Water Electrolytes and Micronutrients

Topic 2

Module 2.5

Phytochemicals

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Learning Objectives

- To learn about the family of phytochemicals and the specific groups;
- To understand actions of phytochemicals in their biological context;
- To learn about the potential of phytochemicals in human health and under clinical conditions.

Contents

Key Messages

- Thousands of phytochemicals exist which can be classified in different families;
- The major action of phytochemicals in plants is protection against reactive oxidative species and insects;
- A couple of experimental and epidemiological studies show data that some phytochemicals may exert beneficial effects in humans against cancer, coronary heart disease and neurodegenerative diseases;
- The data of the studies do not justify a recommendation for the use of single phytochemicals. A general recommendation can be made to increase fruit and vegetable intake as these are the major sources and will help to increase supply;
- Patients having a low intake of fruits and vegetables are at risk of oxidative stress. Specifically polyphenols, involved in the regulation of inflammatory response, are of great importance.

1. Introduction

Phytochemicals are non-nutritive plant chemicals that contain protective, disease-preventing compounds. More than 900 different phytochemicals have been identified as components of food, and many more phytochemicals continue to be discovered today. It is estimated that there may be more than 100 different phytochemicals in just one serving of vegetables.

Whether some of them might be essential is still unknown. For example provitamin A carotenoids become essential for vegans avoiding food of animal origin food (Table 1).

Table 1

Food	Phytochemical(s)
Allium vegetables (garlic, onions, chives, leeks)	Allyl sulfides
Cruciferous vegetables(broccoli, cauliflower, cabbage, Brussels sprouts, kale, turnips, bok choy, kohlrabi)	Indoles/glucosinolates Sulfaforaphane Isothiocyanates/thiocyanates Thiols
Solanaceous vegetables (tomatoes, peppers)	Lycopene
Umbelliferous vegetables (carrots, celery, cilantro, parsley, parsnips)	Carotenoids Phthalides Polyacetylenes
Compositae plants (artichoke)	Silymarin
Citrus fruits (oranges, lemons, grapefruit) Glucarates	Monoterpenes (limonene) Carotenoids
Other fruits (grapes, berries, cherries, apples, cantaloupe, watermelon, pomegranate)	Ellagic acid Phenols Flavonoids (quercetin)
Beans, grains, seeds (soybeans, oats, barley, brown rice, whole wheat, flax seed) Protease inhibitors	Flavonoids (isoflavones) Phytic acid Saponins
Herbs, spices (ginger, mint, rosemary, thyme, oregano, sage, basil, tumeric, caraway, fennel)	Gingerols Flavonoids Monoterpenes (limonene)
Licorice root Green tea Polyphenols	Glycyrrhizin Catechins

2. Major actions of phytochemicals

Most phytochemicals have antioxidant activity and protect plant cells against oxidative damage due to sun light and oxygen exposure.

If they are absorbed intact they may work also as **antioxidants** in the human body reducing the mutagenic effect of free radicals and as a consequence the risk of developing certain types of cancer. Phytochemicals with antioxidant activity are: allyl sulfides (onions, leeks, garlic), carotenoids (fruits, carrots), flavonoids (fruits, vegetables), polyphenols (tea, grapes).

Isoflavones, found in soy, imitate human estrogens and it is claimed that they may reduce menopausal symptoms and osteoporosis.

Indoles, present in cabbages, stimulate enzymes that make estrogen less effective and could reduce the risk of breast cancer. Other phytochemicals, which interfere with enzymes, are protease inhibitors (soy and beans), terpenes (citrus fruits and cherries).

Saponins found in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells. Capsaicin, found in hot peppers, may protect DNA from carcinogens.

Allicin from garlic exerts some anti-bacterial properties.

Some phytochemicals bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls. **Proanthocyanidins** are responsible for the anti-adhesion properties of cranberry. Consumption of cranberries will reduce the risk of urinary tract infections and will improve dental health.

3. Plant sterols

The plant kingdom contains a number of sterols that differ from cholesterol by having ethyl or methyl groups or unsaturation in the side chain. The major ones – **sitosterol**, **stigmasterol** and **campesterol** – can be present in Western diets in amounts almost equal to dietary cholesterol. The most prominent is sitosterol. In the early 1950s it was noted that adding sitosterol to the diet of cholesterol-fed chickens or rabbits lowered cholesterol levels in both species and inhibited the development of atherosclerosis in rabbits. Sitosterol or mixtures of soy sterols were studied extensively as cholesterol-lowering agents between 1950 and 1960. They lowered cholesterol by about 10 percent. This area merits reinvestigation using newer technologies.

In the 1980s it was demonstrated that **sitostanol**, a saturated sitosterol derivative, reduced the absorption of cholesterol and blood cholesterol more effectively than sitosterol and at doses below those of sitosterol. In a recent study, sitostanol was combined or "interesterified" with margarine. The resultant product reduced plasma cholesterol an average of 10.2 percent in a population with mild hypercholesteremia. The sitostanol wasn't absorbed and didn't seem to interfere with absorption of fat-soluble vitamins. In 1999 several companies began marketing margarine and other products containing either stanol or sterol esters. Studies in Finland suggest these products can help lower cholesterol.

Squalene, a sterol precursor also found in plant products, was originally suggested to have a cholesterol-lowering effect. But earlier studies in animals showed that it had no positive influence on atherosclerosis. Sitosterols and squalene are present in both monounsaturated and polyunsaturated vegetable oils and thus may be responsible for some of the variable cholesterol-lowering effects found in studies using these products. This may explain differences seen between various sources and degrees of refinement of olive oil. There are also cholesterol-lowering alcohols in rice bran oils. Several recent studies suggest that rice bran oil lowers plasma cholesterol levels about 7-10 percent in humans.

Finally, **cafestol** is a terpene present in coffee. Some studies have suggested that drinking coffee may be linked with changes in plasma cholesterol that may be explained by the presence of this compound. The manner of preparation may influence the effect of coffee; for example, filtering may remove some cholesterol-raising compounds.

3.1 Plant sulfur compounds

Naturally occurring sulfur-containing compounds (the allium family) are found especially in garlic, onions and leeks, the most prominent of these being garlic. In 2000, the Agency for Healthcare Research and Quality (AHRQ) published an evidence-based "Report on Garlic: Effects on Cardiovascular Risks and Disease, Protective Effects Against Cancer, and Clinical Adverse Effects".

- Thirty-six randomized trials, all but one in adults, consistently showed that, compared with placebo, various garlic preparations led to small, statistically significant reductions in total cholesterol at one month (range of average pooled reductions 1.1 to 15.8 milligrams per deciliter [mg/dL]) and three months (range of 11.6 to 24.3 mg/dL). Eight trials with outcomes at six months showed no significant reductions by the ingestion of garlic compared with placebo. Changes in low-density lipoprotein levels (LDL) and triglycerides mirrored total cholesterol results; no significant changes in high-density lipoprotein levels (HDL) were found;
- Twenty-six small, randomized, placebo-controlled trials, all but one in adults, reported mixed, but never large, effects of various garlic preparations on blood pressure outcomes;
- Twelve small, randomized trials suggested various garlic preparations had no clinically significant effects on glucose in persons with or without diabetes. Two small short trials reported no statistically significant effects of garlic compared with placebo on serum insulin or C peptide levels;

- Ten small, short-duration trials, all but one in adults, showed effects of various garlic preparations on platelet aggregation and mixed effects on plasma viscosity and fibrinolytic activity;
- There were insufficient data to confirm or refute garlic's effects on clinical outcomes such as myocardial infarction and claudication;
- Scant data, primarily from case-control studies, suggest, but do not prove, that dietary garlic consumption is associated with decreased risk of developing laryngeal, gastric, colorectal, and endometrial cancer and adenomatous colorectal polyps;
- Adverse effects of oral ingestion of garlic are "smelly" breath and body odor. Other possible, but not proven, adverse effects include flatulence, esophageal and abdominal pain, small intestinal obstruction, dermatitis, rhinitis, asthma and bleeding.

4. Polyphenols

Polyphenols can be classified into four different groups. Flavonoids, stilbenes, lignans and phenolic acids. Within plants these bioactive constituencies are more or less involved in the defence mechanism against UV radiation and insects. Due to their wide range of effects polyphenols are attractive as food derived drugs and indeed epidemiological studies have shown that populations who consume foods rich in specific polyphenols have a lower incidence of cancer or chronic inflammatory diseases. The mean intake of polyphenols from food is around one gram to day. However depending on the food pattern the intake of different polyphenols vary. For example the consumption of flavonols has been estimated 20-25mg/day in US.

5. Flavonoids

Flavonoids are compounds with varied chemical structures present in fruits, vegetables, nuts and seeds. The major flavonoid categories are **flavonols**, **flavones**, **catechins**, **flavanones** and **anthocyanins**. The main dietary sources of these compounds are tea, onions, soy and wine. The main flavonoid in onions is **quercetin glucoside** and the main flavonoid in tea is **quercetin rutinoside**.

Flavonoid intake has been inversely linked with coronary heart disease in the Zutphen Elderly Study, the Seven Countries Study and a cohort study in Finland. That is, people with a low intake of flavonoid had a higher death rate from coronary heart disease than did those who consumed more flavonoid (about five to six cups of tea per day). It should be pointed out that some flavonoids have toxic effects (gastrointestinal or allergic), especially if taken in large amounts. Systematic work is needed on the major classes of flavonoids to study their structure, effectiveness and potential harmful effects.

The link between flavonoids and atherosclerosis is based partly on the evidence that some flavonoids have antioxidant properties. For example, the **phenolic** substances in red wine inhibit oxidation of human LDL. Flavonoids also have been shown to inhibit the aggregation and adhesion of platelets in blood, which may be another way they lower the risk of heart disease. **Isoflavones** in soy foods have been reported to lower plasma cholesterol and also to have effects similar to estrogen.

The consumption of **isoflavones**, derived from soy products has increased manyfold over the last 25 years. Isolated isoflavones are claimed to have a couple of beneficial effects specifically in the treatment of postmenopausal related problems. The table summarizes recent studies in the field (Espin et al., 2007)

Table 2 Human intervention trials of isoflavon based nutraceuticals

Supplement	Composition	Dose (per day) and assay period	Subjects	Effect	Reference
Red clover extract	Genistein, diadzein, biochinin, formonentin	40 and 80 mg (6 weeks)	27 women	Improvement of arterial compliance. No effect on plasma lipids	Nestel et al (1999)
Soybean Tablet	Isoflavones	80 mg (2months)	20 post-menopausal women	No effect on endothelium function or plasma lipids	Simons et all (2000)
Soy protein extract	Isoflavones	132 mg (3 months)	32 postmenopausal women with diabetes type 2	Improvement of serum lipid profile, glycemic index	Jayagopal et al (2002)
Genistein supplement	Genistein	54 mg (1 year)	30 women	Increase bone mineral density	Moharito et al (2002)
Capsules	Isoflavones	61.8 mg (1 month)	23 peri-menopausal women	Beneficial effects on bone metabolism and on serum lipids	Uesugi et al (2002)
Red clover extract	Isoflavones	86 mg (4 months)	25 pre-menopausal women	No effect on serum lipid profile.No effect on glucose or insulin	Blakesmith et al (2003)
Soy protein extract	Isoflavones (diadzein, genistein, glycetein and their respective glycosides or 69 mg aglycone form by weight	118 mg (3 months)	106 postmenopausal women	No effect on bone resorptions. Decrease of LDL. No effect of HDL and triglycerides	Dalais et al (2003)
Soya supplement	Isoflavones	60 mg (3 months)	33 postmenopausal women	Significant cognitive improvement	Duffy et al (2003)
Red clover extract	Isoflavones	86 mg (1 months)	16 pre and 7 postmenopausal women	Increase in HDL .No effect on cholesterol and triglycerides	Campbell et al (2004)
Red clover tablets	Isoflavones (26 mg biochanin, 16 mg formononetim, 1mg genistein, diadzein	43.5 mg (1 year)	205 women with Wolfe P2 and DY mammographic breast pattern	No increase in mammo-graphic density. No effect on menopausal symptoms	Atkinson et al (2004a)
Red clover extract	Isoflavones	43.5 mg (1 year)	177 perimenopausal women	No effect on serum lipid profile, blood pressure and	Atkinson et al (2004b)

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Capsules	Isoflavones	100 mg (6 months)	30 postmenopausal women	Hypoglycemic	Cheng et al (2004)
Soy extract	Isoflavones	60 mg (6 weeks)	50 postmenopausal women	Cognitive improvement in frontal lobe function.No effect on memory	File et al (2005)
Red clover supplement	Isoflavones	80 mg (90 days)	60 postmenopausal women	Decrease of menopausal symptoms .Positive effect on vaginal cytology and triglycerides	Hidalgo et al (2005)
Soy protein powder	Isoflavones	83 mg (1 year)	150 pateints with adeno matous polyps diagnosed	No reduction of colorectal epithelial cell proliferation and heights of polyps	Adams et al (2005)
Soy extract	Isoflavones	120 mg isoflavone glycoside and aglycone(6 months)	79 postmenopausal women	No effect of vaginal mucosa and endometrium	Kaari et al (2006)
Isoflavone tablet of soya protein	125 mg protein extract with 50 mg isoflavone (35.5 mg genistein and 14.5 diadzein	100 mg and 200 mg (1 year)	43 postmenopausal women	No dose response effect .Prevention of estrogen related bone loss .Lack of undesirable side effect	Hung et al (2006)
Soy protein extract	Isoflavones	97.5 mg and 135.5mg (50 days)	13 postmenopausal women	No effect on bone resorption at any dose	Cheong et al (2007)

A special group of polyphenols are those which are present in grape fruit, the grape procynidines. Table 2 (Espin et al., 2007) lists different studies with these compounds.

Table 3 Human intervention trials of grape procyanidns derived nutraceuticals

Supplement	Composition	Dose (per day and assay period	Subjects	Effects	Reference
Grape seed extract	Oligomeric Procyanidns	200- 300 mg /day, 1 year	3 patients with chronic pancreatitis	Reductions of chronic pancreatitis, vomiting and pain	Banerjee and bagchi (2001)
Grape seed extract	Procyanidns	600mg /day ,long term	17 healthy and hypercholest rolemic humans	Decrase of plasma cholesterol, LDL cholesterol, HDL	Vinson et al (2001)

				cholesterol on hypecholestrol emic subjects. No effect on healthy subjects	
Grape seed extract	Procyanidns	300mg 1 single dose	8 healthy humans	Prevention of post prandial oxidative stress	Natella et al (2002)
Grape seed extract	Procyanidns	300mg/day .1 month	24 heavy smokers	Decrease in TBARS ,no effect on HDL cholesterol , LDL cholesterol ,triglycerides and total cholesterol	Vigna et al (2003)
Grape seed extract	Procyanidns	162 mg/day ,6 months	12 women with chloasma	Reduction and prevention of hyperpigmenta tion	Yamakoshi et al (2004)
Mixture of grape,blueber ry and canberry extract (capsules)	Oligomeric procyanidns	320mg/day	13 premenopaus al women	Reduction of fluid retention	Christie et al (2004)
Grape seed extract	Procyanidns	1g/day,1 month	43 men and women in high cardiovascula r risk	Improvement of flow mediated dilation, no effect on other markers (clotting and fibrinolytic factors, adhesion molecules, serum lipids, urinary isoprostanes, nitric oxide production)	Clifton (2004)
Grape seed extract	Proanthocyan idns	300mg /day.6 months ,Phase 2 trials	66 women with radiation induced breast indurance	No effect on tissue hardness,pain or breast appearance	Brooker et al (2006)

5.1 Anti-inflammatory activities of dietary polyphenols

Different targets are involved in the anti-inflammatory activities of polyphenols: These can be subdivided into targets related to the arachidonic acid dependant pathways such as COX inhibition, LOX inhibition, PLA_2 inhibition. Within the arachidonic acid independent pathways the nitrous oxide synthase (NOS), NFκB and NAG-1 are targets of polyphenols. In addition it has been shown that polyphenols exert effects on human TH1 and TH2 cytokine production (1).

Within the arachidonic dependent pathway polyphenols act in part due to their antioxidative activity. This relationship was investigated more than 20 years ago and a couple of papers presented data on COX-1 and COX-2 inhibition at the transcriptional and encyme level. However, based on

recent research it has become clear that polyphenols act on both pathways as antioxidants and as modulators of gene expression. COX inhibition may account for the anti-inflammatory effects, which reduce prostaglandin synthesis in the arachidonic pathway.

Epigallocatechin gallate, a polyphenol from green tea down regulates COX-2 in TPA stimulated human mammary epithelial cells (2). The underlying mechanism is suggested to be a decrease in the activation of ERK (extracellular signal regulated protein kinase) and p38 MAPK (mitogen activated protein kinase) which are upstream encymes regulating COX-2 expression. EGCG and green tea extracts inhibit IL-1ß-dependent proinflammatory signal transduction and IL-8 gene expression via NF¤B-dependent pathways in cultured respiratory epithelial cells (3). The authors conclude that the use of EGCG and related compounds may represent a novel pharmacological strategy for modulating the effect of inflammation on the NF¤B pathway.

Up to now there are no human studies dealing with the activity of polyphenols during acute or chronic inflammatory response. Castilla and co-workers (3) describe concentrated red grape juice which contains a couple of polyphenols as having an antioxidant, hypolipidaemic and anti-inflammatory effect in both haemodialysis patients and healthy subjects. Total polyphenol concentration is relatively high in red grapes (around 1g/100ml) and contains some interesting compounds like quercetin, catechin, procyanidin, etc. Beside the antioxidant effects the authors evaluated biomarkers of inflammation in plasma after three weeks of supplementation. Adhesion factors VCAM-1, ICAM-1 complement C3 protein and C-reactive protein remained unchanged during the three week study period. In contrast the concentration of MCP-1 decreased progressively. MCP-1 belongs to a group of biomarkers for vascular inflammation, observed in patients with chronic or acute inflammation. MCP-1 correlates with the serum levels of sRAGE (soluble form of the receptor for advanced glycation end products) and is associated with inflammatory markers in patients with Type 2 Diabetes (14). If polyphenols can increase the concentration of sRAGE and MCP-1 this is a clear sign that polyphenols exert anti-inflammatory effects on both an antioxidant and non-antioxidant basis. There is a growing body of evidence that AGEs and the receptor RAGE are implicated in the pathogenesis of ROS-induced vascular complications.

Polyphenols are involved in a variety of reactions during inflammation. Reactive oxygen species (ROS) play a key role in enhancing the activation of NFkB and AP-1 transcription factors, and nuclear histone acetylation and deacetylation in various inflammatory diseases. Such effects have been found to be controlled by the different effects of dietary polyphenols, which may exert very different activities within the inflammatory cascade. The most common activity of polyphenols is their antioxidative action and the modulating effects on NFκB, chromatin structure, glutathione synthesis, nuclear redox factor (Nrf2) activation and glutathione peroxidise activation. As a consequence inflammatory genes in macrophages and different target tissues are regulated. Despite the effect that there are couple of highly interesting results at the moment, there is insufficient data to justify recommending either a single or a specific pattern of a group of different polyphenols in order to reduce inflammation pharmacologically. Further data are needed with respect to dose response, toxicity, bioavailability and distribution before randomised clinical trials with one or more of these polyphenols can be recommended.

6. How can I incorporate more phytochemicals into the diet?

First, it is important for us to become aware of our inadequate consumption of fruits, vegetables, and grains. The average American consumes only one serving of vegetables and one serving of fruit each day (11). In one survey, one in every nine Americans ate no fruit or vegetable on the day they were interviewed (12). Consumption tends to be higher in some European countries.

Increasing the consumption of plant products in one's diet should not be difficult or time consuming. There are plenty of simple strategies for increasing dietary fruits, vegetables, and grains, including the suggestions below:

- Keep fruits and vegetables (fresh, frozen, and canned) stocked and in sight;
- Reach for juice instead of coffee or soda;
- Add chopped fruit to cereal, yogurt, pancakes, muffins, or even a milkshake;
- Snack on fresh chopped carrots, celery, broccoli, cauliflower, and peppers (purchase at a salad bar to save time);
- Add fresh greens, carrots, celery, parsley, tomatoes, and/or beans to your soups;

- Store dried fruit (apricots, dates, raisins, and more) for a quick snack at home or work.

There are also several other easy methods for increasing fruits, vegetables, and grains in your lifestyle. Why not challenge yourself and create one of your own? Good Luck!

7. Are there any negative effects?

Individual phytochemicals are being evaluated for their safety and effectiveness with regard to disease prevention. Although most studies support positive outcomes, there are a few studies involving animals that show possible detrimental effects. These studies involve animals and specific extracted phytochemicals in high dosages. The safety of consuming large amounts of fruits, vegetables, and grains is not presently a concern. The research question being asked is: "Should one increase the intake of a particular plant food containing phytochemicals, and how much should one increase it?" Obviously, like any other newly discovered chemical, there is a need for further investigation for potential health benefits versus risks. Optimal levels of phytochemicals have yet to be determined. In addition, requirements during disease states may differ from those needed for prevention of heart disease and cancer. Individual recommendations in terms of requirements for different genders, age groups, body types, and so forth also need further study.

8. Summary

More than 900 phytochemicals (plant derived bioactive compounds) have been identified and further 900 or more might exist. The major action of phytochemicals is their antioxidative activity. Consequently their frequent intake (e.g. via 5 a day) is related to a decreased risk of developing degenerative diseases (coronary heart disease, neurodegenerative diseases, cancer, etc.). Some phytochemicals show an anti-inflammatory potential which makes them possible future candidates for clinical nutrition.

References

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