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Influence of omega-3 PUFAs on the metabolism of proanthocyanidins in rats

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ABSTRACT

Studies of the bioavailability of proanthocyanidins usually consider them independently of other dietary constituents, while there is a tendency in the field of functional foods towards the combination of different bioactive compounds in a single product. This study examined the long-term effects of ω-3 polyunsaturated fatty acids of marine origin on the metabolic fate of grape proanthocyanidins. For this, female adult Wistar-Kyoto rats were fed (18 weeks) with a standard diet supplemented or not with eicosapentaenoic acid/docosahexaenoic acid (1:1, 16.6 g/kg feed), proanthocyanidin-rich grape seed extract (0.8 g/kg feed) or both. A total of 39 microbial-derived metabolites and 16 conjugated metabolites were detected by HPLC-MS/MS either in urine or in the aqueous fraction of feces. An unexpected significant increase in many proanthocyanidin metabolites in urine and feces was observed in the group supplemented with ω-3 polyunsaturated fatty acids group as compared to the animals fed a standard diet, which contains a small amount of polyphenols. However, proanthocyanidin metabolites in rats given ω-3 polyunsaturated fatty acids and grape seed extract did not significantly differ from those in the group supplemented only with grape seed extract. It was concluded that ω-3 polyunsaturated fatty acids collaborate in the metabolism of polyphenols when present at low doses in the feed matrix, while the capacity of ω-3 polyunsaturated fatty acids to induce microbiota transformations when proanthocyanidins are present at high doses is not relevant compared to that of polyphenols themselves.

1. Introduction

Polyphenols are a large group of compounds found in plant foods that have been shown to have health-related effects in relation to several chronic diseases (Scalbert, Manach, Morand, Rémésy, et al., 2005). Flavanols, included in the family of flavonoids, are among the most studied polyphenols. They range in complexity from monomers, such as (+)-catechin or (−)-epicatechin, to combinations of these structures via different linkages, which gives rise to the corresponding oligomers or polymers- (proanthocyanidins, PAs). Flavanols are found in many common foods, such as grapes, nuts or cocoa, and have been shown to have beneficial effects in relation to different markers of cardiovascular disease. Indeed, the European Food Safety Agency approved a health claim regarding the effects of cocoa flavanols on endothelium-dependent vasodilatation (EFSA, 2006).

A key aspect of the study of PAs is the proper knowledge of their metabolic fate, since they are extensively transformed after ingestion. Dimers and to a lesser extent trimers may be absorbed in the small intestine; the former may be methylated while no post-absorption transformation has been reported for trimers (Monagas et al., 2010). However, most ingested PAs reach the colon, being either extensively depolymerized and absorbed as monomers or metabolized by the gut microbiota (Touriño et al., 2011). Monomers are extensively conjugated in the liver and then circulate in the body before being excreted in urine or accumulated in tissues, or they return to the intestine via enterohepatic circulation. While for those PA transformed by the microbiota, the resulting metabolites are mostly phenolic acids that may be absorbed and follow the same routes as polyphenols absorbed in the small intestine (Mateos-Martín, Pérez-Jiménez, Fuguet, & Torres,

Abbreviations: EC, (epi)catechin; EGC, (epi)gallocatechin; Gluc, glucuronyl group; GSE, grape seed extract; Me, methyl group; MRM, multiple reaction monitoring; PAs, proanthocyanidins; STD, standard diet; Sulf, sulfate group; SPE, solid-phase extraction.

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2012a; Monagas et al., 2010; Urpí-Sardá et al., 2009). Increasing evidence suggests that circulating polyphenol-derived metabolites, especially those produced during colonic fermentation, may be the compounds responsible for the health-related properties of these food constituents (Williamson & Clifford, 2010).

To elucidate the metabolic fate of polyphenols, they are administered alone and in acute doses (Mateos-Martín et al., 2012a; Monagas et al., 2010; Touriño et al., 2011; Urpí-Sardá et al., 2009). In a common diet, however, polyphenols are consumed in combination with other food components, which may have either synergic or antagonistic effects on their bioavailability. Moreover, there is currently increasing interest in the development of functional foods with combinations of bioactive components (Peluso, Romanelli, & Palmery, 2014), which may affect the bioavailability of polyphenols and the health effects derived from them. Therefore, now that the general process of transformation of these compounds has been reported, there is increasing interest in the effects that other food constituents, such as carbohydrates, proteins or dietary fiber, may have on their bioavailability (Bohn, 2004; Zhang et al., 2014).

In relation to dietary fat, studies with animal models (Lesser, Cermak, & Wollfram, 2008) and humans (Guo et al., 2013; Tulipani et al., 2002) have reported that this food constituent increases the bioaccessibility and absorption of certain flavonoids, through different mechanisms. Differential effects of long-chain and medium-chain fatty acids on the bioavailability of polyphenols are probably due to the different metabolic routes that these compounds follow (Lesser, Cermak, & Wollfram, 2006; Murota, Cermak, Terao, & Wollfram, 2013). In contrast, the effects of fatty acids with different degree of unsaturation on the metabolic fate of polyphenols have not been explored. Indeed, studies in this area have only evaluated the differential effects of saturated and monounsaturated fats (Guo et al., 2013; Lesser et al., 2006; Lesser et al., 2008; Murota et al., 2013; Tulipani et al., 2002), and to the best of our knowledge, only one *in vitro study* has considered the effects of a polyunsaturated fat: hazelnut oil (Ortega, Macià, Romero, Reguant, & Motilva, 2011).

Long-chain ω-3 PUFAs of marine origin are a class of bioactive dietary components that have generated a great deal of interest due to their beneficial effects in both animal and human studies, on parameters related to cardiovascular disease (Aguilera, Díaz, Barcelata, Guerrero, & Ros, 2004; Lorente-Cebrián et al., 2013). In a common diet, and in supplements containing different bioactive compounds, polyphenols may be consumed together with ω-3 PUFAs and different interactions may take place (Peluso et al., 2014), which could also affect their metabolic fate. Therefore, the aim of this study was to evaluate the effect that ω-3 PUFAs had on the metabolic fate of grape PAs after long-term in vivo supplementation. To this end, a pilot study was carried out in Wistar-Kyoto rats, and the profile of polyphenol metabolites was measured by targeted HPLC-ESI-MS/MS analysis of urine and the aqueous fraction of feces.

2. Materials and methods

2.1. Chemicals and reagents

The standard diet was Teklad Global 2014 (Harlan Teklad Inc., Indianapolis, IN, USA). Fine Grajfnol® powder, 98% grape seed, was obtained from JF-Natural Product (Tianjin, China), with the following composition: total PAs (UV), ≥95%; oligomeric PAs, ≥60%; procyanidin dimer B_2 (HPLC), ≥1.8%; ash, ≤1.5%; weight loss on drying, ≤5.0%. Porcine gelatin type A 240/260 was from Juncà (Girona, Spain) and the soybean lecithin Topcithin 50 from Cargill (Barcelona, Spain). Oil with an eicosapentaenoic acid:docosahexaenoic acid (EPA:DHA) ratio of 1:1 was obtained by mixing appropriate quantities of the commercial fish oils AFAMPES 121 EPA (A.F.A.M.S.A., Vigo, Spain), EnerZona Omega 3 RX (Milan, Italy) and Oligen liquid DHA 80% (IFIGEN-EQUIP 98, S.L., Barcelona). Soybean oil, obtained from unrefined organic soybean oil (first cold pressing), was from Clearspring Ltd. (London, UK).

Ketamine chlorhydrate was purchased from Merial Laboratorios (Barcelona, Spain) and xylazine from Química Farmaceutica (Barcelona, Spain). Standards of (−)-epicatechin (≥98%), (−)-epigallocatechin (≥95%), 3-hydroxyphenylacetic acid (≥99%), 4-hydroxyphenylacetic acid (≥98%), 3,4-dihydroxyphenylacetic acid (≥98%), 3-hydroxybenzoic acid (≥99%), 4-hydroxybenzoic acid (≥99%), homovanillic acid (≥98%), vanillic acid (≥97%), caffeic acid (≥98%), 3,4-dihydroxyphenylpropionic acid (≥98%), 3-hydroxyphenylpropionic acid (≥98%), 4-hydroxyphenylpropionic acid (≥98%), 3,4-dihydroxybenzoic acid (≥97%), benzoic acid (≥99%), hippuric acid (≥98%), ferulic acid (≥99%), isoferulic acid (≥97%), *p*-coumaric acid (≥98%), *m*-coumaric acid (≥98%), gallic acid (≥97%), enterodiol (≥95%), phenylacetic acid (≥99%), taxifolin (≥85%), and *tert*-butylhydroquinone and formic acid (analytical grade) were obtained from Sigma Chemical (St Louis, MO, USA). Methanol (analytical grade) and hydrochloric acid (≥85%) were from Panreac (Castellar del Vallès, Barcelona, Spain). Acetonitrile (HPLC grade) was obtained from Merck (Darmstadt, Germany). Water for the assay solutions was obtained using a water Milli-Q purification system (Millipore Corporation, Billerica, MA, USA).

2.2. Diets

Four diets were prepared: the standard diet; the standard diet supplemented with ω-3 PUFAs; the standard diet supplemented with grape seed extract; and the standard diet supplemented with both ω-3 PUFAs and grape seed extract. The diets without ω-3 PUFAs were enriched with soybean oil in order to make them isocaloric. All the diets were prepared in-house and included *tert*-butylhydroquinone as an antioxidant, porcine gelatin to promote gelatinization and soybean lecithin as an emulsifier. The mixtures were freeze-dried to obtain pellets that were stored at 4 °C to prevent oxidation and fungal contamination. The composition of each diet, including the supplementations with ω-3 PUFAs and GSE, as well as both the macronutrient and micronutrient profile, is shown in Supplemental Table 1. A mixture of EPA and DHA in a ratio of 1:1 was used, since it has been suggested that each fatty acid may have different health effects (Lorente-Cebrián et al., 2013). This ratio was previously reported as the most beneficial for cardiometabolic risk factors (Lluís et al., 2013; Méndez et al., 2013). The fatty acid composition of the soybean oil and ω-3 PUFA supplement were ascertained by gas chromatography after methylation (Lepage & Roy, 1986) and are provided in Supplemental Table 2. Because PUFAs are extremely susceptible to oxidation and due to the potential toxic effects of lipid oxidation byproducts, the lipid oxidation level was checked throughout the dietary interventional experiment (peroxide values < 5 meq. oxygen per kg of oil).

The doses of ω-3 PUFAs (16.6 g/kg feed) and grape PAs (0.8 g/kg feed) were chosen on the basis of previous studies where similar doses showed beneficial effects (Castell-Auví et al., 2013; Masson et al., 2008) and as realistic doses, i.e., doses which could easily be incorporated into a typical common diet.

2.3. Animals and sample collection

Twenty female, 8- to 9-week-old, Wistar-Kyoto rats (Charles River Laboratories, Wilmington, MA, USA) were housed in cages ($n = 2$–3/cage) under controlled conditions of a 12 h light/12 h dark cycle, temperature of 22 °C ± 2 °C and relative humidity of 50% ± 10%. They had free access to water and pelleted feed (Supplemental Table 1) for 18 weeks after being randomly divided into the four dietary groups: STD group ($n = 5$), given the standard diet supplemented with soybean oil; ω-3 group ($n = 5$), given the standard diet supplemented with ω-3 PUFAs; GSE group ($n = 5$), given the standard diet supplemented with grape seed extract and soybean oil; and ω-3 + GSE group ($n = 5$), given the standard diet supplemented with both ω-3 PUFAs and grape seed