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Sulfur compounds can modulate the bioavailability of flavonols

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Introduction

Biological activities of flavonoids, including the antioxidant effects, greatly depend on their bioavailability (1,2). It has been shown that different dietary quercetin glycosides (from onions and apples) displayed great differences in their absorption kinetics (3). The nature of sugar linked to flavonol appeared to affect the absorption of quercetin. However, the position of substitution seemed to have no influence. In addition, data on the influence of the chemical environment on the bioavailability of polyphenols remained scarce.

The aim of this study was to specify the effect of other bioactive microconstituents, especially sulfur compounds present in large quantities in onions, on the absorption of flavonol glycosides.

Methods

Male 6-weeks old Wistar rats were purchased from Janvier (Le Genest Saint Isle, France). Rats were housed in individual stainless-wire cages, maintained at 22 °C with a 12h light/12h dark cycle. They were fed ad libitum with a semi-synthetic diet and water for one week before the beginning of the experiment. Animals were fasted for 18 hours before and 5 hours after microconstituents administration. Microconstituents were administered to rats (3 per treatment/time) at a unique dose by intra-gastric way:

1. DPDS (dipropyle disulfide), dissolved in corn oil, was orally administrated at a dose of 200 mg per kg body weight (treatment S).
2. Flavonols (quercetin glycosides isolated from onion) as aqueous solution were administrated at a dose of 20 mg/kg body weight (treatment F).
3. The mixture of DPDS and flavonols were administrated altogether at the same dose given in the treatments S and F (treatment SF)

Negative control was included for 3 animals. Blood plasmas were analyzed at different times after administration: 0, 1, 2, 4, 8, 24, 36 et 48 hours. Blood samples were immediately centrifuged and plasmas were stored at -20 °C until analysis.

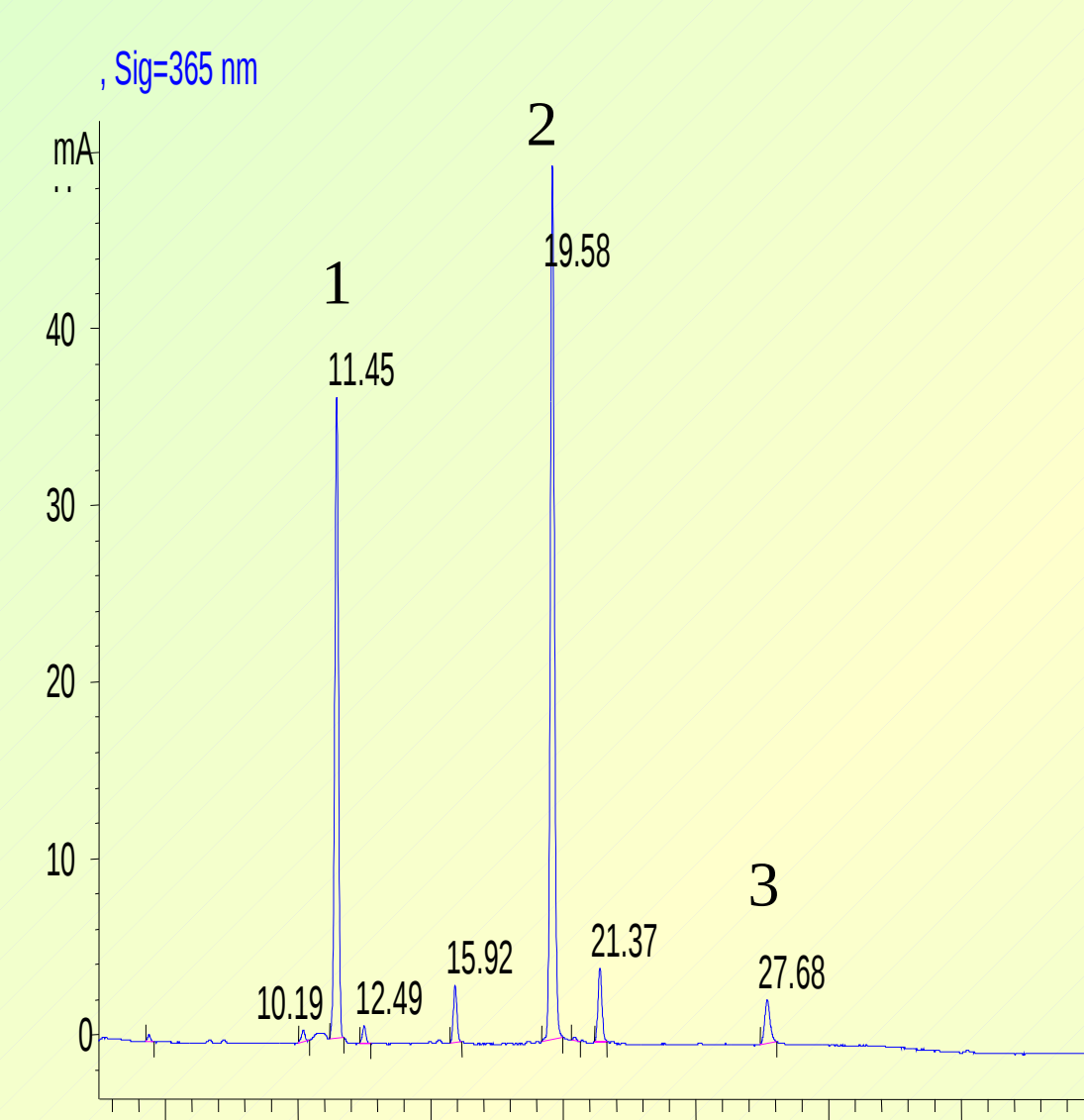
Analysis of plasma flavonols: Blood plasma (500 µl) was extracted by adding 4 volumes of acetone HCl 50 mM containing esculin 2.5 µg.ml⁻¹ as internal standard. The mixture was vortexed for 30 s, sonicated for 30 s, again vortexed for 30 s, and centrifuged for 10 min at 4 °C and 10 000 trs.min⁻¹. Supernatant was evaporated under vacuum and 100 µl of methanol was added to the dry residue. Plasma extracts were analyzed by HPLC/Diode Array Detection (Hewlett Packard 1100) using a column Alltima C18, 5µm (150 × 4.6 mm i.d.) with a guard column Alltima C18, 5 µm (Alltech). The solvent system used was a gradient of A (HCOOH 0.05%) and B (CH₃CN). The best separation was obtained with the following gradient : at 0 min , 10% B ; at 40 min, 40% B ; at 50 min, 50% B. The solvent flow rate was 1 ml.min⁻¹. Flavonols and their conjugates were identified by HPLC/MS (Platforme LCZ, Micromass equipped with a Hewlett Packard 1050 liquid chromatograph) with negative electrospray ionization technique.

Analysis of sulfur compounds: Blood plasma (500 µl) was homogenized with a high-speed blender in distilled water, with 100 µl of an ethanol solution containing p-cymene (0.17 mg/ml), used as internal standard. After adding 0.1 volume trichloroacetic acid 30%, the homogenate was centrifuged and the supernatant was extracted three times with dichloromethane; organic extracts were then concentrated under a nitrogen stream to a final volume of 500 µl. GC/MS was carried out using a Agilent Technologies 6890 gas chromatograph, connected to Agilent Technologies 5973 Mass Selective Detector and equipped with a capillary DB™1701 column (30 m × 0.32 mm I.D., 1 µm film thickness), a splitless injection and an electronic ionisation at 70 eV. Oven was programmed from 35 °C (2 min) to 220 °C at a rate of 5 °C.min⁻¹. The other conditions were as follows: helium (constant velocity fixed at 35 cm/sec) as carrier gas, 200 °C at the injection port. Peak identifications were based on comparison with retention times and spectra of standards.

Statistical analysis was performed with Statview Software (SAS Institute, Cary, NC)

Results and discussion

Flavonol glycosides isolated from onions

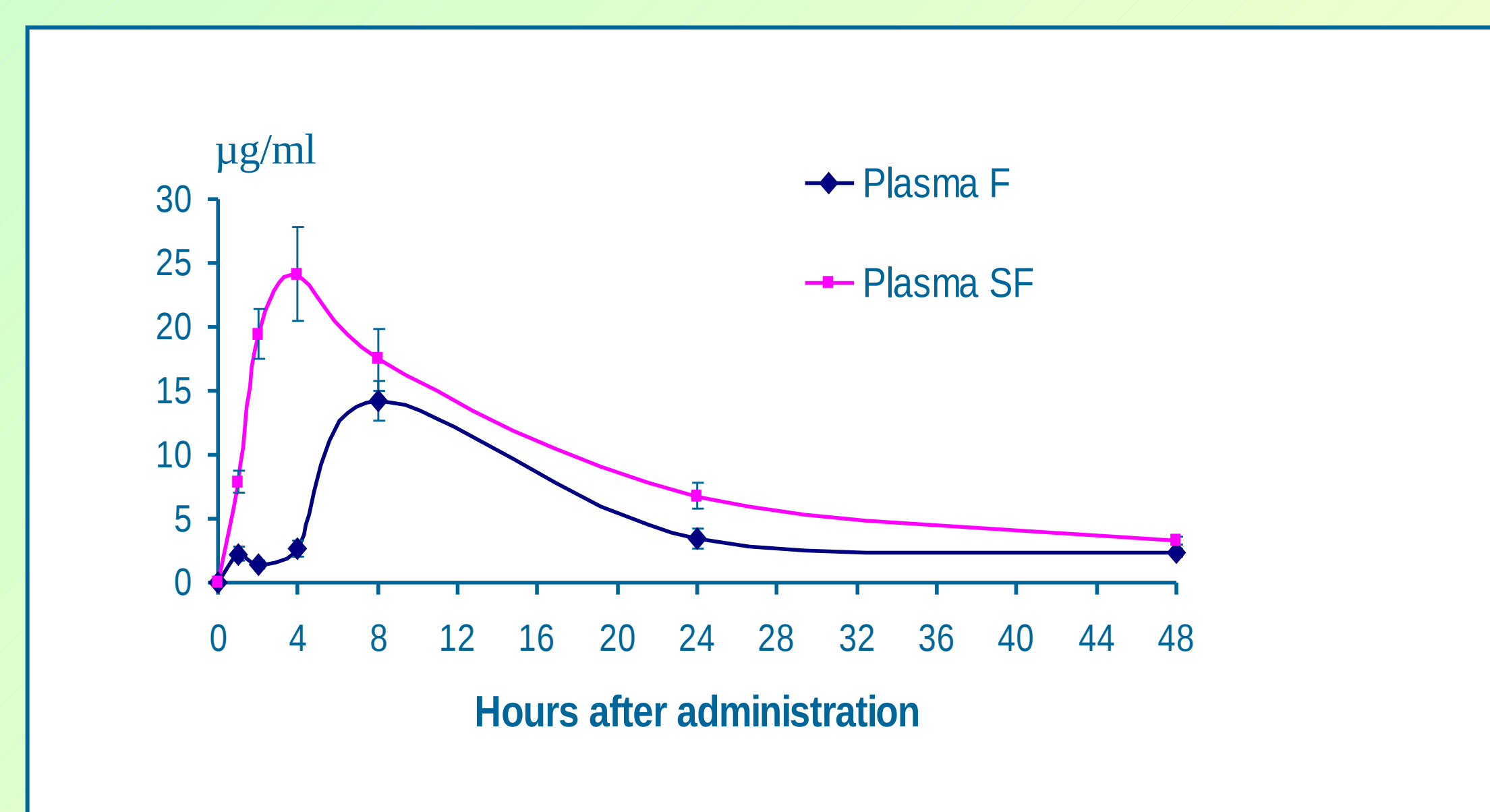


The major compounds were identified as quercetin-3,4'-diglucoside (1) and quercetin-4'-glucoside (2). Quercetin (3) was also detected.

Flavonol metabolites in rat plasmas

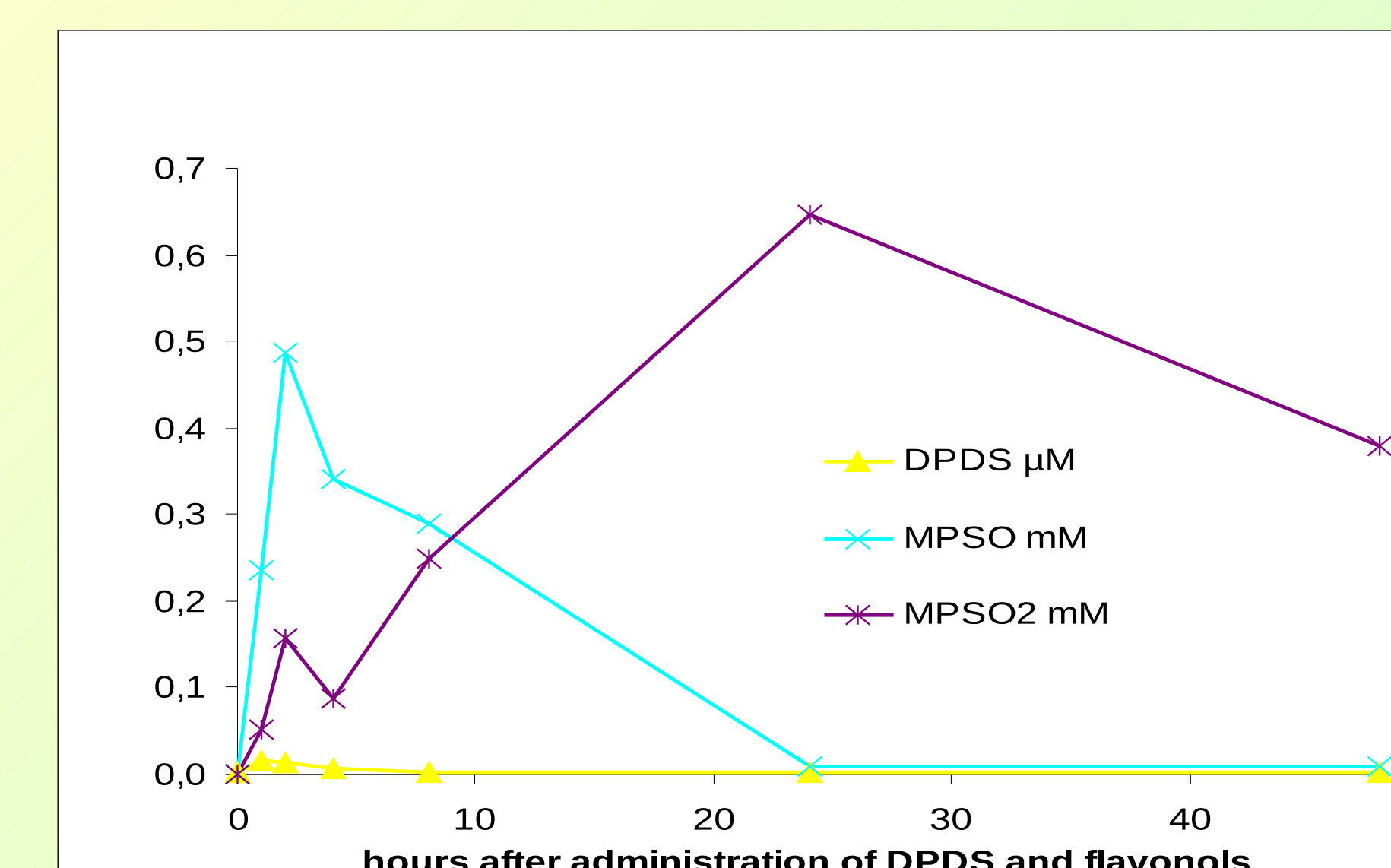
No glycosides were detected in the plasmas of animals supplemented with flavonols and/or DPDS. The major metabolites identified in rat plasmas by HPLC/MS were glucuronides of quercetin and isorahmnetin.

The presence of DPDS changed the absorption kinetics of flavonols



⇒ Flavonols when administrated with DPDS (SF) appeared more rapidly absorbed than administrated alone (F). The maximum was observed at 4 hours for the mixture SF versus 8 hours for flavonols (F).
⇒ The levels of plasmatic flavonols were two-fold higher when rats were supplemented with flavonols and DPDS (SF). After 24 hours, concentrations of flavonols remained higher for the coadministration treatment.

⇒ DPDS appeared in plasmas as different oxidized metabolites, such as MPSO and MPSO2. The plasmatic concentration of MPSO2 was greatly reduced in the presence of flavonols (10 times) and its elimination was slower. This result showed a strong interaction between DPDS and flavonols.



Conclusions

Our results suggested that other phytochemicals present in our diet can influence the bioavailability of polyphenols. The differences related to the bioavailability of flavonols observed after apples or onions ingestion could be due to the chemical environment of food matrix. It could be suggested that sulfur compounds present in onions could prevent the degradation of flavonols through similar mechanisms previously reported for phytochemicals from Citrus that could prevent the oxidation of some drugs (4). Such mechanisms are currently studied at intestinal level with Caco-2 cells.

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