

NEW BIO-MARKERS OF OXIDATIVE STRESS TO HUMANS: A ROLE IN DEVELOPING NEW STRATEGIES FOR HUMAN PROTECTION AGAINST ENVIRONMENTAL (UVA) DAMAGE TO SKIN (PREVENTION BIOMARKERS)

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Section 2: <u>Project Progress Report</u>	NOT CONFIDENTIAL
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Objectives:

Overall the objective of this proposal has been to develop new bio-markers based on changes in gene expression and mitochondrial damage which may be used to monitor exposure and susceptibility to oxidative stress from the environment and to validate this

methodology using the model of acute and chronic skin exposure to the environmental oxidising carcinogen, ultraviolet A radiation. Information has also been sought as to how the most important dietary phenolic compounds are metabolised and which compounds become bioavailable. The major dietary antioxidant phenolic compounds and their metabolites have been examined for a potential protective role in the skin against environmental stress.

Results and Milestones:

We have developed new bio-markers of acute and chronic stress to humans and these have been exploited to monitor changes due to the environmental stress of solar ultraviolet A radiation on the skin. The bio-markers have been designed to have wide applicability and have been tested primarily on cultured human skin cells, fibroblasts and epidermal keratinocytes. The new bio-markers are based firstly on extremely sensitive alterations in the expression of genes (mRNA accumulation and protein synthesis) and secondly on increases in damage to mitochondria. Of the new biomarkers of oxidative stress based on gene expression, the most useful *in vitro* markers of acute oxidative stress appear to be heme oxygenase 1 (involved in inflammation), collagenase (MMP1) and the intracellular adhesion molecule 1 (ICAM1) although several others have been examined. The “common deletion” has been demonstrated to be a powerful marker of mitochondrial damage and has proved robust in human studies as a marker of chronic damage. Compounds from all the major groups of dietary phenolics (flavanols, flavanols, anthocyanidins and hydroxycinnamates) have been examined and that metabolites identified. The phenolics and their bioavailable circulating metabolites have been tested for antioxidant activity and for protection against oxidative damage to skin cells caused by hydrogen peroxide and UVA radiation both in terms of cell viability and activation of specific markers of gene expression. These studies have permitted the complete validation of such biomarkers in skin cells *in vitro* and have highlighted lead compounds for further testing *in vivo*. As an example, epicatechin and its methylated metabolite both protect against oxidative damage to skin cells in a variety of cell damage assays. Metabolites of citrus flavonoids and, in particular hesperetin glucuronide are also protective. This type of information was exploited in a human intervention study comparing a diet either high or low in flavonoid content for its protective properties.

The high flavonoid diet was supplemented with green tea extract (rich in catechins) and oranges (rich in citrus flavonoids). For the human study, volunteers were fed with strictly controlled low or high flavonoid diets in a cross-over design. Skin was irradiated with UVA after a suitable period on each diet. Skin biopsies from irradiated and unirradiated sites were monitored for changes in the oxidative stress markers and for flavonoid content. Although novel chemical entities consistently appeared in the skin of subjects exposed to the high flavonoid diet, these have not yet been definitively identified. However, two of the genes known to be activated by oxidative stress (ICAM-1 and MMP-1) were consistently and significantly activated by UVA stress in the skin of the volunteers on the low flavonoid diet. Of special significance is that this activation was consistently suppressed in volunteers maintained on the high flavonoid diet. The outcomes of this study are therefore of considerable significance. Firstly, biomarkers of oxidative stress developed through *in vitro* models have been clearly validated both in a protection model *in vitro* and *in vivo* in a well-controlled human study. Secondly specific foods containing flavonoid components with protective or potentially protective (through metabolism) properties have been clearly identified. Finally a human study in which volunteers were maintained on controlled flavonoid diets has demonstrated that levels of oxidative stress (as monitored by the new biomarkers) can be influenced by diet.

Benefits and Beneficiaries:

The capability of monitoring changes in the human body as a result of specific food intake, environmental stress and the ageing process has been recognised as a highly desirable technology to have available throughout the European Union. Specifically, the development of biomarkers/bio-indicators has been a priority in the EC Fifth Framework Quality of Life programmes concerned with food, the environment and the ageing process. Improved knowledge on the foods providing optimal health benefits, protection from environmental stress and contributing to healthy ageing will be of benefit throughout the community. This project has provided powerful new bio-markers that will aid in the monitoring process. New information has emerged on the protective effects of metabolites of specific dietary phenolic compounds particularly from the catechin and citrus flavonoid families. In particular the studies on flavonoid metabolites have significantly advanced the field by pointing to the biological relevant molecules and their properties, particularly in terms of protection. The new validated biomarkers will now provide more sensitive and more reliable methodologies for specialised laboratories in Europe (and beyond) to examine new compounds with potentially protective properties in particular by their ability to monitor oxidative stress levels. A human study has been completed which has not only provided initial validation of the new biomarkers of oxidative stress *in vivo* but also that diet can significantly modulate levels of such stress. This information will be of immense value to understanding and promoting healthy diets in the entire European Community and beyond.

Future Actions (if applicable):

The dissemination and exploitation plans will be carried out as detailed in the technological implementation plan.