EUROPEAN RESEARCH ON THE FUNCTIONAL EFFECTS OF ANTIOXIDANTS.

David G Lindsay
Food Science & Technology Department
CEBAS (CSIC)
Apartado de Correos 4195
30080 MURCIA
www.ifr.bbsrc.ac.uk/EUROFEDA

Background
The EUROFEDA project seeks to co-ordinate research on the functional role that dietary antioxidants might play in promoting health, by minimising the damage to cells from oxidative damage. It is achieving this by pursuing the following objectives:

1. setting up a European network for the co-ordination and exchange of information on the functional effects of antioxidants in food;
2. identifying the priority research tasks and, as a result, attempting to stimulate the co-ordination of future research; and
3. disseminating the outputs to end users (consumers, regulatory bodies and industry) thereby assist in encouraging innovation and in improving health.

These objectives are being achieved through the organisation of a series of task group and plenary meetings which are in the process of reviewing:

1. methods for the measurement of DNA damage and repair and other measures of oxidative damage;
2. data on the bioavailability of antioxidants;
3. their effects on gene expression and their possible role in limiting the decline in mitochondrial efficiency with age.

The focus of the meetings will be to produce a review (to be published in Molecular Aspects of Medicine in 2002) which will consider the effects of the following antioxidants and phytochemicals

1. Vitamins C & E
2. Carotenoids
3. Simple and complex phenols
4. Precursors of glutathione and other redox thiols
5. Selenium
6. Antioxidant enzyme inducers (e.g. sulphur compounds in vegetables)

Introduction
Sustained oxidative damage to cell constituents is generally considered to be one of the most important mechanisms leading to the general decline in the function of cells and tissues which often precede
pathological changes. The decline is associated with the ageing process and the onset of degenerative diseases, such as central nervous system dysfunction, immune system decline, and hormonal dysfunction\(^1\). It is clear that aerobic organisms have evolved a sophisticated system of antioxidant defences to minimise such damage. Biochemical studies clearly indicate both direct and indirectly-acting antioxidants play a role in these defences.

Oxidative damage occurs through the generation of a series of reactive oxygen and nitrogen species (ROS). These species can react with lipid membranes, mitochondrial and nuclear DNA, and proteins (e.g. collagen and elastin, enzymes etc.) causing loss of function, cell death or neoplastic disease\(^3\). The mechanisms which give rise to ROS must be tightly regulated given the potential for their toxicity. However the process of regulation is not 100% efficient and oxidised DNA bases, lipids and proteins are formed in increasing amounts as humans age\(^4,5\).

There are strong associations between dietary factors and some degenerative diseases of great public health significance, such as cardiovascular disease and cancer\(^2\). The crucial role that diet may play in determining the pattern and onset of disease is indicated by the very different patterns of cancer and cardiovascular disease that are found amongst first generation migrant populations\(^6\). A very consistent observation that has emerged from these studies is that increasing intakes of fruit and vegetables result in a protection against the risk of cancer and cardiovascular disease\(^7\). This has lead to the development of the hypothesis that these diets are protective because they are rich in antioxidants and neutralise directly, or indirectly, the potential toxicity resulting from the generation of ROS.

There is both direct and indirect evidence for the involvement of mitochondrial damage in neurological disorders, such as Alzheimer’s and Parkinson’s disease and ROS are generated within the mitochondria. In addition there is evidence that excessive intake of calories results in an increase in ROS generation by complexes I and III of the mitochondrial respiratory chain\(^7,8\). It is possible that the diet may play an important role, acting alone or in tandem with other factors e.g. infection, in determining the rate of oxidative damage to cells and the rate of onset of disease in an individual.

The generation of ROS is also believed to underlie more general aspects of ageing, not directly related to disease e.g. the gradual decline in the rate of respiration and loss of immune response\(^9\). The effects of dietary antioxidants on slowing down these processes is a subject where more research could result in major social and economic benefits, if preventative effects were to be demonstrated. The emphasis in this project is to critically review the current evidence on the potential health benefits of dietary antioxidants especially within the range of intakes that could be achieved by individuals consuming a diet rich in fruit and vegetables.

The specific effects of dietary antioxidants, which include the antioxidant vitamins (C and E), the carotenoids, and the vast array of phenolic phytochemicals (with strong antioxidant activities in vitro), have been the subject of detailed studies in EU nutritional research activities. However there is as yet no strong evidence from intervention studies, using single or multiple antioxidants, that there are highly significant effects on disease prevention. Indeed in trials involving the use of beta-carotene supplements the evidence is that health risks may increase\(^11,12\). The importance of identifying which compounds in fruit and vegetables do exert a protective effect against degenerative diseases still remains a distant objective. What is clear is that there are optimal levels of intake of the bioactive constituents of fruit and vegetables, but which matter and how they achieve these effects, at nutritionally-relevant levels of intake, remain major research challenges.

The main issues that remain to be resolved are: -
The development and validation of biomarkers that are clearly related to long-term risks of disease and that enable functional effects to be measured reliably and reproducibly.

An improved understanding of the uptake, tissue and cellular distribution and localisation, and metabolism and turnover of dietary antioxidants and phytochemicals that upregulate antioxidant defences.

The functional effects of antioxidants in the mitochondria particularly in relation to their effects on mitochondrial function and integrity.

The effects of antioxidants on gene expression particularly in relation to the oxidative stress in vulnerable tissues and cells.

The development of in vitro and in vivo systems that are a true reflection of the behaviour of normal human responses to antioxidant phytochemicals and their metabolites at levels of exposure that are relevant to those ingested.

It is clear that many of these goals cannot be met solely through studies on humans given the limitations on studying effects at target tissues and within specialised cells. However animals are often poor substitutes for human responses and no more so than when they are used to study the metabolic effects of the lipophilic antioxidants. New animal model systems and in vitro cell systems are going to have to be developed in order to study dose-response and to know that the functional effects are likely to be those found in humans. Without this information it will neither be possible to understand the functional effects of antioxidants, nor to determine the overall level of intake that will offer optimal protection against the adverse effects of ROS generation. There is likely to be little benefit in undertaking further expensive long-term intervention trials designed to demonstrate the potential for antioxidants to prevent disease unless this work is carried out beforehand.

References
