetable consumption. This bioflavonoid exhibits anti-estrogenic activity (1–3) that may be responsible for the decreased incidence of breast cancer in women consuming a large amount of phytoestrogens (4), and could exert cholesterol-lowering properties by inhibiting cholesteryl ester synthesis (5).

Furthermore, naringenin seems to affect different oxidative processes associated with chronic degenerative diseases. In fact, it partially deactivates the Fenton reaction (6), restores glutathione-dependent protection against lipid peroxidation in \( \alpha \)-tocopherol-deficient liver microsomes (7) and inhibits malonaldehyde production induced either by ascorbic acid in rat brain mitochondria (8) or by autoxidation in rat brain homogenates (9). Naringenin may modulate cytochrome P450-dependent monooxygenase, the primary enzyme involved in the metabolism of many xenobiotics such as drugs, carcinogens and environmental pollutants (10).

Because it has been hypothesized that bioflavonoids activity in vivo is dependent on their incorporation rate into cells and on their orientation in biomembranes (11,12), the finding that naringenin interacts with phospholipid bilayers (9) suggests a possible role in human physiology.

The main sources of naringenin are citrus fruits and tomato (Lycopersicum esculentum) (13,14). In citrus fruits, naringenin is principally present in glycosidic forms such as naringenin-7-neohesperidoside (naringin) and naringenin-7-rutinoside (narinrutin), whereas in tomato, where naringenin is one of the most abundant polyphenols, it is present in the skin as aglycone. The naringenin concentration of tomato is reported to range from 0.8 to 4.2 mg/100 g whole red tomato (14,15). So far, no studies have been conducted to investigate the bioavailability of naringenin from tomato in humans. Given the large daily consumption of tomato and tomato-based products, especially in the Mediterranean region, we investigated naringenin bioavailability in humans after consumption of a meal containing tomato. Because bioavailability is strongly affected by food matrices, we administered cooked tomato paste to obtain results providing information on a frequent dietary habit.

**MATERIALS AND METHODS**

**Chemicals and reagents.** All chromatographic standards, \( \beta \)-glucuronidase and sulfatase were purchased from Sigma (St. Louis, MO). Methanol was obtained from Carlo Erba (Milan, Italy). Orthophosphoric acid (85%) and sodium dihydrogen orthophosphate 1-hydrate were purchased from BDH Italia (Milan, Italy). All chemicals and solvents were of analytical or HPLC grade. Distilled water was purified using a Milli-Q (18 M\( \Omega \)) water purification system (Millipore, Milan, Italy).

**Tomato paste.** Commercial tomato (Lycopersicum esculentum) paste was supplied by Cirio Ricerche (Caserta, Italy). Tomatoes from the same harvest and genotype were used to obtain the paste. The experiment was conducted 4 d after tomato paste bottling.
Study design. Five nonsmoking healthy men gave their signed consent to study participation. The protocol complied with the Helsinki declaration as revised in 1983. The subjects were 24–38 y old, had body weights ranging from 67 to 81 kg and body mass indexes ranging from 22 to 26 kg/m². No subjects were under medical treatment and they did not consume any dietary supplements for at least 2 wk before the study began.

During the 3 d before the study, subjects were asked to not eat citrus fruits and tomatoes, and, in general, to limit their intake of fruits and vegetables. Furthermore, daily food intakes were checked during this period to verify that dietary habits were in compliance with daily nutritional requirements.

A homogenous sauce was prepared by cooking for 10 min at 40°C 150 g of commercial tomato paste (containing 3.8 mg of naringenin) with 30 g of corn oil. A test meal containing 70 g of pasta seasoned with 30 g of corn oil. The same subjects received a control meal containing 70 g of pasta seasoned with 30 g of corn oil.

Sample preparation. Three repeated extractions of food polyphenols were performed as described by Hertog et al. (16) both with and without acid hydrolysis at 70°C to cleave glycosides.

Plasma naringenin was extracted with or without enzymatic hydrolysis of the conjugated forms. To perform the enzymatic hydrolysis, 0.5 mL of an enzyme solution containing 5.5 μL of Helix Pomatia; Sigma), in 0.2 mol/L acetate buffer (pH 5) unconjugated form, 0.5 mL of acetate buffer (pH 5) without enzymes for 45 min. Immediately after incubation, 1 mL of 3 mol/L HCl: was performed with an autoinjector (100-

Mobile phases and elution program have been described (17). The volume injected was 30 μL from Helix Pomatia; Sigma). In 0.2 mol/L acetate buffer (pH 5) without enzymes for 45 min. Immediately after incubation, 1 mL of 3 mol/L HCl: was performed with an autoinjector (100-

RESULTS

A linear relationship between peak height and naringenin concentration was obtained by the calibration experiment. The regression coefficient of peak height (32 μA) vs. concentration (μmol/L) was \( R_{xy} = 0.995 \). The slope and the intercept were 2.85 ± 0.02 μA × L/μmol and 0.3 ± 0.1 μA, respectively. The limit of detection, calculated as the amount of naringenin resulting in a peak height of three times the sd of the baseline noise, was 0.02 μmol/L.

Method validation experiments gave a CV 9.0% and an absolute recovery >80% at every concentration tested (Table 1), sufficient to accept data obtained in this experiment.

The polyphenolic composition of the commercial tomato paste, expressed as mg/100 g of wet weight, was 0.72 ± 0.02 chlorogenic acid (1,3,4,5-tetrahydroxycyclohexane carboxylic acid 3,4-dihydroxycinnamate), 3.12 ± 0.13 rutin (quercetin-3-O-rhamnosylglucoside) and 2.53 ± 0.12 naringenin. Neither naringenin-7-O-rhamnoglucoside nor naringenin-7-O-glucoside was detected in tomato paste, consistent with other studies of the polyphenol composition of tomato (15,18–20). Because the naringenin content of tomato paste was analyzed without acid hydrolysis and heat treatment, the possibility that naringenin in our extracts derived from cyclization of chalconaringenin caused by the extraction procedure, as reported by other authors (21), must be excluded. Extraction after acid hydrolysis at 70°C did not affect the naringenin peak height, confirming the absence of chalconaringenin (21). To verify that the polyphenolic composition of tomato paste was unaffected by cooking, tomato paste samples were spiked with standard solutions and heated at 40°C for 10 min. No changes in polyphenol contents added were detected (data not shown).

<table>
<thead>
<tr>
<th>Concentration (μmol/L)</th>
<th>CV, n = 8</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.37</td>
<td>2.4</td>
<td>80</td>
</tr>
<tr>
<td>0.21</td>
<td>2.1</td>
<td>84</td>
</tr>
<tr>
<td>0.11</td>
<td>8.7</td>
<td>81</td>
</tr>
<tr>
<td>0.07</td>
<td>3.4</td>
<td>80</td>
</tr>
</tbody>
</table>

HPLC, high-performance liquid chromatography.

TABLE 1 Precision and recovery of the HPLC determination of naringenin in plasma
Naringenin was not detected after the men consumed the control meal at any time, whereas after test meal consumption, a significant increment \( (P < 0.05) \) occurred between 0 and 2 h (Fig. 1), followed by a significant decrement \( (P < 0.05) \) between 2 and 4 h. The peak plasma naringenin concentration \( (C_{\text{max}}) \) at 2 h \( (T_{\text{max}}) \) was 0.12 ± 0.03 \( \mu \text{mol/L} \) (Table 2). Unconjugated naringenin was not detected in plasma samples that were not subjected to enzymatic hydrolysis. Although present in tomato paste, neither chlorogenic acid nor rutin or their derivatives, caffeic acid and quercetin, were detected in plasma samples.

### DISCUSSION

The results from various epidemiological studies, designed to assess the relevance of tomato consumption in the prevention of some chronic degenerative diseases, showed an inverse correlation between tomato and tomato-based product intake and risk of cancer at some defined sites (22). Tomatoes are considered one of the most important sources of lycopene, and, so far, their potential anticancer properties have been attributed only to lycopene’s antioxidant activity (22,23). To our knowledge, although plant polyphenols may contribute to the health protection ascribed to fruit and vegetable consumption (24), no studies of polyphenol absorption after tomato or tomato-based product intake have been published. In this study, the bioavailability of all the polyphenols identified in tomato paste was investigated and the suitability of our method for their quantification was verified (data not shown), but only naringenin was detectable in plasma. The test meal contained 1.08 mg of chlorogenic acid, 4.68 mg of rutin and 3.81 mg of naringenin. Although the amount of rutin ingested was the highest, neither rutin nor free quercetin was detected in plasma. Other authors (25) found a low peak quercetin concentration \( (0.3 \mu \text{mol/L}) \) 9 h after ingestion of 202 mg of pure rutin. Given the small amount of rutin in the test meal, it was predicted that no quercetin peak would be detected over the limit of detection \( (0.08 \mu \text{mol/L}) \) if drawing at 9 h was performed.

Chlorogenic acid absorption in humans was observed in a recent study (26) carried out by supplementation of 1 g of chlorogenic acid to healthy ileostomy subjects. Absorption, calculated as difference between the amount of supplement ingested and the amount in the ileostomy effluent, was 33%. The authors concluded that chlorogenic acid is absorbed intact and metabolized extensively in the liver. In our study, calculating 33% of chlorogenic acid ingested amount and dividing this quantity by 3 L of plasma that are normally present in a man of 70 kg, we should have detected a peak ~10-fold higher \( (0.30 \mu \text{mol/L}) \) than our limit of detection for chlorogenic acid \( (0.03 \mu \text{mol/L}) \). However, this calculation is affected by a high grade of approximation because it does not take into account the balance among absorption, distribution, and excretion rates. Furthermore, bioavailability is affected by bacterial degradation in the gastrointestinal tract that might cause an overestimation of absorption in the ileostomy model. Because we considered the possibility that chlorogenic acid was hydrolyzed into caffeic and quinic acids in the gastrointestinal tract, chromatograms were analyzed for caffeic acid. Although our extraction procedure and chromatography were suitable to identify caffeic acid in plasma (data not shown), it was not detected at any time, consistent with another study (26).

The in vivo study here described showed consistent results on naringenin absorption from tomato paste. In tomato paste, only the naringenin aglycone was detected, as in other studies of the polyphenol composition of tomato (15,18–20).

In a study by Erlund et al. (27) orange or grapefruit juices, naturally rich in naringenin glycosides, were administered to humans. They found peaks of naringenin in plasma at 4.8 and 5.5 h after consumption of orange and grapefruit juices, respectively, while, in our case, the \( T_{\text{max}} \) was reached after 2 h. Differences in \( T_{\text{max}} \) between citrus and tomato naringenin could be because of different absorption routes. In fact, absorption of glycosidic forms probably takes place in the distal part of the small intestine, where the enzymes for breaking

### TABLE 2

<table>
<thead>
<tr>
<th>Time, h</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu \text{mol/L} )</td>
<td>( (0.02 ± 0.05)^a )</td>
<td>( (0.12 ± 0.03)^b )</td>
<td>( (0.06 ± 0.03)^a )</td>
<td>( (0.02 ± 0.02)^a )</td>
<td>( (0.02 ± 0.03)^a )</td>
<td>ND²</td>
</tr>
<tr>
<td>( \text{ng/mL} )</td>
<td>( (6 ± 13) )</td>
<td>( (32 ± 8) )</td>
<td>( (16 ± 9) )</td>
<td>( (6 ± 5) )</td>
<td>( (4 ± 9) )</td>
<td>—</td>
</tr>
</tbody>
</table>

¹ Values are means ± so, \( n = 5 \). Those in a row without a common letter differ, \( P < 0.05 \).
² ND = not detectable.
glycosidic linkages are present (28,29), whereas aglyconic forms seem to be absorbed early in the digestive tract (30,31).

We found a C_{max} ranging from 0.07 to 0.12 μmol/L after administration of 3.81 mg of naringenin aglycone with the test meal. We did not find unconjugated naringenin in plasma samples and concluded that naringenin is largely metabolized in the liver and enters the general circulation as the conjugated form. Unfortunately, our chromatography did not permit the detection of unknown peaks that may be conjugated forms. A study in rats (32) showed results in agreement with ours, demonstrating the absence of naringenin as the free form in plasma of rats fed naringenin.

The naringenin plasma level at baseline (T0) was undetectable, as expected, for all subjects except one. The compositional analysis of the diet consumed by this subject during the 3 d before the experiment was conducted did not reveal any anomaly to justify this incoherence, which remains unsolved.

In conclusion, our study demonstrated naringenin absorption from cooked tomato paste in men. Furthermore, because a recent study (33) showed that lycopene administered with some polyphenols enhances its antioxidant properties, our results support the hypothesis that tomato benefits could be attributed to a positive synergistic action in vivo among lycopene and other bioavailable tomato constituents, such as naringenin, rather than to only lycopene properties.

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LITERATURE CITED