Workshop
Protective / preventive role of bioactive food components in human health

Nutraceuticals in Cardioprotection

Prof. Silvana Hrelia
Department for Life Quality Studies
Alma Mater Studiorum-University of Bologna (Italy)

Novi Sad, December 13-14, 2016

Quality of Life

WHO defines Quality of Life as individuals perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns.

The concept of Health-Related Quality of Life (HRQOL) has evolved to encompass those aspects of overall QoL that can be clearly shown to affect health and is related to both chronic diseases and their risk factors.

WHO defines health as "A state of complete physical, mental, and social well-being not merely the absence of disease"
Well-being

The European Observatory on Health Systems and Polices definition of well-being is:

*the emotional, mental, social and spiritual state that permits people to reach and maintain their personal potential in society*

As age increases the role played by health tends to become more and more important until it was almost exclusively.

The health pendulum

Conceptual model of major influences which modulate the ageing trajectory and risk of development of frailty and age-related diseases.

Underpinning the “Health Pendulum” is the hypothesis that interactions between an individual's genotype and his/her environment influence the accumulation of molecular and cellular damage which is responsible for the ageing phenotype and contributes to the development of frailty and age-related diseases.
The desperate need for a definition of aging

Is aging a disease?

- NO, it is separate from age-related diseases
- NO, but it is a risk factor for age-related diseases
- NO, it is the set of precursors of the age-related diseases

Changes in Rankings for 15 Leading Causes of Death, 2002 and 2030

<table>
<thead>
<tr>
<th>Disease or Injury</th>
<th>2002 Rank</th>
<th>2030 Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischaemic heart disease</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Lower respiratory infections</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>COPD</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Perinatal conditions</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Diarrheal diseases</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>8</td>
<td>23</td>
</tr>
<tr>
<td>Trauma, brain, lung cancers</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Road traffic accidents</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Malaria</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>Hypertensive heart disease</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>Self-inflicted injuries</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Stomach cancer</td>
<td>15</td>
<td>10</td>
</tr>
</tbody>
</table>

Heart disease may be a leading cause of death, but that doesn’t mean you have to accept it as your fate. Although you lack the power to change some risk factors such as family history, sex or age there are some key heart disease prevention steps you can take. Don’t smoke or use tobacco. Exercise for 30 minutes on most days of the week. Maintain a healthy weight. Eat a heart-healthy diet.

The analysis of data collected in 12 different studies, which enrolled 280000 subjects revealed that people consuming 3-5 or more than 5 servings/day had a risk reduction for coronary heart disease of 7% and 17%, respectively when compared with subjects who consumed less than 3 servings/day.
Critical review: vegetables and fruit in the prevention of chronic diseases

For hypertension, CHD, and stroke, there is **convincing evidence** that increasing consumption of vegetables and fruit reduces the risk of disease.

There is **probable evidence** that the overall risk of cancer is inversely associated with the consumption of vegetables and fruit.

Data on dementia indicate **possible evidence** for a risk-reducing influence of increased vegetable and fruit consumption.

In addition, there is **possible evidence** that a diet with increased consumption of vegetables and fruit may prevent body weight gain. As overweight is the most important risk factor for type 2 diabetes mellitus, an increased consumption of vegetables and fruit might indirectly reduce the incidence of type 2 diabetes mellitus.

---

Mediterranean Diet

It was first publicized in 1945 by the American doctor Ancel Keys

**Main characteristics:** low consumption of meat and meat products, high consumption of vegetal foods rich in **NUTRACEUTICAL** bioactive compounds.
Mediterranean Diet

The Mediterranean diet involves a set of skills, knowledge, rituals, symbols and traditions concerning crops, harvesting, fishing, animal husbandry, conservation, processing, cooking, and particularly the sharing and consumption of food.

On November 17th 2010, the UNESCO included the Mediterranean Diet on the Representative List of Intangible Cultural Heritage of Humanity during its meeting held in Nairobi, Kenya.

Starting at the base of the pyramid, you’ll find an emphasis on activity and social connections.

Moving upward, you’ll see the core foods that you’ll eat every day: whole grains, fruits, vegetables, beans, herbs, spices, nuts and healthy fats such as olive oil. Fish and seafood are typically eaten at least twice a week, and dairy foods – especially fermented dairy like yogurt and traditional cheese – are eaten frequently in moderate portions.

Eggs and occasional poultry are also part of the Mediterranean Diet, but red meat and sweets are rarely eaten. Water, and wine (for those who drink) are typical beverages.
**Nutraceuticals**

- **Nutraceutical**, a term combining the words “nutrition” and “pharmaceutical”, is a food constituent that provides health and medical benefits, including the prevention and treatment of disease.

- The term nutraceutical was originally defined by Dr. Stephen L. DeFelice, founder and chairman of the Foundation of Innovation Medicine (FIM), Crawford, New Jersey (USA).

---

*Eat the colours of life*
Institutions like FAO and WHO recommend a daily intake of at least 400 grams of fruits and vegetables a day, if possible in 5 different servings.

Cardiovascular diseases

More than any other disease, the etiology of cardiovascular disease reveals many risk factors that are amenable to nutraceutical intervention. Cardiovascular disease (CVD) is now the leading cause of death globally and is a growing health concern.

Lifestyle-related conditions, such as obesity, hyperlipidemia, type 2 diabetes, and hypertension, are also widespread and becoming more prevalent globally. Although the traditional cardiovascular risk factors have been extensively investigated, dietary factors are also important in the pathogenesis of CVD and may to a large degree determine CVD risk factors such as blood pressure and dyslipidaemia, but have been less extensively investigated.
Cardiovascular risk factors

Although the traditional cardiovascular risk factors have been extensively investigated, dietary factors are also important in the pathogenesis of CVD and may to a large degree determine CVD risk factors such as blood pressure and dyslipidaemia, but have been less extensively investigated.

Whilst epidemiological studies have identified a relationship between diet and CVD, there is still considerable scientific uncertainty about the relationship between specific dietary components and cardiovascular risk.

It has been suggested that oxidative stress is involved in the etiology of several chronic diseases including CVD, diabetes, stroke, some cancers, and neurodegenerative disorders. Observational, prospective cohort studies suggest that a higher dietary intake or supplementation of antioxidants is associated with a lower risk of CVD and mortality, but the evidence from clinical trials is still largely negative.

The conflicting results between the protective effects of antioxidants as part of dietary intake and the lack of effectiveness of single antioxidant supplementation in trials has led to a focus on whole foods or modified diets as protective against CVD.
Elderly people had lower circulating antioxidants and higher lipid peroxide levels than younger subjects, suggesting that advanced age is associated with systemic oxidative status.

The literature highlights that the ageing process and the pathogenesis of age-related diseases are influenced by oxidative stress, inflammation status, and gut microbiota. Thus, an healthy personalized dietetic approach specifically formulated for elderly people, with a defined pattern of nutrients, may represent a key strategy to improve the ageing process considering functional foods and/or nutraceutical supplements.

The project allowed to analyse TAA, SOD, CAT, GR, GST, GPx activities and GSH levels in three hundred elderly European people (average age 70.43±3.9 y).
What we have yet to discover?

- Epidemiological Studies
- Nutraceuticals as beneficial compounds against cardiovascular diseases
- Supplementation studies in humans and animals

**Molecular Mechanisms?**

Flavonoids may reduce the risk of death from coronary heart disease and cancer

- Hertog et al. (2003) Lancet

Do Flavonoids Reduce Cardiovascular Disease Incidence or Mortality in US and European Populations?


The “Quercetin” case

- B-ring catechol group
- Un-saturation in the C-ring
- Carbonyl group
- Presence of transition metal ion binding functions

**Powerful scavenger of ROS and RNS:**

- Fenton systems: Fe$^{2+}$ Cu$^+$ and peroxide ONOO$^-$ and HOCl
- Metal chelators
Major metabolites present in human plasma following Q ingestion

Quercetin (Q)

\[ \text{OH} \]

\[ \text{OH} \]

\[ \text{OH} \]

\[ 0.1-5 \]

\[ 0.9-5 \]

\[ \text{Plasma concentration} \]

\[ \mu M \]

\[ 0.9-2 \]

\[ 0.1-3 \]

3'-O-Me-quercetin (3MeEC)

\[ \text{OH} \]

\[ \text{OH} \]

\[ \text{OH} \]

\[ \text{C} \]

\[ \text{H}_3 \]

\[ 0.1-3 \]

Quercetin-7-O-\(\beta\)-D-glucuronide (Q gluc)

\[ \text{OH} \]

\[ \text{OH} \]

\[ \text{OH} \]

\[ \text{C}_\text{O} \]

\[ \text{O} \]

\[ 0.9-5 \]


Bioavailability

Uptake/association of quercetin, 3'-O-methyl quercetin, and 4'-O-methyl quercetin with H9c2 cells

Protecting the heart through activation of the MAPK PI3K–Akt and Erk 1/2 signaling pathway

Signalling through the PI3K–Akt and/or the MEK1/2–Erk 1/2 cascades results in:

1. phosphorylation and inactivation of caspses 3 and 9, which inhibits apoptosis;
2. phosphorylation and inactivation of the pro-apoptotic proteins BIM, BAX, BAD and p53, one consequence of which is to prevent the release of mitochondrial cytochrome c in response to an apoptotic stimulus.

Possible Mechanisms of Q Bioactivity

Activation of Akt

Activation of ERK 1/2
Q modulates gene expression profile in cardiomyocytes

Hierarchical clustering display of data in cardiomyocytes treated with Q based on the significant 91 genes. Each gene is represented by a single row of colored bars. The red color indicates upregulation of the gene expression, and the green color denotes the downregulation of the gene expression compared with the controls.

**Cluster 2:** genes critically involved in antioxidant/detoxification mechanisms related to cell survival: NQO1, HO-1, TR, GSTa3, and GSTp2 underlying a common functional regulation.


Q reduces inflammatory responses in cardiac cells

Effect of Q on iNOS expression in LPS-stimulated cardiomyocytes

Proposed mechanisms underlying the cardioprotective effects of Q

exerts its cardioprotective effects via:

- its well-known antioxidant activity
- through the modulation of intracellular signalling pathways (Akt, ERK1/2), and caspase-3 activity
- through changes in gene expression and a strong up-regulation of phase 2 enzymes, highlighting Q ability to act also with an indirect antioxidant mechanism
- through reduction of inflammatory responses by inhibiting iNOS induction in LPS-stimulated cardiac cells, reducing NO production, and counteracting LPS-induced apoptosis

The Sulforaphane case

**Glucosinolate:**
- Glucoraphanin

**Isothiocyanate:**
- Sulforaphane
The Sulforaphane case

Comparison of the protective effects of steamed and cooked broccolis on ischemia–reperfusion-induced cardiac injury

Salinsa Maitere1,2, Anvar Leel1, Dipak Roy1, Hiranay Gangopadhyay3, Utpal Raychaudhuri3 and Dipak K. Das4

(a)

(b)

Central IVH  Steamed broccoli  Cooked broccoli

Nrf2  HIF-1α  HO-1  GAPDH

SOD1  iNOS  EDOD2  Cu/ZnSOD
Nrf2 is impaired in the aging heart

- Nrf2 levels in the nuclear extracts of myocardium are lower in aged (>23 months) than young (2 months) mice.
- Nrf2-ARE-binding efficacy was significantly reduced (0.6 fold) in the aging heart.
- Immunofluorescence analysis using anti-Nrf2-Ab revealed decreased cytosolic and nuclear Nrf2 in old versus young myocardium.


These observations suggest that enhancing Nrf2 function and endogenous cytoprotective mechanisms by SF, may combat age-induced ROS and protect the myocardium from oxidative stress diseases.

Proteomic profiling of SF-treated cardiomyocytes

A 2-DE-based proteomics approach was employed to profile the proteins affected by SF, leading to the identification of 41 distinct proteins with altered expression, which were associated with diverse biological functions.

Angeloni et al., PloS One, 2013
Carbonyl and glycateive stress

An imbalance between pro-glycangent species and anti-glycangent enzymatic defenses, in favor of the pro-glycangent agents. Reactive carbonyl compounds are constantly produced by the metabolism of carbohydrates, lipids, and aminoacids.

MG is a potent dicarbonyl glycating agent formed by the degradation of triose-phosphate that accumulates in OS conditions and reacts with proteins, DNA and lipids to form AGE directly and relatively rapidly.

AGEs

AGEs are generated in the late stages of Maillard reaction in foods and biological systems. These products are mostly formed by the reactions of reducing sugar or degradation products of carbohydrates, lipids, and ascorbic acid.

AGEs exist in high concentration in foods, processed by common methods such as dry heat. Food processing - dry heat, ionization, or irradiation accelerates the generation of new AGEs whether done at industrial or commercial levels.

Heat and dehydration are also common in home cooking.

Human and animal studies demonstrated that about 10 % of AGEs contained in a meal can be absorbed into the circulation, of which two-thirds remain in the body for 72 hours, so they stay long enough to promote OS, more AGEs, and potentially more tissue injury.
Glycation reactions also occur endogenously in all tissues and body fluids under physiological conditions and AGEs exist in relatively low concentrations in most of the biological systems.

AGES levels increase in diabetes and also normal aging process. Glucose is the most frequently found reducing sugar and also the most investigated carbonyl precursor under physiological conditions.

Physiologically formed AGES are defined as the non-enzymatic reaction of glucose, α-oxoaldehydes, and other saccharide derivatives, with proteins, nucleotides, and lipids, in the human body.

Receptors for AGES (RAGEs) were believed to play a critical role in AGES related biology and the pathology associated with diabetic complications and aging disorders.

Consequently, defense mechanism against the Maillard reaction such as the deglycation of protein-bound Maillard products and the detoxification of dicarbonyl compounds to non-reactive compounds would be beneficial.
**Effects of AGEs in vivo**

- AGE accumulation and ROS affecting cell dysfunction & death
- Deposition of β-amyloid protein impairing hypothalamic signaling
- Appetite regulation alterations in reward pathways
- Addiction-like neuroadaptations

**Dietary AGEs and their dicarbonyl precursors**

**The Glyoxalase system**

\[
\text{CH}_2\text{COCHO} \quad \text{Methylglyoxal}
\]

\[
\text{Glyoxalase 1} \quad \text{GSH} \quad \text{CH}_3\text{CH(OH)CO-SG} \quad \text{S-D-Lactoylglutathione}
\]

\[
\text{Glyoxalase 2} \quad \text{CH}_3\text{CH(OH)CO}_2^- + \text{H}^+ \quad \text{H}_2\text{O}
\]

MG is detoxified by the glyoxalase system. The major physiological substrate for Glo1 is MG and this accumulates markedly by GSH depletion.

OS is inextricably linked to glycation because the depletion of GSH in OS also decreases the activities of Glo1 and increases MG concentration and associated glycation reactions.

Glycation of proteins by Glo1 substrates may also increase ROS production, a further cause or contributory factor to OS.
Towards a nutraceutical approach

Ellagitannin oligomers and a neolignan from pomegranate arils and their inhibitory effects on the formation of advanced glycation end products
Hideyuki Ito, Peng Li, Mayuko Koriyama, Atsufumi Nagai, Noritsa Nishida, Tadashi Yoshida

Sulforaphane reduces advanced glycation end products (AGEs)-induced inflammation in endothelial cells and rat aorta
T. Matsui, N. Nakamura, A. Ojima, Y. Nishino, S. S. Yamagishi

Betain reduces the accumulation and cross-links of collagen in high-fructose-fed rat heart through inhibiting non-enzymatic glycation
Jiayuan Han, Chang Tan, Yihang Wang, Shuklin Yang, Delong Tan

Novel Targets of Sulforaphane in Primary Cardiomyocytes Identified by Proteomic Analysis
Cristina Angiolini, Silvia Turroni, Laura Bianchi, Daniela Fabiani, Elisa Moresi, Marco Malaguti, Emanuela Leocardi, Tullia Maroldi, Luca Birri, Patrizia Brigida, Silvana Irela
Proposed mechanisms underlying the cardioprotective effects of SF

SF exerts its cardioprotective effects via:

- **GLO1**
- **β-actin**

**Graphs:***
- Cell viability (% of control)
- Pro-Caspase 3
- Active Caspase 3
- β-actin
- µg AGE/mg protein

**Proposed mechanisms:**  
SF exerts its cardioprotective effects via:

1. **Degradation of advanced glycation end products (AGEs):**
   - SF degrades AGEs, reducing their accumulation.

2. **Regulation of apoptosis:**
   - SF activates pro-Caspase 3, leading to the activation of Caspase 3.

3. **Stabilization of β-actin expression:**
   - SF stabilizes β-actin expression, maintaining cell integrity.

These mechanisms contribute to the cardioprotective effects of SF.
New nutritional strategies intend to identify nutraceutical bioactive compounds with the ability to directly target and enhance intrinsic cytoprotective mechanisms.

Individual nutraceuticals can have greater or lesser effects on specific Nrf2- and inflammation-related genes in various tissues and experimental models.

Only using a combination of nutraceuticals, as those naturally present in fruits and vegetables, it would be possible to modulate the greatest diversity of Nrf2- and inflammation related genes in the greatest number of tissues to achieve the most dramatic protective effects against oxidative damage, toxicants, and inflammation, and to provide the most robust preventive/protective and anti-aging benefits.

There is intriguing but not yet compelling evidence that relatively small amounts of certain of the dietary nutraceuticals may lower risk of coronary heart disease mortality in European and US countries.

More research is needed to establish that cardioprotective relationships exist with these bioactive compounds and, if they prove to be protective, what consumption levels may be required to achieve health benefits.

Future studies are needed that allow for more direct comparison of research findings using more complete and comprehensive nutraceutical databases, more standardized and comprehensive dietary assessment methods, more information on the age, sex, health status and other characteristics of populations studied, more complete cardiovascular outcome measures, and longer lengths of follow-up that will allow for more direct comparison of research findings.
The Med-Italian Diet

Italians do EAT better

Thanks for your attention

Silvana Hrelia
Department for Life Quality
silvana.hrelia@unibo.it