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Unravelling of the health effects of polyphenols is a complex puzzle complicated by metabolism

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Plant metabolism creates complex mixtures of polyphenols in plant foods. Epidemiology and human trials reduced this complexity, by studying specific foods; subclasses of polyphenols; individual polyphenols, or total antioxidant capacity (TAC). This implies the following assumptions: (1) a limited number of potent polyphenols exists; (2) well-defined natural potent mixtures of polyphenols exist; (3) polyphenols share a common biological activity (e.g. antioxidant activity). To find potent polyphenols (1st assumption), *in vitro* screening has been widely applied, but most published results are of limited use because metabolism, changing biological activity profoundly, has frequently not been considered. The abundant anecdotal evidence for natural potent mixtures of polyphenols (2nd assumption) on the internet is very hard to verify. Additionally, cross-cultural studies have revealed the potency of e.g. cocoa. Polyphenols share the antioxidant phenolic group which inspired researchers to measure their antioxidant activity, thus greatly reducing complexity (3rd assumption). Unfortunately, the elegant antioxidant hypothesis has to be rejected, because poor absorption and extensive metabolism annihilate any contribution to the endogenous body antioxidants. In conclusion, the above assumptions are hard to verify, and no quick answers are to be expected. Future research should focus on structure–activity relations at nanomolar levels and explore metabolomics.

Introduction

Nutritional epidemiology has established a large body of evidence which shows that specific foods and nutrients or dietary patterns are associated with cancers, cardiovascular diseases, and diabetes [1,2], and with intermediate outcomes such as weight gain, blood pressure and insulin resistance [3,4]. There is increasing support from well-designed randomised trials that show the benefits of e.g. healthy dietary patterns and fruit and vegetable consumption [5,6]. It is estimated that diets low in vegetables and fruits are responsible for 1.5–4% of the global disease burden [7]. The US Healthy People 2020 (HP2020) objectives [8] recommend that citizens increase their intake of fruit and vegetables to reduce disease risk.

Apart from vegetables and fruits, also beverages like tea, red wine, and cocoa have been associated with disease reduction, especially cardiovascular diseases (CVD)[1] [9–13]. All these foods have in common that they are rich sources of polyphenols. Flavonoids, a subclass of the polyphenols, are generally considered as the active constituents of certain plants and spices that have been used for thousands of years in traditional Eastern medicine [14]. It has been demonstrated that flavonoids affect inflammatory cells and the activity of many enzyme systems *in vitro*, and some evidence was found for *in vivo* effects [14]. However, Middleton rightfully was quite critical about the relevance of these effects, because major questions about how flavonoids can enter organ cells were not answered yet. Especially one aspect of polyphenols, their inherent antioxidant properties, attracted much interest. A paper describing a screening method for the antioxidant activity of dietary and plasma antioxidants, including polyphenols, soon became the most cited paper in the polyphenol and health field[15]. The popularity of the antioxidant hypothesis, proposing that dietary antioxidants might enhance the antioxidant defence system of the body, really boosted polyphenol health research over the last twenty years.

Plant metabolism creates complex mixtures of polyphenols

Polyphenols exist as very complex mixtures of related compounds in plant foods. Most of these compounds share a common origin in the plant: the amino acid phenylalanine. Phenylalanine is deaminated to cinnamic acid, which then enters

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[1] *Abbreviations used:* TAC, total antioxidant capacity; CVD, cardiovascular diseases; FMD, flow mediated dilation; EFSA, European Food Safety Authority.

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the phenylpropanoid pathway, a biosynthetic route present in almost every plant (Fig. 1) [16]. A key step in this metabolic route is the introduction of one or more hydroxyl groups into the phenyl ring, thus producing phenols. As a result, phenolic compounds have a common building block in their carbon skeleton: the phenylpropanoid unit C_6–C_3, a phenylring with a 3 carbon side chain. Plant metabolism uses this building block to produce the very large variety of (poly)phenols which can be classified into a number of subclasses with simple to complex structures. Cinnamic acids (C_6–C_3), benzoic acids (C_6–C_3 and C_6–C_1), flavonoids (C_6–C_3–C_6), stilbenes (C_6–C_2–C_6), coumarins (C_6-C_3), and lignans (C_6–C_3–C_3–C_6) have simple to rather simple structures, whereas in proanthocyanidins ((C_6–C_3–C_6)$_n$) and lignins ((C_6–C_3)$_n$) the common building blocks are connected to form oligomeric or polymeric structures (Fig. 1). Within each subclass of (poly)phenols plant metabolism produces a variety of compounds, e.g. by adding additional hydroxyl groups and frequently a whole array of sugars, mostly monosaccharides and disaccharides at different positions in the polyphenol structure. Flavonoids identified and described in plants amount to almost 4700 different structures [17,18]. So, plant metabolism produces very complex mixtures of polyphenols in plants.

An important question is whether this overwhelming complexity of polyphenols in plants is really relevant for our diet. Starting in the early nineties [19], polyphenol content values have been determined in foods to produce food composition tables which have been used to calculate dietary intake of polyphenols. Subsequently, these data were used in epidemiological studies, and the first study of this kind determined the cardiovascular effects of a subclass of the flavonoids [20]. Food contents of other polyphenols have been published since, and compilations of these literature data in comprehensive food data bases have been performed by USDA [21,22] and INRA, which developed the web-based Phenol-Explorer (http://www.phenol-explorer.eu) [23]. The USDA databases only contain flavonoid data as aglycones. However, Phenol-Explorer contains data of the original sugar-conjugated flavonoids and of other polyphenols, and demonstrates that the diet also contains complex mixtures of polyphenols: 518 different polyphenols have been identified and quantified in 455 foods.

Approaches followed to deal with polyphenol complexity

Thus, humans are exposed to complex mixtures of polyphenols. To tackle this complexity, epidemiology as well as human trials on the health effects of polyphenols have followed similar approaches. At the highest level of complexity, dietary patterns or intake of vegetables and fruits have been related to health outcomes [6]. However, these kind of studies will only show that polyphenols might play a role, but certainly will give no clues on the identity of the polyphenols relevant for health. Therefore, reduction of complexity is necessary, and has been achieved in a number of ways: by studying more specified foods (e.g. tea, cocoa or berries) [9,12,24]; polyphenol classes (e.g. flavonoids, lignans) [25,26] or subclasses (e.g. flavonols, flavan-3-ols) [24,27,28], total antioxidant capacity (TAC) [29]; or very seldom, individual polyphenols (e.g. quercetin, naringenin, hesperetin) [30]. Implicitly, a number of assumptions are made here: (1) there is a limited number of potent polyphenols; (2) well-defined natural potent mixtures of polyphenols exist; (3) particular classes or subclasses of polyphenols share a common biological activity (e.g. antioxidant activity). In the following, these assumptions will be studied in more detail, trying to find out whether they have really helped to find the most interesting polyphenols for health.

There is a limited number of potent polyphenols (1st assumption)

How can we find these potent polyphenols? An approach followed by many researchers in the past three decades is to expose animal and human cell lines to individual polyphenols and measure a relevant response of these cells. The purpose of these *in vitro* screening tests is to discover structure–activity relations of polyphenols, which might guide the quest for potent polyphenols. *In vitro* screening is efficient, and high-throughput systems are able to screen many compounds in a short time. A PubMed search showed that from 1980 – May 2013, more than 14,000 papers have been published on *in vitro* screening of polyphenols and the number of papers is still growing. *In vitro* screening using cell lines has made extensive use of both polyphenol aglycones and sugar conjugates in the low micromolar to millimolar range [31]. Sugar conjugates are the typical forms that exist in plants. However, after ingestion, dietary polyphenols appear in the circulation not as aglycones or sugar conjugates, but only as metabolites (except for anthocyanins which may appear as sugar conjugates in blood), and their presence in plasma after dietary intake rarely exceeds micromolar concentrations [31]. So, in the majority of these studies, exposures were performed with non-physiologically high concentrations of non-metabolised forms that are not physiologically relevant either. Mammalian metabolism affects the phenolic OH groups which are methoxylated, or conjugated with glucuronic acid or sulphate. These reactions affect the typical biological properties of the phenolic groups of the polyphenols, and as a result, their biological activity will be profoundly changed as is apparent from many examples [32–36]. In addition, non-metabolised forms such as aglycones may enter cells more readily, while

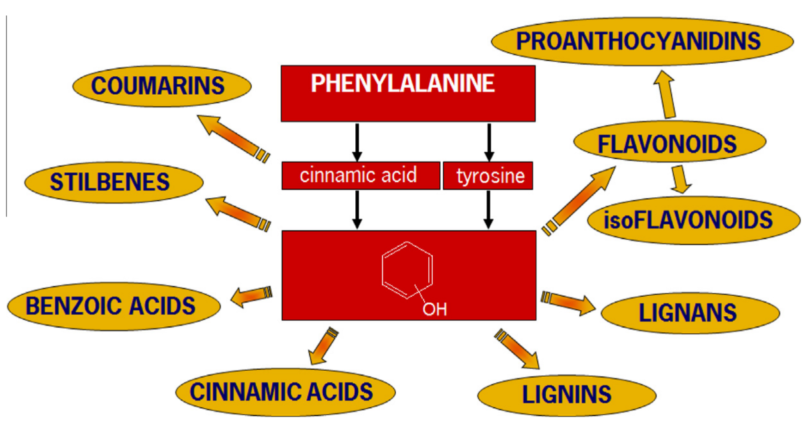

Fig. 1. Plant metabolism, the phenylpropanoid pathway: the origin of polyphenol complexity.