

Accelerated Antioxidant Bioavailability of OPC-3[®] Bioflavonoids Administered as Isotonic Solution

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The degree of absorption of bioflavonoids, a diverse and complex group of plant derived phytonutrients, has been a frequent debate among scientists. Monomeric flavonoid species are known to be absorbed within 2 h. The kinetics of plasma reactive oxygen species, a reflection of bioactivity, of a commercial blend of flavonoids, OPC-3[®] was investigated. OPC-3[®] was selected to compare absorption of an isotonic flavonoid solution vs tablet form with the equivalent amount of fluid.

In the case of isotonic OPC-3[®] the reactive oxygen species of the subject's plasma decreased significantly ($p < 0.05$), six times greater than OPC-3[®] tablets by 10 min post-consumption. After 20 min the isotonic formulation was approximately four times more bioavailable and after 40 min twice as bioavailable as the tablet, respectively. At time points 1 h and later, both isotonic and tablet formulations lowered oxidative stress, although the isotonic formulation values remained significantly better throughout the investigation period of 4 h. These findings point to a dramatically accelerated bioavailability of flavonoids delivered in an isotonic formulation. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: flavonoid bioavailability; OPC-3[®]; antioxidant; isotonic formulation.

INTRODUCTION

Dietary flavonoids are now appreciated to have a multitude of health benefits, including antioxidant activity in humans. The absorption of flavonoids in humans is very complex, involving interaction between flavonoids, other food components, duodenal uptake mechanisms and the gut micro-flora. Monomeric flavonoid constituents such as anthocyanins, catechin, quercetin and hesperitin appear in the blood stream and their metabolites subsequently appear in the urine within the first 2 h (Davalos *et al.*, 2006; Kanaze *et al.*, 2007; Grimm *et al.*, 2006). Flavonoids such as the oligomeric proanthocyanidins (OPCs) in Pycnogenol[®] require bacterial metabolism prior to complete absorption into the blood stream which requires up to 8 h (Grimm *et al.*, 2006).

Prior to absorption, digestive enzymes and gastric fluids are needed to create an isotonic solution for the ingested food. An isotonic solution triggers osmo-receptors, located at the distal end of the stomach, which opens the duodenal sphincter, releasing the digested isotonic contents into the duodenum. Administration of dietary flavonoids already in an isotonic solution is expected to accelerate the absorption into the small intestine while reducing the time the nutrients stay in the stomach.

As flavonoids from plant extracts represent complex mixtures of compounds, it is technically very demand-

ing to identify and quantify these compounds and their metabolites in the blood after consumption. Therefore, we chose to investigate the resultant antioxidant effects for comparison of the bioavailability of a complex flavonoid mixture delivered either as isotonic solution or tablet form.

MATERIALS AND METHODS

Subjects. Ten borderline healthy subjects (5 men and 5 women), aged 24–45 years (mean age 29.8 years; SD 6.3), all non-smokers, were recruited presenting with elevated blood plasma oxidative stress as established by means of a D-ROM test. The subjects did not take medication or dietary supplementation for the 3 weeks prior to the study.

Plasma free radical testing. The oxidative stress status of patients was investigated by quantifying direct reactive oxygen metabolites (D-ROM) using the free radical analytical system (FRAS) (Diacron, Grosseto, Italy). In brief, the assay estimates hydroperoxides in a small blood sample (20 µL) after incubation in buffer solution together with a chromogenic agent. Photometric analysis provides oxidative stress status in Carr units, with 1 Carr unit corresponding to 80 µg H₂O₂/dL. Values above 300 Carr units suggest oxidative stress (Komatsu *et al.*, 2006).

Study design. A mixture of 400 mg flavonoids (OPC-3[®]), consisting of equal amounts of extracts derived from

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French maritime pine bark Pycnogenol[®], grape seed, bilberry, citrus and red wine were obtained from nutraMetrix[®], Division of Market America, Greensboro, NC, USA, as previously described (Cesarone *et al.*, in print). These were provided in a sealed foil pouch and after mixing with 180 mL water yielded an isotonic solution. In addition, the OPC-3[®] mixture was provided as a standard compressed tablet which was consumed with 180 mL water.

All ten subjects received at 9 am on an empty stomach a single portion of the isotonic OPC-3[®] liquid formulation and at another time the tablet equivalent with a 1 week wash-out period between investigations. At baseline, before consumption of the test items and subsequently after various time points the oxidative stress status of patients was investigated and used as parameter for the bioavailability of consumed antioxidant flavonoids. The direct reactive oxygen metabolites were quantified as described above.

Statistics. The measured changes in plasma free radical values between groups were assessed using non-parametric tests (Chi squared test) and the analysis of the variance (ANOVA). The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Within 10 min post-consumption a significant lowering of the subject's plasma free radicals was apparent with the isotonic OPC-3[®] formulation. In contrast, no effect resulted from consumption of the same flavonoids in tablet form at this time (Table 1). With flavonoids delivered in isotonic formulation, a 10% plasma radical reduction had set in after 20 min as opposed to the 40 min time period required for the same effect in the case of flavonoid tablets.

The bioavailability gap between isotonic OPC-3 liquid formulation and the OPC-3 tablets narrowed gradually with time as expected (Fig. 1). Both formulations should yield the same absolute bioavailability, but with accelerated uptake in the case of the isotonic delivery form. Interestingly, until the end of the investigation period of 4 h, the plasma free radical values always remained

Table 1. Blood oxidative stress in Carr units, mean values, standard deviation and statistical significance between groups

Time	Tablets	Isotonic formulation	p value
Baseline	422 (34)	430 (26)	n.s.
10 min	426 (33)	403 (33)	<0.05
20 min	411 (39)	388 (14)	<0.05
30 min	389 (43)	378 (39)	<0.05
40 min	380 (24)	340 (42)	<0.05
50 min	341 (34)	305 (29)	<0.05
60 min	288 (39)	269 (25)	<0.05
70 min	286 (53)	266 (14)	<0.05
80 min	288 (43)	246 (43)	<0.05
1½ h	289 (21)	258 (41)	<0.05
2 h	278 (29)	266 (27)	<0.05
2½ h	285 (38)	259 (33)	<0.05
3 h	277 (33)	249 (28)	<0.05
3½ h	278 (23)	250 (19)	<0.05
4 h	273 (12)	248 (33)	<0.05

lower in the case of the isotonic OPC-3 liquid formulation compared with the corresponding values found for the OPC-3 tablet.

In addition, the plasma free radical response, following consumption of the isotonic OPC-3 formulation as shown in Fig. 1, suggests a biphasic absorption model. This may be due to the diverse range of molecular sizes of flavonoid molecules present in OPC-3, with monomeric and dimeric species being absorbed quickly and having an immediate effect on oxidative stress reduction. The second phase may be due to the digestive breakdown and absorption of the larger molecules, maximizing in this study at around 70 min.

In conclusion, the findings demonstrate that the flavonoid mixture provided in isotonic OPC-3[®] is significantly more bioavailable in humans, in terms of antioxidant activity than an equivalent mixture in tablet form. Furthermore, the isotonic OPC-3[®] formulation resulted in a more potent antioxidative effect than an equivalent mixture in tablet form.

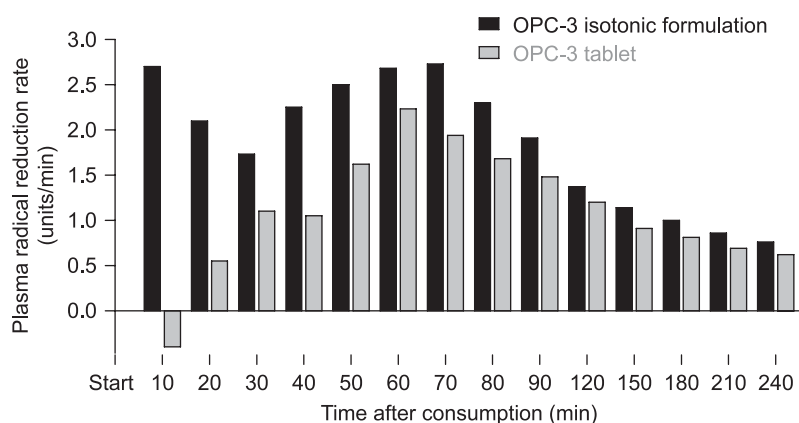


Figure 1. Lowering of plasma oxidative stress in 10 subjects subsequent to oral consumption of an isotonic liquid flavonoid formulation OPC-3[®], or the same flavonoid composition administered as tablet. The bars depict the rate of change of blood plasma free radicals relative to the elapsed time after consumption.

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