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Add polyphenol for better sugar control

How do polyphenols lower the Glycaemic Index of foods?

Team 1574
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Abstract

The metabolic syndrome is a condition associated with several health problems, which results in high health costs in developed and developing countries. Therefore, it is currently receiving a lot of attention publically, as well as in the scientific world. Indeed, the number of studies about the metabolic syndrome has increased considerably in the last years. One of the potential methods to aid against the prevalence of the metabolic syndrome is the use of polyphenols. Polyphenols consists of several hydroxyl groups and aromatic rings and can mostly be found in plants, especially in the peels. More than 8000 polyphenols structures have been identified and they can differ in stability, bioavailability and physiological functions. Polyphenols are present in many food sources such as herbs, berries, wine, seeds and vegetables with flavonoids being the most commonly present polyphenols.

The bioavailability of polyphenols is mainly determined by their mechanical structure, but can be positively or negatively influenced by the food it is consumed with. Only 5 to 10 % of the polyphenols is absorbed in the small intestine. The rest is transported to the colon where they are metabolized by the microbiota and undergo conjugation, by which they can be highly modified from the original polyphenols. The polyphenols that are not metabolized in the colon are excreted. The potential mechanisms by which polyphenols can have beneficial effects are by inhibition of α-glucosidase and α-amylase, the inhibition of glucose transport by inhibition of GLUT2 and SGLT1, improvement of the pancreatic β-cells, as well as an effect on lipid metabolism and reactive oxygen species (ROS). The GI lowering effect of polyphenols has only been confirmed *in vitro* and therefore the focus of Royal Cosun should be on the benefits for lipid metabolism over the GI lowering effect. Our advice is to add polyphenols (flavonoids) to their sugar products. As it will probably not decrease the GI, it still has a beneficial health effect making the sugar less unhealthy.

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Introduction

The metabolic syndrome is a complex condition, associated with several health problems like abdominal obesity, elevated blood pressure, elevated fasting plasma glucose, high serum triglycerides and low high-density lipoprotein levels. The prevalence of the metabolic syndrome is increasing in both developed and developing countries and high rates of obesity and diabetes mellitus type 2 are associated with high health care costs [1]. Diets rich in vitamins and minerals are changing into an increased intake of energy-dense foods that are high in carbohydrates and fat in combination with a decrease in physical activity [2]. One of the ways in which this emerging problem may be dampened is by the use of diets with a low Glycaemic Index (GI). The GI measures how fast and how much a certain foods raise glucose levels. Besides, it is also of importance to take the different fates of carbohydrates into account. Fructose, the carbohydrate which causes a sweet taste, is stored as liver fat when consumed in excessive amounts. A more fatty liver will drive insulin resistance [3].

In the last decade, the use of polyphenols is becoming more popular in both the scientific as well as the commercial food producing world [4]. Among scientists, the aims are to establish evidence for the effects of polyphenols on human health and to identify the polyphenols which are the most potent in their effects. Polyphenols are large, heterogeneous phytochemical compounds which are thought to play a role in lowering the GI and are found in plant-based foods. More information has to be gathered on the nature and distribution of polyphenols in the human diet. Epidemiological studies may subsequently be able to relate the intake of polyphenols to disease outcomes.

Royal Cosun is a company which is interested in this problem and asked us, as an academic consultancy team, to give more insight in this topic. We therefore aim to give more insight into the types and bioavailability of polyphenols and their potential mechanisms on glucose and lipid metabolism. Besides these insights, we will provide Royal Cosun an advice on the use of polyphenols in their products and side-streams and include an extensive discussion.

Classes of polyphenols een duidelijk verhaal

To understand the potential health effects and mechanisms of polyphenols, it is important to get an overview of the classes of polyphenols. Therefore, this chapter will describe the known classes of polyphenols, the polyphenol content in food and the dietary intake of polyphenols.

Polyphenol structures consist of several hydroxyl groups on aromatic rings and have been identified in plants, including in plants which can be used as a food source. Polyphenols can be classified based on the number of phenol rings present and the structural elements that connect these phenol rings [5]. Classes of polyphenols differ in stability, bioavailability and physiological functions [6] and today, more than 8000 phenolic structures are identified [7-9]. Four main classes of polyphenols can be distinguished: phenolic acids, flavonoids, stilbenes and lignans. Polyphenols may be conjugated with carbohydrates, or bound to organic acids and with themselves. The majority of polyphenols in plants are conjugated with different sugar units at different positions, which is known as glycosylation [6].

In the first class, the phenolic acids, the two most important subclasses are hydroxybenzoic acids and hydroxycinnamic acids (**Figure 1**). Hydroxybenzoic acids have a C1-C6 backbone, while hydroxycinnamic acids have a C3-C6 backbone [6, 10]. Phenolic acids are often found in bound form, only acid or alkaline hydrolysis or enzymes can release them freely [11-13]. Less research has been conducted on the hydroxybenzoic acids, since they are found in only a few edible plants and the content of hydroxybenzoic acids in these plants is lower than hydroxycinnamic acids [14, 15]. Hydroxycinnamic acids are more common and found in all parts of fruit, yet the highest concentrations are present in the outer layer of ripe fruits. This high concentration declines during ripening. Caffeic acid and ferulic acid are hydroxycinnamic acids, of which caffeic acid is the most prevalent phenolic acid. Ferulic acid, present in cereal grains, is the most prevalent phenolic acid and is found in the outer layers of the food source.

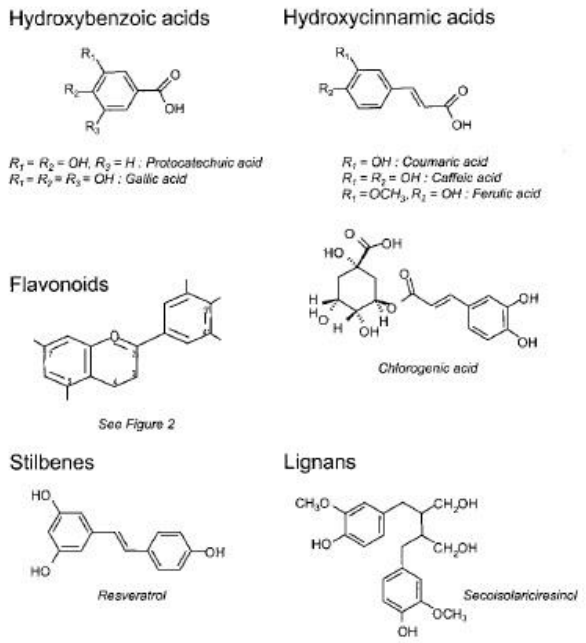

Figure 1 Chemical structures of polyphenols. Reprinted from "Polyphenols: food sources and bioavailability" by C. Manach, A. Scalbert, C. Morand, C. Rémésy and L. Jiménez, 2004, Am J Clin Nutr , 79, p 728. Copyright 2004 by American Society for Clinical Nutrition

The flavonoids (**Figure 1** [4]) have a C6-C3-C6 structural backbone and half of the so far discovered polyphenols belong to this class [10]. Rings in the chemical structure can be attached at different places, on which a distinction between subclasses of flavonoids can be made [4, 6]. The flavonoids are divided into six subclasses (**Figure 2** [4]): flavonols, flavones, isoflavones, flavanones, anthocyanidins and flavanols. The latter subclass also contains the catechins and proanthocyanidins.

Flavonols are the most common flavonoids found in foods. Quercetin and kaempferol are two examples of flavonoids. The concentration of flavonols in food is low and the biosynthesis needs sunlight, which explains why the concentrations of

flavonols are the highest in outer layers of fruits and vegetables [16, 17]. To give an example, cherry tomatoes have a higher flavonol content than standard tomatoes since the skin is a larger part of the whole fruit in cherry tomatoes. The second subclass of flavonoids consists of the flavones, which consist of glycosides of luteolin and apigenin. Parsley and celery are examples of flavone containing food sources. Flavanones are the third subclass of flavonoids and are found in high concentrations in citrus fruit. This subclass is glycosylated by a disaccharide. The solid parts of citrus fruit have the highest flavanone concentration, which clarifies why orange juice contains less flavanones compared to the normal orange [18]. Soya is the main source of the fourth subclass of flavonoids, namely isoflavones. Genistein, daidzein and glycitein are isoflavones present in soya. The total concentration of isoflavones in soya differs per geographical area, growing conditions and processing.

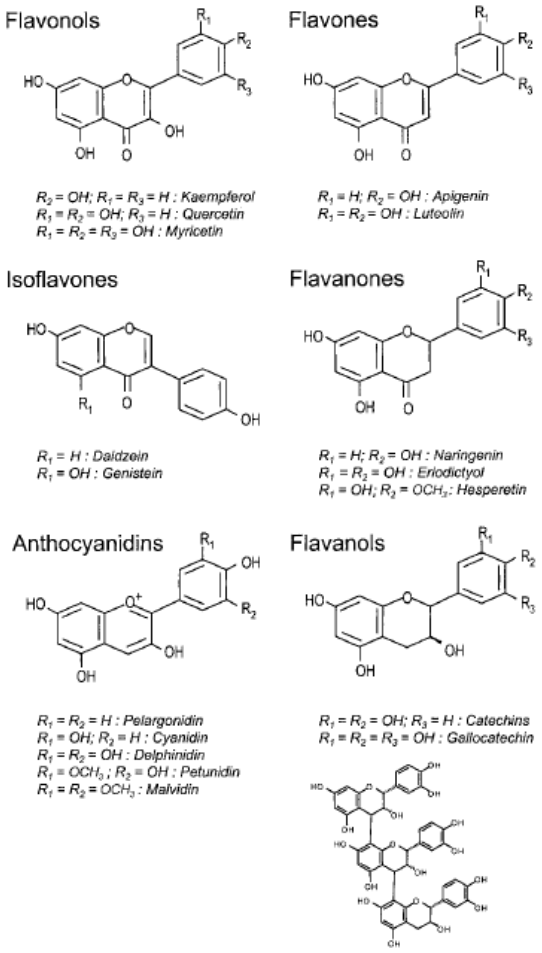

Figure 2 Chemical structures of flavonoids. Reprinted from "Polyphenols: food sources and bioavailability" by C. Manach, A. Scalbert, C. Morand, C. Rémésy and L. Jiménez, 2004, Am J Clin Nutr , 79, p 728. Copyright 2004 by American Society for Clinical Nutrition

The last two subclasses of flavonoids are the flavanols and the anthocyanins. Flavanols exist in two forms: as monomers (catechins) or as polymers (proanthocyanidins). In green tea and chocolate, catechins are the most abundant. Catechins and epicatechins are the main flavanols in fruit as well. Given that the flavanols are not glycosylated in foods, flavanols differ from other flavonoids and this difference influences their uptake in the GI tract [4]. Proanthocyanidins are bound as polymers between the C4 and C8 or C6 backbone. The exact polymerization degree of proanthocyanidins is not studied very often [19]. The determination of the proanthocyanidin content in foods is difficult, since these flavanols have many diverse structures and molecular weights [20].

The last subclass of flavonoids includes the anthocyanidins, which exist in both coloured and uncoloured forms. They are glycosylated and stabilized by the formation of complexes with other flavonoids, a process which is known as copigmentation. This subclass of flavonoids is merely found in red wine, though they are most common found in fruits. Food contents of cyaniding, one of the anthocyanidins are proportional to the colour intensity and increase when the fruit ripens.

The third class of polyphenols, the lignans (**Figure 1**), are metabolized in the body by microflora in the intestine. Metabolism of lignans results in higher enterodiol and enterolactone concentrations in plasma and urine. In plasma and urine higher levels of these substances are measured compared to the intake of

these polyphenols. Therefore, there might be other precursors in the diet which are also metabolized to enterodiol and enterolactone [21]. Linseed, also known as flaxseed, is the main dietary source of lignans and contains around a thousand times more lignans compared to other sources such as cereals, fruits and vegetables [22]. The main lignan which is present in linseed is secoisolariciresinol [14].

Stilbenes form the fourth class of polyphenols (**Figure 1**). Resveratrol is a stilbene which is studied more than once for its anticarcinogenic properties. It is found in low concentrations in red wine. The nutritional intake of stilbenes is very low, which makes any protective effect of this class of stilbenes unlikely [23-25].

Given that most knowledge exists about these four classes, they are the most valuable for Royal Cosun at this moment. The online database Phenol-Explorer [14] gives a clear, scientific overview of the polyphenol classes. It is regularly updated and may be useful for Royal Cosun. Besides the four classes of polyphenols described, more types of polyphenols exist (**Figure 3**). The Phenol-Explorer acknowledges two more classes: "other polyphenols" and a very small class of "non-phenolic acid metabolites". In addition, more subclasses of flavonoids are added to this database.

In the online database of Phenol-Explorer around 500 polyphenols are identified, whereas in literature already 8000 polyphenols are identified [6]. The website uses a critical analysis before including new polyphenols in the database. The data is based on scientific literature and quantitative studies contribute to aggregation. In 2013 the last update is done, covering 161 polyphenols or groups, before and after processing [26].

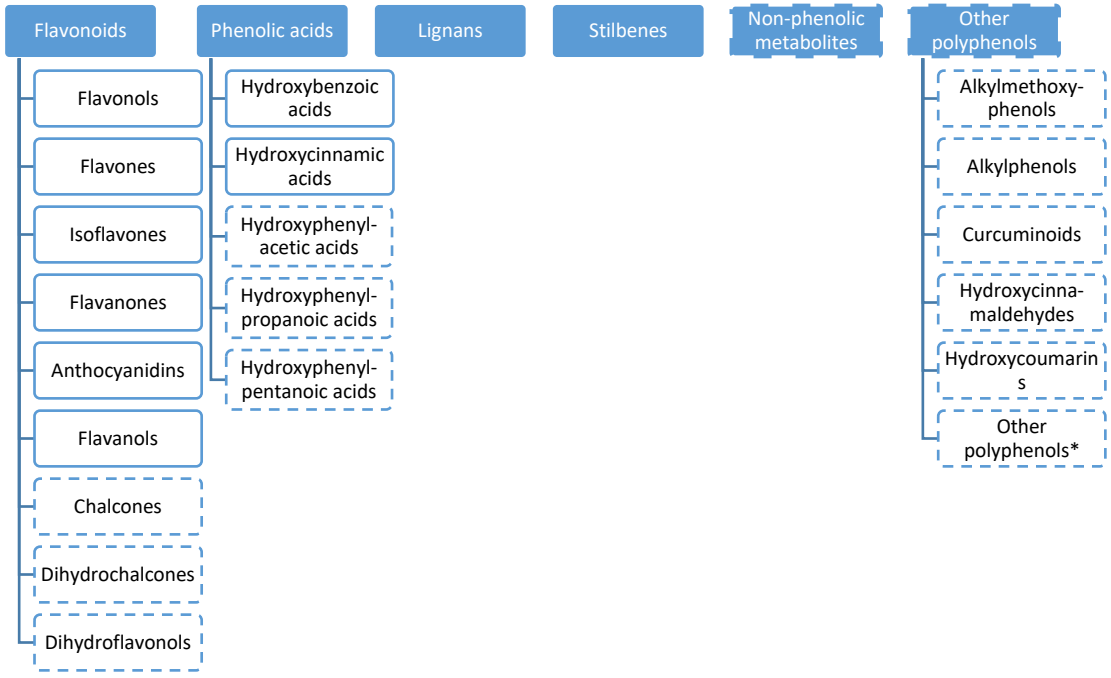

*Figure 3 The four main classes of polyphenols. Additional classes and subclasses according to Phenol-Explorer marked with a dashed line. * indicates there are more subclasses of other polyphenols, which can be find on Phenol-Explorer.*

The database is widely accepted and cited by 380 articles on Scopus over the last five years. Besides the polyphenol forms found in food, the database also contains metabolites and the foods in which the polyphenols can be found. Each polyphenol is presented on name, molecular weight, chemical formula and structure [26].

Polyphenol content in foods

In 2010, Pérez-Jiménez [5] identified the hundred richest dietary sources of polyphenols by using the Phenol-Explorer database [14]. Determination of the total polyphenol content in food is difficult, which is mainly due to the diversity in polyphenols [27, 28]. The hundred dietary sources with the highest contents of polyphenols were listed. The food group with the highest number of foods is the seasoning group (spices and herbs), followed by seeds and vegetables. Cloves contain the highest polyphenol content, namely 15.000 milligram per 100 gram of cloves. Rosé wine contains the lowest polyphenol content (7.8 milligram per 100 millilitre). The amount of polyphenols in a food serving was determined as well. This list, containing 89 foods, differs from the first, since serving sizes varies. For example, herbs are consumed in very little quantities, whereas fruit juices and alcohol are more commonly consumed. A ranking of food sources on polyphenol content may help in prioritize which food groups need more investigation in the future.

The polyphenol content of plants is affected by many factors. Environmental factors are one of these. Exposure to light and degree of ripeness are two environmental factors which contribute to the content of polyphenols in plants. Differences are observed between the degree of ripeness and the polyphenol content in plants in various classes of the polyphenols [29]. The concentration of phenolic acids lowers during ripening, whereas anthocyanin concentrations become higher. The phenolic acids are also vulnerable to stress: the more stress, the higher the phenolic acid content in for example strawberries [30]. Nowadays, it is still a challenge to determine all variables and their weights which induce stress for each family of plants. Besides environmental factors, also storage may influence the polyphenol content of foods. Polyphenols that are able to oxidize will form polymerized substances during storage, which subsequently will lead to beneficial or harmful changes to consumer acceptability. An example of a beneficial change is black tea; an example of a harmful change is browning of fruit. Cold storage does not seem to change the polyphenol content of apples and pears [31-33].

Next to environmental factors and storage, also methods of culinary preparation affect the polyphenol content of foods. As already mentioned before, most polyphenols are present in the outer layers of foods. Therefore, simply peeling fruits and vegetables can result in a lower polyphenol concentration. The same applies for potatoes; most polyphenols are found in the skin of the potatoes and therefore no phenolic acids are present in French fries [34]. Heat will affect the polyphenol content as well. Thermal processing increases the level of free polyphenols, probably due to the breakdown of nonextractable polyphenols. Polymerization and oxidation reactions may also underlie cause this increase in free level of polyphenols [35, 36]. On the other hand, boiling vegetables, cooking in a microwave and frying will result in a decrease in polyphenol content [37]. Therefore, steam cooking is a preferable culinary method to prepare foods. However, flavonoids seem to be more resistant against mechanical processing compared to other classes of polyphenols [38].

The last factor which influences polyphenol content of foods is industrial food processing, for instance during the production of fruit juices. Stabilization steps during this process aim at the removal of flavonoids, which are responsible for discoloration of the juice willen ze niet juist de kleur bewarenin hun sap ?. Polyphenols are known to cause colour [4]. Another example is the refinement of palm oil, which leads to a decrease in polyphenol content by 23% [39, 40]. Milling of whole grains results in a higher phenolic acid content than milling which results in white flour [41]. To understand the bioaccessibility of polyphenols in the body, we need to know more than only the polyphenol content in foods. In addition, the chemical and enzymatic actions need to be investigated in future research [42].

The number of factors which can influence the concentration of polyphenols in food and the large number of existing polyphenols makes it impossible to make a food-composition table for polyphenols. A food-composition table could help to calculate an individual's polyphenol intake from dietary questionnaires and to relate these intakes to diseases in epidemiological studies. In appendix A, a table is provided in which the polyphenol content of different food sources is shown.

Dietary intake of polyphenols

Information on dietary intake of polyphenols worldwide is lacking, however variability in polyphenol intake is enormous. The main reason for this variability is individual food preferences, which also go along with the geographical area individuals live. For example, since citrus fruits are basically the only source of flavanones, these polyphenols are likely to have a high intake in Southern Europe, where these fruits are highly consumed. The same accounts for soya intake in Asia [43-45], as individuals who live in America or Europe only consume a fraction of the amount of isoflavones Asian individuals consume. However, women who are following phytoestrogen replacement therapy in America or Europe increase their isoflavone intake due to the soya extract capsules [46]. In the Netherlands, consumption of monomer flavonols is high due to coffee and tea. In addition, other sources of polyphenols are consumed, such as chocolate, apples and pears [47]. The intake of hydroxycinnamic acids varies greatly with coffee consumption, since this is the main dietary source for this class of polyphenols [34].

Manach *et al.* [4] suggests that the total polyphenol intake reaches probably 1 g/d in people who eat several servings of fruit and vegetables every day. This is also supported more recently by Ovaskainen *et al.* [48]. A diet without polyphenols is basically impossible to achieve.

Bioavailability of polyphenols

Bioavailability is the fraction of an ingested nutrient or compound that reaches the systemic circulation and the specific sites in the human body where it can exert its biological action [49]. In other words, it means how much of the amount of polyphenols one ingests is able to exert its beneficial effect in target tissues in the body. Each food product contains a mixture of polyphenols and they can cause synergistic effects, therefore it is difficult to get final conclusions on the bioavailability and bioactivity of single phenolic compounds.

Several factors affect the bioavailability of dietary polyphenols in humans. In **Table 1** [50] an overview of the main factors is given.

Table 1
Main factors affecting the bioavailability of dietary polyphenols in humans. Reprinted from "Bioavailability of the Polyphenols: Status and Controversies" by M. D'Archivio, C. Filesi, R. Varì, B. Scazzocchio, R. Masella, 2010, Int J Mol Sci, 11, p. 1324. Copyright by the authors; licensee Molecular Diversity Preservation International, Basel, Switzerland.

External factors	Environmental factors (*i.e.*, sun exposure, degree of ripeness); food availability
Food processing related factors	Thermal treatments; homogenization; liophylization; cooking and methods of culinary preparation; storage
Food related factors	Food matrix; presence of positive or negative effectors of absorption (*i.e.*, fat, fiber)
Interaction with other compounds	Bonds with proteins (*i.e.*, albumin) or with polyphenols with similar mechanism of absorption
Polyphenols related factors	Chemical structure; concentration in food; amount introduced
Host related factors	Intestinal factors (*i.e.*, enzyme activity; intestinal transit time; colonic microflora). Systemic factors (*i.e.*, gender and age; disorders and/or pathologies; genetics; physiological condition)

In the previous chapter, the external factors and food processing related factors were discussed. In this chapter we will address food related factors, polyphenol related factors and host related factors.

Food related factors

Food matrix; presence of positive or negative effectors of absorption

Previous studies have shown that combinations of food compounds may affect the bioavailability of polyphenols, both in a positive and negative way.

For instance, the direct interaction between polyphenols and components in food, that is binding to proteins and polysaccharides, seems to affect the absorption. Likewise indirect effects of diet on gut physiology (intestinal fermentations, pH, biliary excretion, transit time, etc.) may also have an important role in absorption of polyphenols [51, 52].

To give an example, a study of McDougall *et al.* [53] showed that ice-cream added to raspberry juice reduced the total content of anthocyanins available in serum, whereas another study [54] revealed that the bioavailability of galloylated green tea catechine (EGC, EGCG, EC, and ECG) was increased by addition of bovine, soya- and rice milks, citrus juices or ascorbic acid.

Other studies have shown that the dietary fat content also affects the bioavailability of polyphenols. For example, a higher fat in cocoa content increases the concentration of procyanidin during *in vitro* digestion which subsequently leads to greater stability of procyanidins. This stability effect could be related to the enhancement of micellar structures within the small intestine which probably protects the procyanidin molecules from being enzymatically degraded during digestion [55].
Lastly, it has been suggested that dietary fibres have a negative effect on polyphenol absorption due to formation of gel, increased viscosity or the binding and entrapping of polyphenols [42].

Polyphenols related factors

Chemical structure

The chemical structure of the compound is one of the main factors influencing bioavailability. The chemical structure refers to the degree of glycosylation, acylation or polymerization, but also to their basic structures (i.e., benzene or flavone derivatives), conjugation with other phenolics, molecular size, and solubility [7].
In foods most of the polyphenols exists as polymers or in glycosylated forms, so with an attached sugar group. This sugar group is called glycine; the non-sugar group is called aglycone. These natural forms of polyphenols cannot be absorbed in the human body and have to be hydrolysed by the intestinal enzymes or by colonic microbiota before absorption is possible [50].

Host related factors

Intestinal absorption and the role of gut microbiota

In **Figure 4** [56], an overview of the absorption and metabolic pathways of polyphenols is displayed. After ingestion, oral saliva may modify polyphenolic structures. However, this capability to transform the native structures of polyphenols is rather weak [57]. In addition, the fate of polyphenols in the stomach is not yet clear, but assumed is that most of the polyphenols resist hydrolysis in the stomach by acid and arrive intact in the duodenum [58]. Aglycones, together with some other glucosides, are the only polyphenols which can be directly absorbed in the small intestine since the absorption of polyphenols is influenced by the way they are glycosylated [59]. Alternatively, some other polyphenols should reach the colon where they are hydrolysed by microbiota before they can be absorbed. [51, 52]. Given the smaller exchange area and a lower density of transport systems in the colon, absorption of polyphenols occurs less readily in the colon than in the small intestine. So, polyphenol absorption in the colon is slower and less efficient compared to the small intestine [4]. In the small intestine, two mechanisms are involved in the transport of glucosides into enterocytes, however the relative contribution of these mechanisms for the various glucosides is still unclear. The first mechanism concerns the sodium-dependent glucose transporter-1 (SGLT1) by which the glucosides could be transported into the enterocytes [60]. After being transported, they could be hydrolysed inside the cells by a cytosolic β-glucosidase (CBG) enzyme [61]. The other mechanism of transport of glucosides into enterocytes involves the lactase phloridzine hydrolase (LPH), a β-glucosidase enzyme of the brush border membrane of the small intestine. LPH catalyses extracellular hydrolysis of some glucosides, in other words, the release of sugar groups, which results in the formation of aglycones. These aglycones diffuse across the brush border in the small intestine [62]. However, research has shown that the substrate specificity of LPH can vary significantly

across the types of polyphenols. For example, only glucosides were efficiently hydrolysed by LPH, independently of the aglycone part of the glucoside.

Absorption of different types of polyphenols may also differ because of their polymeric nature and molecular weight. For example, proanthocyanidins, one of the most abundant dietary polyphenols, has a high molecular weight and therefore its absorption is limited through the gut barrier. Consequently, proanthocyanidins may only exert activity facilitated by phenolic acids produced by microbial degradation or local activity in the gastrointestinal tract. In addition, oligomers larger than trimers are likewise not expected to be absorbed in the small intestine in their native forms. *In vitro* studies have shown that only dimers and trimers of flavanols are able to cross the epithelium layer of the small intestine [4, 63]. Therefore, oligomers are likely to be transported to the colon where they are absorbed.

It has been estimated that only 5-10% of the ingested polyphenols are actually absorbed in the small intestine [57]. The remaining unmodified polyphenols that are not absorbed in the small intestine reach the colon. There, the colonic microbiota hydrolyses glycosides into aglycones and degrade them to simple phenolic acids [64, 65]. The activity of the microbiota is very important for the polyphenols to be biologically active, because they produce active metabolites [50]. The microbial metabolites are absorbed and are transported to the liver and small intestine where they undergo other structural modifications, due to the conjugation process [66].

The level of biotransformations that polyphenols undergo along the gastrointestinal tract is dependent on two main factors. The first factor is the specific structural subfamily of the polyphenol, because its chemical structure determines which transformations it can undergo by gut microbiota species. The second factor is richness of intestinal microbiota of individuals. Some specific biotransformations can be carried out by a wide range of gut microbial species and genera (e.g. deglycosylations), but other more specific chemical reactions on polyphenols require the presence of particular species or strains that contain genes coding for specific enzymes (as those responsible for intestinal generation of urolithins or (S)-equol). Marín *et al.*[56], Setchell *et al*. [67] and Lu *et al.* [68] found that only 30–40% of the Western people excrete significant quantities of equol after consumption of isoflavones, while this percentage in Japanese man is about 60%, according to a study of Morton *et al.* [69]. So, a great inter-individual variability exists in active metabolite production.

Concerning the influence of the chemical structure, different properties of the polyphenols play a role. For example, the type of initial glycosylation pattern of flavonols affect their degradation rates in the gut. Besides, the type of glycosidic bond (C- or O-glycosides) has influence on their degradation rates. Metabolism of a C-glycosidic bond seems to be much slower than the hydrolysis of an O-glycosidic bond. Due to the slow degradation, these compounds may be more bioavailable, because they have greater opportunity to be absorbed than the ones that are faster degraded in the colon [70]. Once flavonols have been metabolized into aglycones, they are extensively degraded by other colonic microbiota, producing simpler phenolic compounds which will be absorbed in the colon [56]. Furthermore, flavanones seem to be more bioavailable than other related flavonoids such as flavonols. A reason for this can be that these compounds are less degraded than other flavonoids by colonic microbiota and therefore are more available for absorption [71, 72]. Anthocyanins on the contrary do not seem to undergo extensive metabolism of the native glycosides to glucuronic-, sulfo- or methyl derivatives, and therefore their

bioavailability is very low [71]. So, only a small part is absorbed in the small intestine and large amounts will enter the colon. There they are deglycosylated by gut microbiota and able to be absorbed [73]. To conclude, intestinal transformations by microbiota and enterocyte enzymes are crucial for polyphenols in order to be absorbed at enterocyte and colonocyte levels.

Conjugation

After absorption the aglycones are subjected to three types of conjugation: glucuronidation by UDP-glucuronyltransferases(UGTs), sulfation by sulfotransferases (SULTs), and methylation by catechol-Omethyltransferases (COMTs) [74]. Glucornidation, sulfation and methylation represent three major classes of phase II metabolism.

UGTs can be found in many tissues and catalyse the transfer of a glucuronic acid from UDP-glucuronic acid to polyphenols. Glucuronidation of polyphenols first occurs in the enterocytes after which conjugation follows in the liver [75-77]. SULTs catalyse the transfer of a sulfate moiety from phosphoadenosine-phosphosulfate to a hydroxyl group on polyphenols. Sulfation occurs mainly in the liver [78]. COMT catalyses the transfer of a methyl group from adenosylmethionine to polyphenols that contain a diphenolic moiety, like quercetin, cyaniding, catechin, and caffeic acid. COMT is present in many tissues, but its activity is highest in the liver and the kidneys [79, 80]. Conjugation produces from some dietary polyphenols active metabolites, but it also increases the molecular weight and water solubility of the metabolites [81]. The nature and ingested dose of a substrate determine the relative importance of the three conjugation types. In general, sulfation is a higher-affinity, lower-capacity pathway than glucuronidation, thus an increased ingested dose causes a shift from sulfation to glucuronidation [82]. Besides, this balance between sulfation and glucuronidation also seems to be affected by species and sex, as was found in studies with mice and rats [83]. The capacity and efficiency of the conjugation process is high, since the concentration of free aglycones in plasma is usually very low after the intake of a nutritional dose [50]. Tea catechins are an exception, as their aglycones can account for a significant proportion of the total amount in plasma (up to 77% for epigallocatechin gallate) [84]. Dietary polyphenols are largely modified and consequently the compounds that reach cells and tissues are chemically, biologically and, in many instances, functionally different from the original dietary form [50].

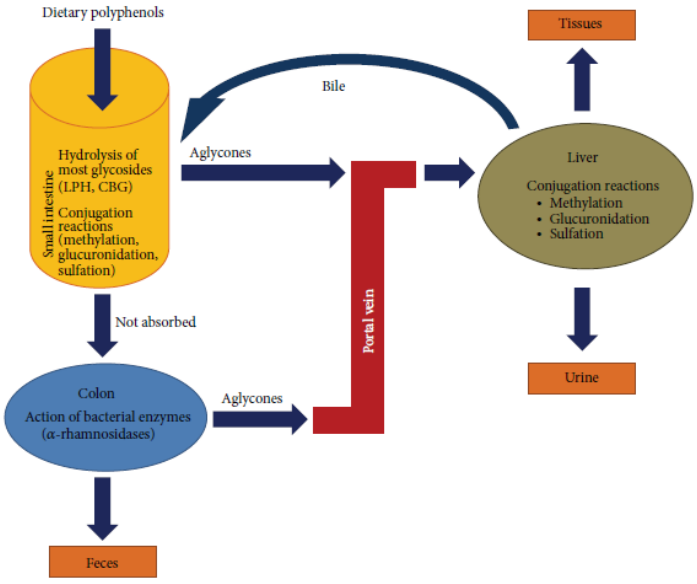

Figure 4 Absorption and metabolism routes for dietary polyphenols and their derivatives in humans. Reprinted from "Bioavailability of Dietary Polyphenols and Gut Microbiota Metabolism: Antimicrobial Properties" by L. Marín, E.M. Miguélez, C.J. Villar and F. Lombó, 2015, BioMed Res Int, p 3. Copyright 2015 by Laura Marín et al.

Tissue distribution and plasma concentrations

The consumed polyphenols first enter the plasma and tissues of the gastrointestinal tract. This was demonstrated by several animal studies [85-88] that used radiolabelled polyphenols doses, highest levels of radioactivity were found in blood, stomach, intestine and liver. However, other studies detected polyphenols in an extensive amount of various tissues in mice and rats with the use of HPLC analysis. These tissues include: brain [89, 90], endothelial cells [91], heart, kidney, spleen, pancreas, prostate, uterus, ovary, mammary gland, testes, bladder, bone, and skin [86, 92-94]. The concentrations obtained in these tissues ranged from 30 to 3000ng aglycone equivalents/g tissue depending on the dose administered and the tissue considered [4]. After uptake in the gastrointestinal tract, polyphenols and their metabolites enter the systemic circulation via the portal vein and the liver. Today, it is still not sure whether some polyphenols accumulate in specific target organs. In addition, the nature of the metabolites present in tissue may differ from metabolites in plasma due to the specific uptake or excretion of metabolites in tissues or due to intracellular metabolism. Plasma concentrations reached after polyphenol consumption, vary highly according to the nature of the polyphenol and the food source [4]. For example, since flavonoids are extensively metabolised in the body, mainly conjugates of flavonoids are found in plasma after ingestion of flavonoid containing foods ranging between nM and low µM concentrations [95]. For isoflavones, which are considered to be the best absorbed flavonoids, plasma concentrations of 1.4-4 µmol/L are reached after 6 to 8 h in adults who consume soya derivates containing about 50 mg of isoflavones. The plasma levels of anthocyanins are even lower: after ingestion of ≈ 110-200 mg anthocyanins, peak plasma concentrations are in the range a few tens of nanomoles per liter and occur after 30 min and 2 h after consumption [96, 97].

Elimination

The absorbed metabolites of polyphenols can be excreted either via the biliary or by the urinary tract. The large conjugated metabolites are often eliminated in the bile, whereas the monosulfates, i.e. the smaller conjugates, are most likely to be excreted in urine. When the conjugated metabolites are secreted in the bile, intestinal bacteria containing β-glucoronidases release free aglycones. Subsequently, the aglycones can be reabsorbed resulting in the enterohepatic cycling. Due to the reabsorption, a second plasma peak of the conjugated polyphenols can occur [4, 51]. The unabsorbed metabolites are eliminated in the faeces [56].

Human studies have shown that the total amount of excreted metabolites is more or less correlated with maximum plasma concentrations of intact polyphenols [4]. When the excretion of the intact polyphenol is low and the absorption is quite high, this indicates extensive metabolism. For example, while quercetin glucoside absorption is rather high (up to 50%), the excretion of intact quercetin in urine is low. This means the extent of metabolism by which quercetin is modified is high [51]. Low urinary values of excreted polyphenols may also indicate pronounced biliary excretion [4].

Comparative bioavailability of polyphenols

A compilation of 97 bioavailability studies is shown in **Table 2** [71]. Data of well-characterized polyphenol sources were converted to single 50-mg doses of aglycone equivalents, showed that gallic acids, which are phenolic acids, are the best absorbed polyphenols, followed by the isoflavones. Maximal plasma

concentrations (C_{max}) for the metabolites were 4 µmol/L and 2 µmol/L respectively (**Table 2**). Anthocyanins and proanthocyanidins are absorbed very poorly, resulting in C_{max} values of 0.03 and 0.02 µmol/L respectively. The compilation also shows that the time to reach C_{max} (T_{max}) is shorter for the polyphenols which are absorbed in the small intestine than for the polyphenols absorbed in the colon after hydrolyzation by the microbiota. These polyphenols, including gallic acid, quercetin glucosides, catechins, free hydroxycinnamic acids, and anthocyanins, reach their C_{max} in about 1.5 hour, whereas polyphenols like rutin and the flavanones hesperidin and naringin, reach their C_{max} at 5.5 hour. This supports that the mechanisms of uptake in the small intestine are more effective than in the colon, as described before. Surprisingly, chlorogenic acids, which are also absorbed in the colon, reach their C_{max} already after 1 hour. However, this might be due to the fact that the one study they used for this polyphenol in this compilation, chlorogneic acid was provided as a liquid to fasted volunteers which might accelerates the absorption kinetics. The relative urinary excretion was used to estimate minimal absorption rate. However, the polyphenols which are highly excreted in bile, like genistein, may be underestimated when using this method. Though, the urinary excretion values were consistent with plasma kinetic data for most polyphenols. With respect to the elimination half-lives, it appears that catechins, gallic acid, and flavanones have short half-lives ranging between 1-4 hours [71].

Table 2

Compilation of pharmacokinetic data from 97 bioavailability studies. Reprinted from "Bioavailability and bioefficacy of polyphenols in human. I. Review of 97 bioavailability studies" by C. Manach, G. Williamson, C. Morand, A. Scalbert, and C. Rémésy, 2005, Am J Clin Nutr, 81, p 238S. Copyright 2005 by American Society for Clinical Nutrition

	T_{max}		C_{max}		AUC		Urinary excretion		Elimination half-life	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
	h		*µmol/L*		*µmol h/L*		*% of intake*		*h*	
Daidzin	6.3 ± 0.6	4.0–9.0	1.92 ± 0.25	0.36–3.14	21.4 ± 6.5	2.7–38.6	42.3 ± 3.0	21.4–62.0	5.3 ± 0.8	3.4–8.0
Daidzein	4.9 ± 1.0	3.0–6.6	1.57 ± 0.52	0.76–3.00	12.2 ± 2.9	7.5–17.4	27.5		8.5 ± 0.8	7.7–9.3
Genistin	6.5 ± 0.6	4.4–9.3	1.84 ± 0.27	0.46–4.04	23.7 ± 6.7	6.2–45.1	15.6 ± 1.8	6.8–29.7	7.8 ± 0.7	5.7–10.1
Genistein	4.1 ± 0.6	3.0–5.2	2.56 ± 1.00	1.26–4.50	19.8 ± 6.5	10.4–32.2	8.6		7.1 ± 0.3	6.8–7.5
Glycitin	5.0		1.88 ± 0.38	1.50–2.26	7.9		42.9 ± 12.0	19.0–55.3	8.9	
Hesperidin	5.5 ± 0.1	5.4–5.8	0.46 ± 0.21	0.21–0.87	2.7 ± 0.7	1.9–4.1	8.6 ± 4.0	3–24.4	2.2	
Naringin	5.0 ± 0.2	4.6–5.5	0.50 ± 0.33	0.13–1.50	3.7 ± 1.5	0.9–7.0	8.8 ± 3.17	1.1–30.2	2.1 ± 0.4	1.3–2.7
Quercetin glucosides	1.1 ± 0.3	0.5–2.9	1.46 ± 0.45	0.51–3.80	9.8 ± 1.9	5.7–16.0	2.5 ± 1.2	0.31–6.4	17.9 ± 2.2	10.9–28.0
Rutin	6.5 ± 0.7	4.3–9.3	0.20 ± 0.06	0.09–0.52	2.9 ± 0.9	1.6–5.5	0.7 ± 0.3	0.07–1.0	19.9 ± 8.1	11.8–28.1
(Epi)catechin	1.8 ± 0.1	0.5–2.5	0.40 ± 0.09	0.09–1.10	1.1 ± 0.3	0.5–2.0	18.5 ± 5.7	2.1–55.0	2.5 ± 0.4	1.1–4.1
EGC	1.4 ± 0.1	0.5–2.0	1.10 ± 0.40	0.30–2.70	2.0 ± 0.8	1.0–3.6	11.1 ± 3.5	4.2–15.6	2.3 ± 0.2	1.7–2.8
EGCG	2.3 ± 0.2	1.6–3.2	0.12 ± 0.03	0.03–0.38	0.5 ± 0.1	0.2–0.9	0.06 ± 0.03	0.0–0.1	3.5 ± 0.3	2.5–5.1
Gallic acid	1.6 ± 0.2	1.3–1.5	4.00 ± 0.57	2.57–4.70			37.7 ± 1.0	36.4–39.6	1.3 ± 0.1	1.1–1.5
Chlorogenic acid	1.0		0.26				0.3			
Caffeic acid	1.4 ± 0.6	0.7–2.0	0.96 ± 0.26	0.45–1.35			10.7			
Ferulic acid	2.0		0.03				27.6 ± 17.6	3.1–61.7		
Anthocyanins	1.5 ± 0.4	0.7–4.0	0.03 ± 0.02	0.001–0.20			0.4 ± 0.3	0.004–5.1		
Proanthocyanidin dimers	2.0		0.02 ± 0.01	0.008–0.03						

[1] All data were converted to correspond to a supply of 50 mg aglycone equivalent.

T_{max}, time to reach C_{max} AUC, area under the plasma concentration-time curve EGC, epigallocatechin.

Mechanisms

General carbohydrates digestion primarily takes place in the intestine, in which the jejunum and ileum are the most important parts. In the intestine, carbohydrates will be broken down into glucose, which will be subsequently broken down by brush border enzymes of opgenomen in bloed ?. Polyphenols can influence glucose digestion in several ways (**Figure 5**), which will briefly be discussed in this chapter. Besides describing the effects of polyphenols on glucose metabolism, lipid metabolism will also be discussed, as well as the production of reactive oxygen species.

Inhibition of enzymes involved in glucose metabolism in the intestine

Carbohydrates that reach the intestine are broken down by several enzymes into glucose. The main enzymes responsible for this are α-glucosidase and α-amylase. They hydrolyse glucose linked to the starch and as a result glucose is released. Polyphenols could possibly influence starch breakdown by inhibition of the α-glucosidase and α-amylase enzymes. It has been shown *in vitro* that several polyphenols inhibit one or both enzymes [98, 99]. In a recent *in vitro* study by Striegel *et al*. [98] the inhibitory effect of polyphenols on those two enzymes in black tea water extract (WHT) and black tea pomace were studied. The water extract was used to create a low molecular weight phenolic enriched fraction (LMW) and a high molecular weight enriched fraction (HMW), as well as an hydrophobic fraction (HBBT). It was observed that the HMW was the most bioactive against α-glucosidase with an IC_{50} of 8.97 µg/ml. The IC_{50} is the concentration of an inhibitor where the response time of the normal process is reduced by half. However, the HBBT fraction was more bioactive against α-amylase (IC_{50} = 0.049 mg/ml). The black tea pomace had a significant inhibitory effect on α-glucosidase (IC_{50} = 14.72 µg /ml), but lower effect on α-amylase (IC_{50} = 0.21 mg/ml). These results indeed suggest that there exists an inhibitory effect of polyphenols on α-glucosidase and α-amylase. McDougall *et al.* [100] also showed the same inhibitory effect on α-amylase, as well as an inhibitory effect on protease activity and gastrointestinal lipase activity *in vitro,* which could influence carbohydrate digestion as well.

The main groups of polyphenols that show this inhibitory effect on α-glucosidase and α-amylase *in vitro* are flavonols [99, 101], including quercetin and catechins [102, 103]. Several *in vitro* studies also showed that the inhibitory effect was observed using polyphenolic extracts from food, such as tea, which is explained above [98], wine [53, 104] and fruits and berries [53]. In the study by McDougall *et al.* [53], it was shown that all fruit and berry extracts had an inhibitory effect on at least one of the two enzymes. However, the difference in polyphenolic concentration between the extracts with the highest and lowest effect was very high. Blueberries and black current, which contained the most anthocycin polyphenols, showed the highest inhibitory effect on α-glucosidase, while strawberries and raspberries, with the highest amount of phenolic acids, showed the highest effect on α-amylase. Removal of these tannins from the extract also stopped the inhibitory effect on α-amylase by the strawberry extract. The results from this study show that different polyphenols may influence different steps of the starch digestion.

If the same inhibitory effect on carbohydrate breakdown by polyphenols can be observed *in vivo* as observed *in vitro*, then the consumption of certain polyphenols may result in a lower blood glucose level, as glucose will be broken down in a slower rate. In an *in vivo* study by Hanamura *et al.* [105] it is indeed observed that there is a reduction in blood glucose levels in mice when fed a diet of polyphenols from

the Acerola fruit. It is suggested that the mechanisms involved are α-glucosidase inhibition and uptake transporter inhibition, which will be explained below. However, one should be careful drawing conclusions from this because in *in vivo* studies, given that other factors could interact with these results.

Uptake transporter inhibition

The hydrolysis of carbohydrates to monosaccharides is completed by brush border enzymes, allowing the absorption in the lumen by the enterocytes. Monomeric sugars require particular carriers to cross the phospholipidic membrane of the cells [106]. The most of these carriers are active, which means that they require energy in order to absorb molecules. These carriers can be facilitated by a sodium co-transporter (SGLT1), or by an independent sodium co-transporter (GLUT). The mechanism of sugar transport in the enterocyte is shown in **Figure 5**.

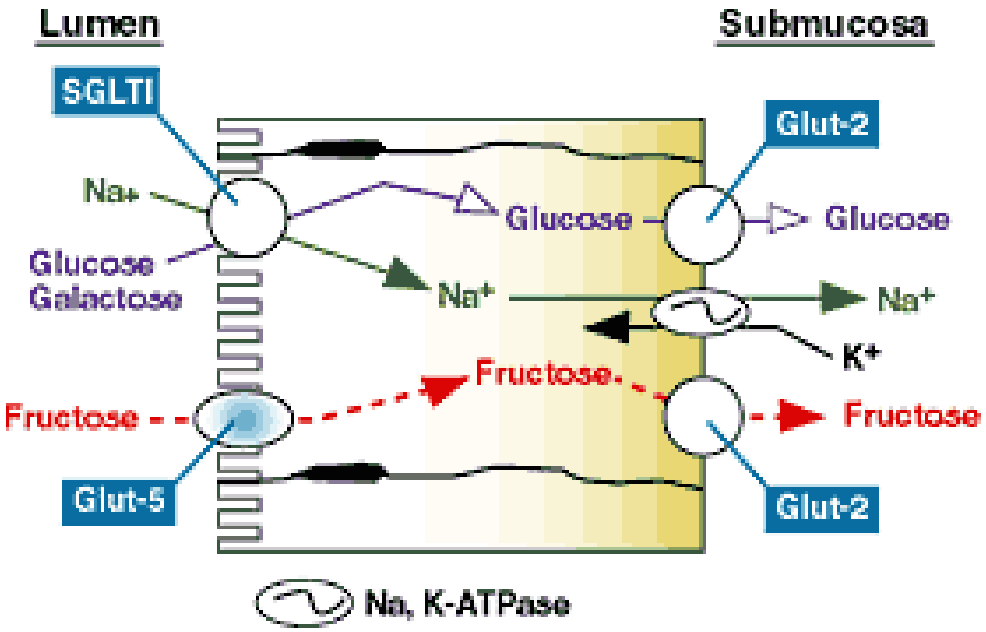

Figure 5 Mechanism of sugar transport. Reprinted from cellinteractive.com, by UCLA Center for Human Nutrition.

The kinetics of these carriers could influence the GI. When the activity of these carriers is high, more sugars can cross the intestinal membrane after which they are subsequently released in the blood stream, increasing the postprandial GI. Thus, by inhibiting the activity of SGLT1 and GLUT2, less sugar will be absorbed from the intestine and the glucose level in the blood will decline. *In vitro* studies demonstrated that the kinetics of this transporter could be reduced by the presence of some particular polyphenols. A study performed on Caco-2 cells [107], suggests that some polyphenols, present in tea leafs, particularly the non-glycosylated family, including catechin, epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC), could inhibit SGLT1 and GLUT2 transporters. Indeed, it was observed that all of these polyphenols caused a significant reduction in glucose uptake under sodium-dependent conditions, however under sodium-free conditions, only EGCG, EGC and ECG caused a significant reduction in glucose uptake. In the same study two other classes of polyphenols were studied: flavonoid glycosides and aglycones. It was shown that the flavonoids

(neohesperidin, dihydrochalcone and phloridzin) had significant inhibitory effects on SGLT1. However, in the case of aglycones (phloretin, apigenin and quercetin and myricetin), there was only a significant inhibition effect observed for GLUT2. In addition, relevant inhibition effects on SGLT1 activity from ECG and EGCG were also found in another study performed on rats [108] and this seems to confirm a correlation of this particular class of polyphenol with sugar carriers.

A study done with brush border-membrane vesicles from the jejenum of pigs [109] showed significant inhibitory effects of quercetin-3-O-glucoside and quercetin 4'-O-glucoside on the Na-dependent glucose transporter 1 (SGLT1). Quercetin is considered as a competitive inhibitor [58]; in other words quercetin binds to SGLT1 in the same binding site of the substrate. Since it cannot be absorbed by the small intestine *in vivo*, the kinetics of the transport of glucose in the lumen cells are reduced.

However, it should be noted that the polyphenols compete with sugars. Given that the sugars are present in a much higher concentration than the polyphenols, we suggest that the inhibitory effect on the GLUT carriers is very minor as the polyphenols are outcompeted by the sugars. Wel een effect bij lage suikerconcentraties ?

Pancreatic β-cells

After a meal, insulin secretion is increased in the body which enables it to absorb glucose. Insulin is released by pancreatic β-cells and thereby glucose will be taken up by the GLUT2 transporters. Glucose taken up by the transporter undergoes phosphorylation, which generates an increased ATP concentration in the cells. This increased level of ATP causes the inactivation of ATP-sensitive K-channels on the cell membrane, which in turn leads to depolarization of the membrane. This depolarization induces the opening of calcium channels and therefore a flow of Ca^{2+} into the cells is generated. The rise in Ca^{2+} concentration promotes the release of insulin by the pancreatic β-cells [110]. Continued high levels of glucose can lead to the dysfunction of the pancreatic β-cells, which is associated with development of diabetes type 2 [111]. As mentioned above, polyphenols show an inhibitory effect on the glucose uptake by GLUT2 transporters, which is the start of the mechanism of insulin release by the pancreatic β-cells.

To illustrate, in a study of Prince *et al*. [112], normal and Streptozoticin (STZ) induced diabetic rats were fed a diet which contained the flavonoid rutin for 45 days. It was shown that there was a significant lowering effect in plasma glucose and a significant increase in insulin levels, as well as restoration of glycogen content in the pancreas in the STZ diabetic rats. However, the same effects were not observed in the normal rats without diabetes.

In addition, in a study performed by Kobori *et al.* [113], STZ diabetic mice were given a diet with either 0.1 or 0.5 % of quercetin. Again, a significant lowering in blood glucose levels and improved plasma insulin levels were observed. In this study, it is suggested that the positive effect of quercetin is due to the suppression of STZ-induced alteration on gene expression, primarily the suppression of the cyclin-dependent kinase inhibitor (Cdkn1a). This enables the recovery of cell proliferation and therefore might improve liver and pancreatic function.

These results show that polyphenols may increase the health of diabetic rats, as it improves liver and pancreatic function, improve plasma insulin levels and lowers blood glucose levels. However, in non-

diabetic rats, it remains unclear if polyphenols help in the prevention of diabetes. Therefore, future *in vitro* research should focus on healthy rats instead of rats with diabetes.

In **Figure 6** [114], an overview of the possible sites of action of polyphenols on glucose digestion is shown, as discussed in this chapter.

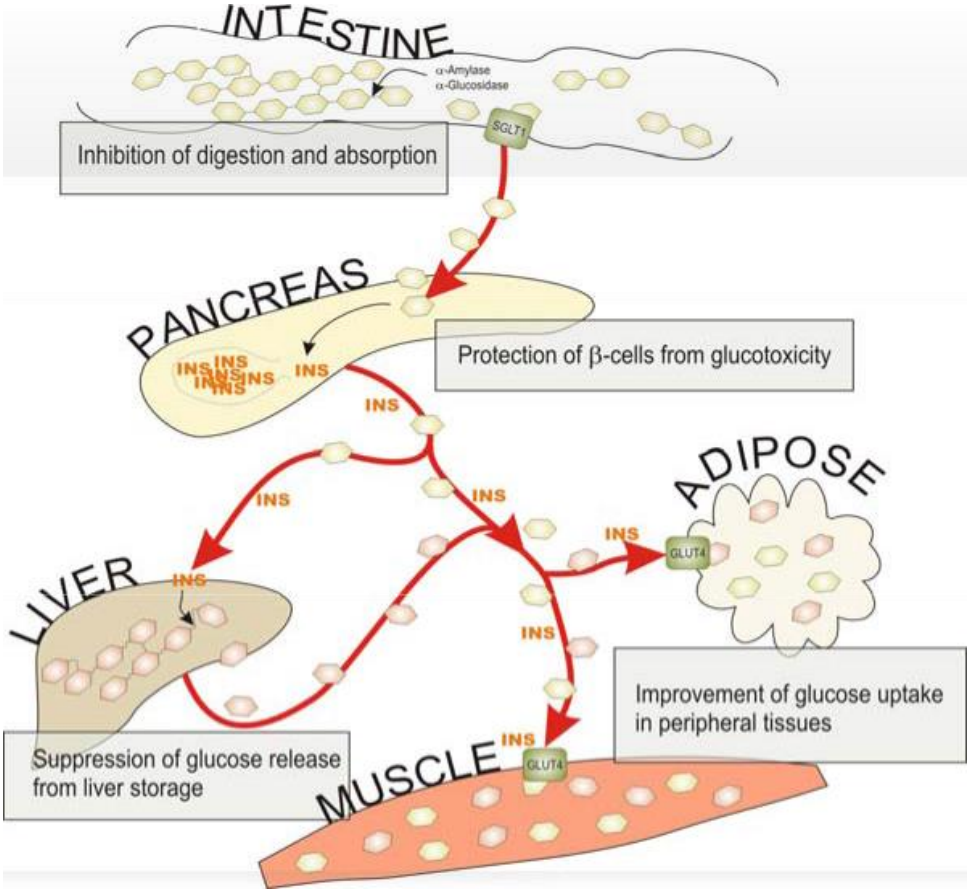

Figure 6 Potential sites of action of dietary polyphenols on carbohydrate metabolism and glucose homeostasis. Reprinted from "Impact of Dietary Polyphenols on Carbohydrate Metabolism" by K. Hanhineva, R. Törrönen, I. Bondia-Pons, J. Pekkinen, M. Kolehmainen, H. Mykkänen and K. Poutanen, 2010, Int J Mol Sci, 11, p. 1367. Copyright 2010 by the authors.

Effects on liver function and lipid metabolism

During fasting, the liver is an essential organ. It regulates blood glucose levels to maintain the body in homeostasis. After food consumption, it can be used or stored in our body. During fasting, triglycerides are produced in the liver, which are subsequently released into the bloodstream. They can also be converted into cholesterol. In obese people, more food gets converted into triglycerides and cholesterol, which leads to hypertriglyceridemia; a condition in which there is an excess of the triglycerides in the bloodstream. Many studies indicate that some polyphenols can affect glucose metabolism in hepatic tissue [115-122]. In this chapter the influence of polyphenols on hepatic function will be discussed with examples from animal studies.

An animal study from Wolfram *et al.* [122] showed that epigallocatechin gallate (EGCG), a catechin from green tea, has a decreasing effect on blood glucose levels and on hepatic triglycerides. They suggest that a possible mechanism of catechins comprises the induction or repression of the expression of genes coding for specific enzymes. In most cases, induced expression is dominant over repression. It was shown that supplementation with EGCG increases expression of hepatic glycogenic enzymes, like glucokinase. Glucokinase is an enzyme that facilitates phosphorylation of glucose to glucose-6-phosphate [123] and can be found in the liver, pancreas, small intestine, and brain tissues. In the pancreas, glucokinase regulates insulin secretion, so mutations in glucokinase genes can lead to hyper- as well as hypoglycaemia [124]. The enzyme plays a pivotal role as a "glucose-sensor" in recognition of blood glucose levels in the bloodstream and regulation of carbohydrate metabolism. When the enzyme is working properly, blood glucose homeostasis is maintained. The study of Osbak *et al. [124]*, detected a decreased expression of other gluconeogenic enzymes, for example of phosphoenolpyruvate carboxykinase (PEPCK). PEPCK is one of the essential enzymes in gluconeogenesis, which facilitates conversion of oxaloacetate into phosphoenolpyruvate and carbon dioxide. In gluconeogenesis glucose is formed from metabolic precursors. Overexpression of PEPCK could lead to symptoms of type II diabetes mellitus and similar conditions. According to Wolfram *et al.* [122], polyphenol supplementation leads to a decreased endogenous liver glucose production and to an increased glucose-induced insulin secretion.

The mechanism of genistein and daidzein, two examples of soya isoflavones, has also been linked lower blood glucose and hepatic triglyceride concentrations [120]. This study was done in db/db mice. The db/db mouse is an animal model used for obesity and diabetes studies. In these mice leptin receptor for the fat cell-specific hormone leptin, known as satiety hormone, is deficient due to a point mutation in the gene for the leptin receptor [125]. Lower blood glucose and hepatic triglyceride concentrations were found in a study using non-obese diabetic mice as well [117]. It was also concluded that soya isoflavones reduce activity of glucose-6-phosphatase (G6Pase) and hepatic PEPCK [120]. G6Pase hydrolyses glucose-6-phosphate into free glucose and into a phosphate group. Activity of this enzyme is likewise reduced when isoflavone supplementation is applied to another mice model, as was found by Cederroth *et al*. [126]. Glucokinase activity was increased, therefore these two studies suggest that soya isoflavones genistein and daidzein suppress liver glucose output [120, 126].

Hesperidin and naringin are flavonoids which can be found in citrus fruits. A study of Jung *et al.* [118] suggests that these flavonoids influence glucokinase expression in the liver. An increase in mRNA levels coding for this enzyme was observed after supplementation with hesperidin and naringin [119]. Decreased expression of PEPCK and G6Pase were observed upon naringin supplementation.

Besides, other mechanisms of soya supplementation were studied by Cederroth *et al.* [126]. They found overexpression of peroxisome proliferator–activated receptor (PPARγ) and of the PPAR co-activator (PGC-1α) in liver, muscle and in white adipose tissue. PGC-1α is a transcription co-activator that plays a crucial role in the regulation of cellular energy metabolism, stimulation of mitochondrial biogenesis and in the regulation of both carbohydrate and lipid metabolism. Its expression and activation is stimulated by AMP-activated protein kinase (AMPK) [127]. PGC-1α overexpression resembles training, which results in increased fat oxidation and increased oxidative capacity [128]. **Figure 7** [128] shows how PGC-1α works as co-activator and binds to transcription factors. When these transcription factors bind to DNA, transcription of different proteins involved in fatty acid oxidation and mitochondrial biogenesis will be induced.

Furthermore, Cederroth *et al.* [126] suggests that PPARγ overexpression may lead to improved fatty acid beta-oxidation, the process in which fatty acids are broken down to produce energy [126, 127]. When mice were fed with soya, there was a significant increase in phosphorylation and gene expression of AMPK and acetyl-CoA carboxylase, both essential enzymes in β-oxidation. Moreover, genes involved in peroxisomal fatty acid oxidation and mitochondrial biogenesis were overexpressed, serum insulin levels were lowered and more glucose was absorbed into the skeletal muscle tissue [126].

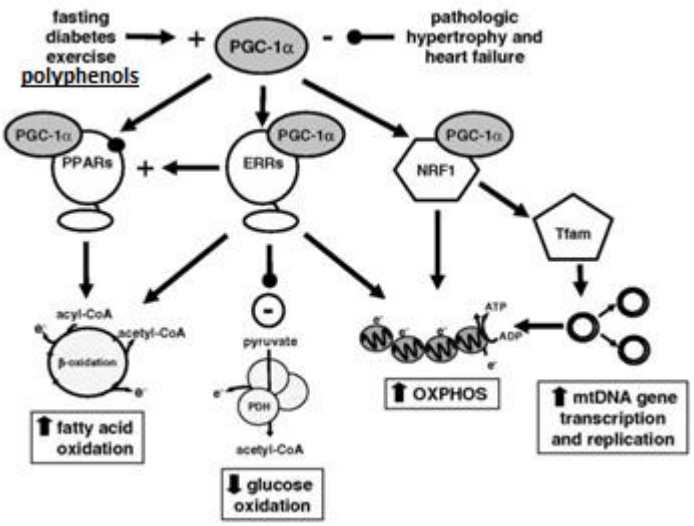

Figure 7 Influence of PGC-1α on transcription factors and its effects on metabolism.

So, it is assumed that some isoflavones, for example genistein from soya, can stimulate fatty acid oxidation in the skeletal muscle [126, 129]. In the study of Palacios-González *et al.* [129], it was reported that genistein fed rats showed overexpression of genes involved in fatty acid oxidation in the skeletal muscle through AMPK phosphorylation [129]. When AMPK is phosphorylated, it becomes active [130]. AMPK has several functions, it stimulates hepatic fatty acid oxidation and ketogenesis, it inhibits cholesterol synthesis, lipogenesis, and triglyceride synthesis, it stimulates skeletal muscle fatty acid oxidation and muscle glucose uptake, and it modulates insulin secretion by pancreatic beta-cells [131]. Several other studies found activation of AMPK by polyphenols [126, 127, 130, 132, 133].

Cederoth *et al.* [126] found overexpression of PGC-1α after soya consumption. This gene is involved in mitochondrial biogenesis. PGC-1α activity is highly regulated by post-translational phosphorylation and acetylation, which is primarily controlled by SIRT1 [134]. It has been shown (*in vitro*) that several polyphenols can activate SIRT1 and polyphenols may induce mitochondrial biogenesis via this mechanism [134]. Resveratrol, quercetin, hydroxytyrosol, isoflavones (like daidzein, genistein, and fromononetin) and EGCG all have been shown to be involved in the SIRT1/PGC-1α pathway and increased mitochondrial biogenesis [135-141]. A study with humans of Taub *et al*. [142] showed that administration of an epicatechin-rich cocoa to patients with type 2 diabetes and heart failure stimulated mitochondrial biogenesis in skeletal muscle. This stimulation occurred via increased expression of SIRT1 activated PGC-1α [143]. Another *in vivo* study has shown that curcumin up-regulates PGC-1α in the brain of (senescence-accelerated) mice [144]. Thus, polyphenols belonging to stilbenes, flavonols, phenolic alcohols, isoflavones, flavones and flavan-3-ols seem to induce mitochondrial biogenesis. However, most data is obtained from *in vitro* studies and little evidence is present from *in vivo* studies. The studies available are still insufficient to conclude on the actual potential that polyphenols may induce mitochondrial biogenesis in humans [134].

Polyphenols and ROS production

Polyphenols may accelerate pro-oxidant reactions *in vitro*, which is discussed in this chapter followed by the influence of reactive oxygen species (ROS). Most studies conducted on this topic so far focused on

the polyphenols present in green tea, namely the flavonoids compounds EGC and EGCG. Sang *et al.* [145] showed that these polyphenols are unstable and in the human body undergo auto-oxidation which ultimately results in the production of ROS. The same study also showed that the concentration of the present EGCG, the pH of the system, the incubation temperature and the presence of oxygen are important determinants of stability of EGCG.

EGCG and EGC can react with H_2O_2 which leads to oxidation of the A ring of both compounds (**Figure 8** [16]). Subsequently, decarboxylation will follow to form one oxidation product of EGC and two oxidation products of EGCG [146]. It has also been shown that transition metals play an significant role in the formation of H_2O_2 mediated by ECGC [147]. In the presence of iron or copper, such reactions, also known as Fenton reactions, produce hydroxyl radicals (HO•) [148]. Hydroxyl radicals are capable of reacting with organic matters like carbohydrates, lipids, amino acids and nucleic acids [149]. It is stated that green tea polyphenols do not induce DNA damage mediated below concentrations of 10 µM, but rather prevent DNA damage caused by hydrogen peroxide in a dose dependent manner.

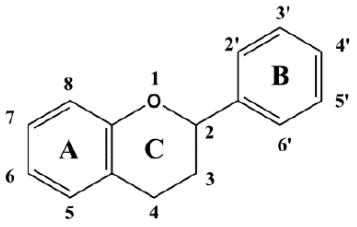

Figure 8 The basic chemical structure of the main flavonoids compounds. Retrieved from "Flavonols and flavones in food plants: A review" by K. Herrmann, 1976, Int. J. Food Sci. Technol. 11, 433–448. Copyright by author.

Influence of ROS

ROS may have beneficial roles in the body, also known as mitohormesis. It is suggested that they induce molecular mediators of insulin sensitivity and antioxidant defence mechanisms of the human body [150]. In a human clinical trial study of Ristow *et al.* [150], it is suggested that there is an essential role for exercise-induced ROS formation in promoting insulin sensitivity in humans. They found an induction of several molecular regulators of insulin sensitivity, including PPARγ and its two co-activators PGC1α and PGC1β, which regulate the insulin-sensitizing gene expression. Besides, they found induction of SOD1, SOD2 and glutathione peroxidase, which are molecular mediators of endogenous ROS defence [150].

Other studies using animal models support the finding that increased ROS formation counteracts insulin resistance [151, 152]. Besides, Patti *et al.* [153] found that decreased PGC1 expression may be responsible for decreased expression of NRF-dependent genes, leading to the metabolic disturbances characteristic of insulin resistance and diabetes mellitus. Lastly, an *in vitro* study of St. Pierre *et al.* [154] showed significantly increased expression of PGC-1α and PGC-1β upon H_2O_2 treatment.

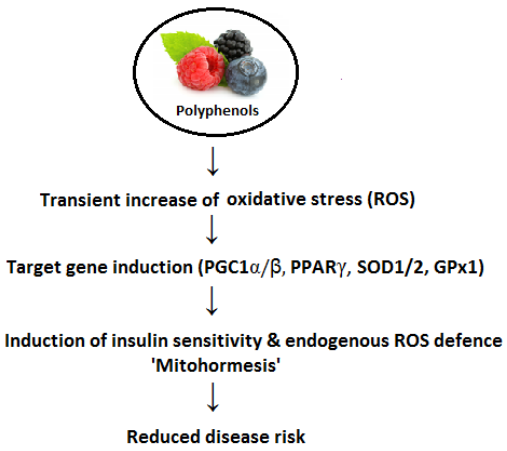

Figure 9 Effects of ROS on disease risk.

To conclude, there could be a potential role for ROS in insulin sensitivity via the activation of PGC1 genes. Besides, PGC1 genes are involved in mitochondrial biogenesis. Therefore, ROS may increase mitochondrial biogenesis via this mechanism. This will result in improved oxidative capacity and lipid oxidation, so more energy is burned. In **Figure 9** the effects of ROS on disease risk are summarized.

Discussion

In the last few decades, the interest in polyphenols greatly increased as it could be a potential beneficial source in fighting metabolic diseases. However, polyphenols are also a widely discussed topic with many different views. In the following discussion we will go into the controversies surrounding polyphenols.

Studies

Methodology of studies is important when evaluating strength of studies. Therefore, the impact factor of scientific journals will be discussed, followed by the advantages and disadvantages of different study designs.

Impact factor

The impact factor of scientific journals is an indication for the average number of citations to recent articles published in that journal. The average impact factor for the scientific journals used in this review is 5,27. The journals with the highest impact factor were Nature, Journal of American Medical Association and Cell.

Cross-cultural studies

In vitro and *in vivo* studies are not the only types of studies that show health benefits of polyphenol. Cross-cultural studies might give insight in this topic as well. One of the most relevant examples of a cross-cultural study is the "French Paradox"; an epidemiological study in the north of France where the local diet is rich of saturated fats. A low incidence of coronary heart disease (CHD) was observed compared with other countries with similar diet, such as in the U.K. (**Table 3**) [155].

Table 3 Age standardised coronary heart disease (CHD) mortality and event rate in selected European regions (men, aged 35–64 years). Reprinted from "THE FRENCH PARADOX: LESSONS FOR OTHER COUNTRIES" by J. Ferrières, 2004, Heart, 90, p. 108. Copyright by the author.

Population	Official CHD mortality rate per 100000*	CHD mortality per 100000†	Coronary events per 100 000‡
Glasgow (UK)	332	365	777
Belfast (UK)	280	279	695
Lille (northern France)	89	172	298
Strasbourg (north eastern France)	80	141	292
Toulouse (south western France)	53	91	233
Barcelona (north eastern Spain)	63	76	210

*Based on death certificate enumeration.
†Fatal events included definite, possible, and unclassifiable (mainly sudden deaths with no available diagnostic information) coronary deaths.
‡Coronary events included non-fatal events (definite myocardial infarction) and fatal events.

The same study suggests that the low incident of CHD is due to the consumption of red wine. Indeed, a moderate alcohol consumption is negatively associated with CHD [156]. In particular in red wine, this negative correlation is stronger with respect to other alcoholic drinks [157], due to the presence of catechins [158]. Another cross-cultural study, done with Kuna Indians in San Blas Islands (Panama), seems to confirm the negative correlation of polyphenols and CHD [159]. In this epidemiological study, it was shown that in that population, the increase of blood pressure due to an increasing age is very low. The cause of a lower blood pressure in older individuals might be due to a higher consumption of cocoa

(an average of 5 cups per day). One of the first hypothesis, which might explain this situation, attributed this found result to genetic factors. This theory was disproved by the fact that when San Blas Islands habitants moved to the city and subsequently changed their diet, their age-related blood pressure increased [158]. A high consumption of cocoa, which is rich in flavonoids, might therefore lead to a lower incidence of CHD.

Epidemiological studies are very useful to observe certain relationships (cause and effect) and by this steer future research. However, it is important that the epidemiological studies are confirmed *in vitro* and *in vivo* to really verify the observed relationships on an individual level, as it is not sure if the observed cause and effect is due to the variable proposed in the studies or due to other cultural differences.

In vivo/in vitro

Many studies have been conducted on polyphenols, but it is important to distinguish the *in vivo* and *in vitro* studies; *in vivo* studies are performed directly on humans or animals while *in vitro* studies are a simulation of the digestive process performed in the laboratory. Most of the studies performed on polyphenols are *in vitro* studies, which are very useful to understand certain mechanisms of polyphenols. Moreover, they can be performed fast, they are relatively inexpensive and it is easy to control variables when compared to *in vivo* studies. However, the exact mechanisms observed *in vitro* often do not represent the mechanisms *in vivo*. When performing an *in vitro* study, the interactions between the real digestive system and polyphenols are not measured. Most of the *in vitro* studies on polyphenols did not take this interaction into account. It is shown that phenolic OH groups are methoxylated, or conjugated with glucuronic acid or sulphate by the intestinal metabolism affecting their biological properties and activity [158, 160-162]. Moreover, the dose used in *in vitro* studies often does not reflect the same amount one could consume in real life hoeveelheid kan “geregeld” worden door concentreren / isoleren van polyfenolen, because the amount used in the studies is higher, causing an overestimation of the outcome.

Thus, when looking at *in vitro* studies of polyphenols, one should be aware of this since the results from *in vitro* studies may not correspond to what is observed *in vivo*. *In vivo* studies have some limitations as well; in particular in animal studies physiological differences between laboratory animals and humans are clearly observed [10]. However, human studies are very hard to conduct, because they are more expensive, it is often difficult to find enough willing test subjects and ethics should be taken into account. Thus, to get an overview of a certain mechanism, it is necessary to conduct both *in vitro* and *in vivo* studies.

Low concentrations of polyphenols in diet compared to in vivo studies

hoeveelheid kan in een diet “geregeld” worden door concentreren / isoleren van polyfenolen The difference in the amount of polyphenols used *in vitro* and *in vivo* studies has been discussed previously. However, there is often also a large difference between the doses of polyphenols used in *in vivo* studies on mice and the dose humans consume with a normal diet [95, 163]. While most studies mention this point in their discussion, it is still important to be aware of this difference in doses. Hoek-van den Hil [95] uses doses of 350 to 500 mg/kg bodyweight/day of flavonoids in the experiments performed on mice. In

contrast, the intake of flavonoids of humans in a normal diet is between 1.5 and 15 mg/kg bw/day [4]. Thus, in this study, the amount used on mice is between 200 and a 1000 times higher than normal human dietary intake and 15-100 times higher than human intake from the use of flavonoid supplements [95].

While studies with such high doses are very useful to show significant effect of polyphenols, it remains uncertain if the doses consumed by humans in a normal diet will show the same effects. The most potent way to gain more insight on these matters is by conducting human studies using isolated compounds at dietary levels. In two studies using this methodology, they found a possible link between polyphenols and endothelial function [164, 165]. However, as with most human studies, the samples sizes used were rather low. In conclusion, *in vivo* studies on mice are very useful to gain insight in certain effects or mechanisms of polyphenols, but the doses used are often much higher than humans consume in their normal diet. The effects observed in these studies may thus not be observed in humans. Therefore, it is necessary to be careful when drawing conclusions from studies with non-pharmacological doses.

Uptake of polyphenols

As we described in the bioavailability chapter, the concentrations of most polyphenols do not reach a higher concentration than 2 µmol/L in the blood plasma. However, epidemiological and cross-cultural studies do indicate a beneficial effect of polyphenols. Although many *in vitro* and animal studies also show positive results, it is not yet known how polyphenols effect the human health, because it seems impossible with such a low uptake. Some studies [166, 167] suggest that the uptake should be higher. Polyfenolen kunnen ook werkzaam zijn zonder in het bloed opgenomen te zijn, zoals de remming van enzymen of invloed op / van micro-organismen, fermentatie, vorming metabole producten in de darm

Soya foods are rich of isoflavones, a group of flavonoids. In those studies it is reported that soy-isoflavones bind to the estrogen receptor (ER), which indicates that isoflavones compete with the endogenous estrogens. The affinity of the endogenous estrogen binding with the ER is very high. Thus, to compete with estrogen, the affinity of the isoflavones must be very high. This is just a hypothesis, but it may be of importance to take into account when studying the mechanism of polyphenols. . The results in this study indicate that isoflavones do have a bioactivity that influences processes in the body which suggests that the uptake of isoflavones can be higher than the known concentrations to indeed be effective [166, 167].

An explanation for this higher uptake could be related to the metabolites of polyphenols. As discussed in the bioavailability chapter, the microbiota in the intestine convert polyphenols into active metabolites. The metabolites are the bioactive forms of polyphenols. A study on black tea extracts suggests that metabolites of polyphenols are present in higher concentrations in blood plasma than dietary polyphenols [168]. The high concentration of metabolites makes the effects likely to occur. In the study of Duynhoven *et al.* [168], the concentrations of conjugated metabolites and catabolites were measured in blood plasma. The plasma the concentration of the polyphenols in black tea (catechin, EGC, EGC and EGCG) is 0.008 µmol/L. It only takes two hours before this concentration reaches its maximal level. The maximum plasma concentrations of the metabolites, which are called valerolactones, are higher, up to 0.034 µmol/L and stay in the blood up to six hours. Remarkable are the concentrations of catabolites of

phenolic acids (pyrogallol-sulfate and hippuric acid). These reach a maximal concentration of 2 - 2.6 µmol/L and remain in the plasma up to 30 hours [168]. The metabolites of polyphenols can be found in the blood plasma at a 325 times higher concentration than the polyphenols themselves. Besides that, the metabolites circulate about 10 times longer than dietary polyphenols. These bioactive metabolites can therefore reach a high steady-state if the precursors are consumed on daily basis. Knowing this, it is likely that the metabolites are responsible for the beneficial effects instead of the polyphenols in the form they are consumed.

In vitro studies often use dietary polyphenols as a sample and very high polyphenol doses are needed in order to be able to see significant results. It is suggested for *in vitro* studies to work with metabolites in a lower concentration, to find results which are closer to the reality of a human daily diet. More research on the mechanism of the metabolites could be useful in understanding the uptake and beneficial effects of polyphenols.

Another speculation concerning the uptake of polyphenols concerns the storage of wine in oak barrels. When wine is stored in these barrels, the polyphenols in wine will form polymers. Polymers are not easily absorbed in the intestine, they are converted into aromatic acids and reach the colon. This is the same mechanism which is also observed in tea. This hypothesis concerning storage in oak barrels definitely needs more research.

Adverse effects of polyphenols

Polyphenols might have beneficial effects, however an overload of polyphenols, due to a supplementation, can cause some side effects. High intake of polyphenols can reduce the bioavailability of iron. Indeed, polyphenols bind to iron which results in forming an insoluble compound that cannot be absorbed from the intestine [106]. The inhibitory effect of polyphenols on iron absorption is confirmed by an human study of Hurrell *et al.* [169]. In this experiment, iron absorption was calculated after receiving different beverages rich of polyphenols such as black tea, red wine and cocoa. The results of this study show significant reductions of iron absorption after ingestion of each beverage, compared with the control. Black tea showed the highest inhibitory effect. Thus, people who have a higher risk of iron deficiency such as pregnant woman, vegetarians and people in growth should be aware to not reach an overload of polyphenols. Iron is not the only micronutrient from which the absorption can be influenced by polyphenols. Isoflavones, in particular genistein, might have some goitrogenic capacity. In other words, isoflavones may influence the iodine utilisation in the body [106]. An *in vitro* and *in vivo* study [170] showed that genistein could inhibit the activity of rTPO, which is the enzyme responsible of the production of thyroxine (T4) or triiodothyronine (T3), the thyroid hormones. These hormones are combined with iodine responsible for the regulation of metabolism. However, human studies still fail to demonstrate that isoflavones can improve the risk for hypothyroidism [95].

Others enzymes could be inhibited by a high presence of polyphenols as well. For example, gallic acid can have some inhibitory effects on protease enzymes and in particular the collagenase enzyme [171]. The amino acids which form proteins, can only be absorbed in the intestine in monomeric or in dimeric form by specific transporters. Therefore, they need hydrolysation by the protease enzyme [106]. The inhibition of protease enzymes can reduce the uptake of some amino acids in the intestine because

there are no specific carriers for long chains of amino acids. Reducing the uptake of a particular class of amino acids, such as the essential amino acids, could lead some side effects in our protein turnover or in the function of some structural proteins, enzymes or transporters [106].

Besides these above mentioned adverse effects, some studies indicate that quercetin could have genotoxic effects. High intake of quercetin has been associated with an increased incidence of cancer. The GI tract and the liver are most likely to be affected first by polyphenols. However other studies, which used the same dose of quercetin, did not show any adverse effects [95].

Advice for Royal Cosun

Side-streams and type of polyphenols

Since polyphenols can be found in almost all fruits and vegetables we recommend Royal Cosun to focus on these side-streams that would be the most suitable for them based on the financial favourability. When choosing the right side-product, it should be kept in mind that the highest concentration of polyphenols can be found in the outer-layers of plant products. Hence, we advise to choose a side-stream with the highest surface-to-volume ratio

Polyphenols add colour to the fruits and vegetables. Therefore, we advise Royal Cosun to use crops with darker or red colorations, as these contain higher amounts of polyphenols. Based on the literature we have studied, examination of polyphenol content in red potato peals, tomatoes, strawberries, pears, parsley, celery, apples, onions and berries would be recommended for Royal Cosun. Furthermore, other side-streams should be checked for their polyphenol content as they could be useful as well. A method that can be used for this is mass spectrometry.

Polymers are not suitable to use as a source for polyphenols. In addition, conjugated polyphenols have the most potent metabolic effects and therefore we advise Royal Cosun to use conjugated polyphenols [50]. Another important requisite is that the polyphenols should not oxidize easily, because they form polymerized substances during storage, which could lead to beneficial or harmful effects in consumers [31-33, 172].

Processing

Special attention should be paid to the processing of the polyphenols as differences in their glycosylation, acylation or polymerisation partly determine their absorption and metabolism [7]. In nature polyphenols are often glycosylated, this can change with different types of processing - cutting the peal, cooking, heating, fermentation, or just usual methods of culinary preparation [42]. For example, a lot of polyphenols are eliminated from the source during boiling. We would recommend steaming as a culinary method. Because certain processing methods may cause a reduction of polyphenols in food sources, there should be paid extra attention on how side-streams are processed. Influence of environmental factors (ripeness, sun exposure), food processing effects (thermal treatment), absorption effects, interactions with other compounds and their influence and host related factors [50] should be examined in the lab on the particular chosen polyphenol or mixture *in vitro* as well as studied *in vivo*. Also the storage of foods can influence the polyphenols, since they are sensible to heat, oxygen and sunlight. Thus, to keep a constant level of polyphenols, foods should be stored in a covered and environment without oxygen.

Adding to food

It is essential to know that the combination of polyphenols with certain food compounds may affect the bioavailability and absorption of polyphenols [51, 52]. The effects can be both positive and negative for our health. For example, dietary fibers, like mentioned in the bioavailability chapter, can have a negative effect on absorption of polyphenols in the human body [42]. Thus, we suggest that Royal Cosun studies

those interactions before the polyphenols are added to certain food compounds and the product becomes commercially available.

Even though there is almost endless choice of polyphenols we would recommend flavonoids due to the amount of published literature about their bioavailability, mechanisms and the fact that they are the most present polyphenols in our diet. For their synthesis sun light is essential and concentrations are, as expected, highest in outer layers. Two examples are kaempferol and quercetin [16, 17]. One should also pay attention to the factors like degree of ripeness, storage, stress etc. For example polyphenol content is not changed if we store apples and pears in the cold [31-33, 172].

Royal Cosun and its customers should be aware that, if a biological effect of polyphenols in the human body is desired, polyphenols should be consumed over a longer period of time. Additionally, flavonoids should not be consumed with vitamin C, as flavonoids have a pro-oxidant function, which is reduced by vitamin C [173].

While referring to the effect of polyphenols, host related factors are important. However, the role of gut microbiota in metabolising polyphenols is still not entirely clear [50]. To understand more about the mechanism of polyphenols, we advise Royal Cosun to look into the metabolites.

Glycaemic Index or lipid metabolism

Primarily based on the above described research found in literature, we recommend Royal Cosun to advertise about the beneficial effect of polyphenols on lowering the lipid metabolism and not on the GI lowering capacity, since this was only proven in in vitro ~~*in vivo*~~ studies.

Conclusion

The highest source of polyphenols can be found in peel of fruits, in particular in blueberries; this can be a good starting point for isolation of polyphenols. However, it is necessary to consider various variables that could reduce the benefits of polyphenols. The most important variables refer to their processing, as indeed some phases of the production process or storage can reduce the polyphenol level in foods.

As mentioned above, the GI lowering effect of polyphenols is not entirely clear. Some evidence has been found in *in vitro* studies, but there is a lack of confirmation of *in vivo* studies concerning this matter. For that reason, we advise Royal Cosun to focus on the beneficial effects of polyphenols on lipid metabolism, as these effects seem more promising. Cosun wil natuurlijk ook erg graag suiker een gezonder / minder kwalijk imago geven, dus als advies wellicht: wanneer suiker met een lagere GI gewenst is zal dit bewezen moeten worden en verder uitgebreid onderzoek moeten plaatsvinden op GI gebied e.d. waarbij in vivo onderzoek een noodzaak is.

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Appendices

Appendices A Food sources of polyphenols

This appendix contains a table of the main food sources of polyphenols [5, 14]. This list is based on the content of polyphenols provided by one serving. The products of the assortment of Royal Cosun are marked in grey. Of these products the types of polyphenols are presented as well, especially those polyphenols mentioned in the review. Although is it stated that the food sources in **Table 1** are rich of polyphenols, this much be investigated in Royal Cosun's own products, since the polyphenol content of foods is susceptible to many factors.

Table 1 Food servings providing at least 1 mg polyphenols with their polyphenol content (mg per serving), sorted on decreasing polyphenol content. Products in grey are part of the assortment of Royal Cosun.

Food	***Serving*** [a] ***(g)***	***Polyphenols*** [b]	***Polyphenols AE*** [b]	**Types of polyphenols**
		Content	***Content***	
Black elderberry	145[c]	1956	1196	Anthocyanins, Flavonols (quercetin)
Black chokeberry	145[c]	1595	1114	
Blackcurrant	145[c]	1092	689	Anthocyanins, Flavanols (catechin, epicatechin), Flavonols, Hydroxycinnamic acids, Hydroxybenzoic acids
Highbush blueberry	145[c]	806	425	
Globe artichoke heads	168	436	259	
Coffee, filter	190	408	209	
Lowbush blueberry	145[c]	395	714	
Sweet cherry	145[c]	394	209	Anthocyanins, Flavanols (catechin, EC, ECG, EGC), Hydroxycinnamic acids
Strawberry	166[c]	390	340	Anthocyanins, Flavanols (catechin, EC, EGC, EGCG), Flavonols, Hydroxybenzoic acids, Hydroxycinnamic acids, Stilbenes (resveratrol)
Blackberry	144[c]	374	260	Anthocyanins, Flavanols (catechin, EC, EGC), Flavonols, Hydroxybenzoic acids, Hydroxycinnamic acids
Plum	85	320	242	Anthocyanins, Flavanols (catechin, EC) Flavonols, Hydroxycinnamic acids
Red raspberry	144	310	154	Anthocyanins, Flavanols (catechin, EC), Flavonols (quercetin), Hydroxybenzoic acids, Hydroxycinnamic acids
Flaxseed meal	20[d]	306[e]	244[e]	
Dark chocolate	17	283	275	
Chestnut	19	230	231	
Black tea	195	197	175	
Green tea	195	173	164	
Pure apple juice	248	168	151	Phloretin, Flavanols (catechin, EC), Flavonols (quercetin), Hydroxybenzoic acids, Hydroxycinnamic acids (caffeic acid)
Apple	110	149	147	Flavanols (catechin, EC, ECG), Flavonols, Hydroxycinnamic acids
Whole grain rye bread	120	146[e]	146[e]	
Hazelnut	28[d]	138	138	
Red wine	125	126	117	
Soya yogurt	125	105	53	
Cocoa powder	3	103	99	
Pure pomegranate juice	150	99	50	Anthocyanins, Flavanols (catechin), Flavonols (quercetin), Hydroxybenzoic acids, Hydroxycinnamic acids (caffeic acid)

Soya flour	20[d]	93	53	
Black grape	54	91	113	Anthocyanins, Flavanols (catechin, EC, ECG, EGC), Flavonols, Hydroxycinnamic acids, Stilbenes (resveratrol)
Black olive	15	85	48	
Pure grapefruit juice	150	79	39	Flavanones (hesperidin, naringin), Flavonols (quercetin), Other (phlorin)
Pure blood orange juice	154	71	31	
Milk chocolate	32	75	75	
Spinach	59	70	40	Flavonols
Pecan nut	15	69	69	
Prune	32	62	32	
Redcurrant	144	62	119	Anthocyanins, Flavanols (catechin, EC, EGC), Flavonols, Hydroxybenzoic acids, Hydroxycinnamic acids, Stilbenes (resveratrol)
Soya, tempeh	40	59	40	
Peach	99[d]	59	52	Flavanols (catechin), Hydroxycinnamic acids
Soya tofu	130	54	32	
Green olive	15	52	35	
Black bean	35	52	14	
Red onion	30	50	30	
Green grape	54	48	46	Flavanols (catechin, EC, ECG, EGC) Flavonols, Hydroxycinnamic acids, Stilbenes (resveratrol)
White bean	35	44	11	
Chocolate beverage with milk	187	39	39	
Roasted soya bean	15	37	40	
Potato	128	36	23	Hydroxycinnamic acids (caffeic acid)
Shallot	32	36	21	
Soya milk	187	34	20	
Red chicory	14	33	18	
Broccoli	72	33	15	
Soya meat	40[d]	29	19	
Whole grain rye flour	20	29[e]	29[e]	
Pure pomelo juice	154	27	12	
Nectarine	99	25	20	
Green chicory	14	23	16	
Pear	138	23	15	
Beer	574	22	22	
Yellow onion	30	22	15	Flavonols (quercetin), Hydroxybenzoic acids
Apricot	65	22	10	Flavanols (catechin, EC), Flavonols, Hydroxycinnamic acids
Asparagus	75	22	8.6	
Quince	100	19	12	
Almond	10	19	0.8	
Whole grain wheat flour	20	14[e]	14[e]	
White wine	125	13	10	
Rosé wine	125	12	10	
Dark beer	574	10	5.2	
Extra virgin olive oil	16	10	4.8	
Soya bean sprout	60	9.3	6.0	
Carrot	54	7.6	3.5	Hydroxybenzoic acid, Hydroxycinnamic acid (caffeic acid)
Bilberry	145[c]	7.4	7.4	Flavonol (quercetin) Hydroxycinnamic acid (ferulic acid) Stilbenes (resveratrol)
Pure lemon juice	15	6.3	1.8	Flavanones (hesperidin) Flavones, Other (phlorin)
Red lettuce	24	5.4	3.4	

Soya cheese	40[d]	4.9	3.1	
Green bean	60	4.8	3.4	
Curly endive	14	3.4	2.0	
Cauliflower	38	2.7	2.7	
Peanut roasted dehulled	40	2.6	2.6	
Rapeseed oil	16	2.5	2.5	
Pumpkin	60	2.5	2.0	
Pasta	60	2.5	2.5	
Banana	97	2.5	2.5	Flavanols (catechin, EC, EGC), Hydroxybenzoic acids
Endive (escarole)	14	2.5	1.5	
Tomato	50	2.1	1.2	
Green lettuce	24	1.9	1.1	
White onion	30	1.6	1.0	Flavonols (quercetin)
Refined oat flour	20	1.6[e]	1.6[e]	
Refined wheat flour	20	1.2[e]	1.2[e]	
Pomegranate	100	1.1	1.1	Flavanols (catechin, EC, EGC)
Sweet green pepper	20	0.9	0.5	Flavones, Flavonols, Hydroxycinnamic acids

Abbreviation: AE, (polyphenols as) aglycone equivalents.

[a] From the Food Standards Agency, UK (Food Standards Agency, 2002), except for values marked with a superscript.

[b] Sum of individual polyphenols determined by reverse-phase high-performance liquid chromatography (HPLC) and proanthocyanidins oligomers determined by direct-phase HPLC.

[c] From Wu et al. [174]

[d] From Cassidy et al. [175]

[e] Polyphenol content determined by chromatography after hydrolysis of the glycosides and esters.

EC= Epicatechin, ECG=Epicatechin gallate, EGC=epigallocatechin, EGCG=epigallocatechin gallate