Within a very busy time period of preparing the second annual report for the European Commission and shortly before our General Assembly Meeting 2008 in Liverpool the present project brochure was written and printed. The second year of LYCOCARD yielded a lot of substantial results, e.g. concerning delivery of lycopene to cells or metabolism of lycopene. Some of these results were already submitted as papers to scientific journals for publication. Many partners presented their results at scientific conferences or at industrial meetings. As part of LYCOCARD's dissemination work the news platform "Tomato+Health" was launched in October 2007. A first electronic newsletter was published in March 2008. Another important goal of LYCOCARD is to encourage the network between scientists from different European countries. Thus, it was good to see the increased number of training activities (summer school, workshops, etc.) during the second year. Especially the exchange of young scientists between the different countries is a great chance for them to improve their capabilities and to make Europe a strong scientific unit. LYCOCARD presents 2nd year results in Toronto (8-11 June 2008) at the 8th World Congress on the Processing Tomato and in Okinawa (22-27 June 2008) at the 15th International Symposium on Carotenoids. Thus, the progress of the project is shown globally. We are surprised and pleased that an increasing number of people from various backgrounds can hardly wait to get to know the final results in 2011.

Research activities and smaller meetings formed a good group of interesting people who will meet again as a whole in Liverpool. Some highlights from the second year are presented below.
LYCOPENE, ITS METABOLITES AND ITS OXIDATIVE/DEGRADATIVE PRODUCTS: BIOLOGICAL ACTIVITIES IN IN VITRO MODELS

DR. GORDON LOWE

2007-2008 Project Pillar Leader of the in vitro studies within LYCOCARD.

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There exists considerable epidemiological evidence supporting a relationship between an increased consumption of tomatoes and tomato products with a decreased risk of cardiovascular disease (CVD). Among the many studies, some simply compare dietary intake of tomatoes, plasma or tissue concentrations of lycopene (the red coloured bioactive pigment of tomatoes) with various biomarkers related to atherosclerosis or CVD. Consequently, the main objectives of the in vitro work packages of LYCOCARD are directed toward the evaluation of mechanisms for the uptake of lycopene, its transport within plasma and its role in protecting against the development of atherosclerotic plaques. The primary avenue of investigation within the in vitro section has focussed on cell culture, the oxidation of isolated Low Density Lipoproteins (LDL), the synthesis and isolation of lycopene isomers and their respective metabolites and finally the analysis of lycopene metabolites to investigate their biological activities in various cell types. Approaching month 24 of the project it is evident that progress is being made in all the areas mentioned above.

- **Intestinal Uptake of Lycopene.** Several membrane transporters have been identified and are thought to be responsible for the uptake of lipids and carotenoids into human enterocytes. The incorporation of β-carotene into enterocytes by specific membrane transporters has been demonstrated, but the mechanism for lycopene has yet to be elucidated. LYCOCARD studies over the last two years in one work package have indicated that more than one specific transporter may be needed for the uptake of lycopene from the human gut.

- **Oxidation of LDL.** One of the initial events of atherosclerosis is believed to be the oxidation of LDL. This may occur in the circulation or in the sub-intimal space of an artery. LDL largely transports cholesterol to the tissues and contains several lipid soluble antioxidants including vitamin E, β-Carotene (all-E)-lycopene and lycopene (Z)-isomers. One major risk factor for CVD is cigarette smoking. Experimental results obtained within the last year have indicated that cigarette smoke, and other well defined free radical generators depleted lycopene and its (Z)-isomers within isolated human LDL. Alongside this lycopene depletion, cholesterol is oxidised to 7-ketocholesterol and this compound was shown to have a profound effect on the cell signalling and apoptotic pathways of THP-1 cells. The inclusion of lycopene in these studies reversed the effect indicating another protective role of the carotenoid.

- **Lycopene Isomers and Metabolites.** The lycopene molecule has a long 40-carbon open ended structure and during oxidation the long polyene chain can be cleaved or isomerized via dehydrogenation/hydration yielding (Z)-isomers. The (Z)-isomers of lycopene are located in LDL particles and in the tissue of the body. Their chemical nature and antioxidative properties have not been fully investigated. Isolation and distribution of the isomers in parallel with the development of novel in vitro tests will hopefully yield more information on the role of (Z)-isomers as antioxidants. The degradative pathway of lycopene is very complex and involves the formation of (Z)-isomers, epoxides and lycopens to name but a few compounds. One suggestion is that lycopene is chemically modified to produce shorter chain compounds that retain biological activity. In this respect, the researchers working on LYCOCARD are in a unique position, with one collaborative partner having the expertise to chemically synthesise these components and distribute them to other partners for biological testing.

- **Biological Activity of Metabolites.** Lycopene has been tested using an adipose cell line and in HEK cells. In the former case no effect was seen, whilst in the latter a non-significant increase in gene expression was observed.

- **Inherent Difficulties.** In vitro studies are largely hampered by the nature of lycopene as a substance: it is very insoluble in aqueous media and many organic solvents and will form aggregates readily. It is vital that lycopene can be delivered to cells in culture. Several methods have been proposed for delivering lycopene or its (Z)-isomers to cells in culture. All the methods have been assessed and the outcome indicated that either the use of the solvent tetrahydrofuran or inclusion of lycopene or its isomers in micelles provide the most effective method of delivery to cells.

**IN-VITRO STUDIES HAVE BEEN ORGANISED INTO THE FOLLOWING WORK PACKAGES (WP):**

**WP 1.** Fractionation and isolation of lycopene (Z)-isomers  
(Dr. V. Böhm, Jena University, Germany).

**WP 2.** Isolation of lycopene metabolites from aecellular models and/or by organic synthesis  
(Dr. C. Caris-Veyrat, INRA Avignon, France).

**WP 3.** Determination of the antioxidant activity of (Z)-isomers and metabolites of lycopene in different model systems  
(Dr. V. Böhm, Jena University, Germany).

**WP 4.** Transactivation of nuclear hormone receptor pathways by lycopene, lycopene (Z)-isomers and lycopene metabolites  
(Dr. R. Rühl, Debrecen University, Hungary).

**WP 5.** Evaluation of nuclear receptors target gene expression in the presence of lycopene (Z)-isomers and metabolites  
(Dr. R. Rühl, Debrecen University, Hungary).
Study of the involvement of transporters for lycopene absorption
(Dr. P. Borel, INRA/INSERM/Marseille University, France)

WP 7.
Effects of lycopene, lycopene (Z)-isomers and lycopene metabolites on adipocyte differentiation
(Dr. P. Borel, INRA/INSERM/Marseille University, France)

WP 8.
Establishment of methods to deliver lycopene, its (Z)-isomers and its metabolites alone and in combination with cigarette smoke condensate (tar), cholesterol and oxidised LDL into cultured cells (Dr. V. Stangl, Charité Berlin, Germany).

WP 9.
Study of the effects of lycopene on the oxidative status of vascular cells exposed to tar or to cholesterol/oxysterols/oxidised LDL (Dr. P. Palozza, Catholic University of Rome, Italy).

WP 10.
Study of the effects of lycopene on cell growth and differentiation of vascular cells exposed to tar or to cholesterol/oxysterols (Dr. P. Palozza, Catholic University of Rome, Italy).

WP 11.
Characterisation of intracellular lycopene metabolites and oxidation products formed by the interaction of lycopene with cigarette smoke condensate or cholesterol (Dr. P. Palozza, Catholic University of Rome, Italy).

WP 12.
Comparative studies of the effects of lycopene, extracts from fresh tomatoes and lyophilised tomatoes, lycopene (Z)-isomers and other tomato carotenoids (β-carotene and phytofluene) on redox status and cell growth of vascular cells exposed to cigarette smoke condensate and cholesterol/oxysterols (Dr. P. Palozza, Catholic University of Rome, Italy).

WP 13.
Characterisation of oxidation products of lycopene and apolipoprotein B 100 in oxidized LDL (Dr. G. Lowe, Liverpool University, UK).

WP 14.
Evaluation of lycopene effects on endothelial function (Dr. V. Stangl, Charité Berlin, Germany).

Finally, the *in vitro* studies have indicated that lycopene and its (Z)-isomers may have a role in protecting human LDL from oxidation. However, free radicals and reactive species can cause the chemical cleavage (and possibly isomerisation) of lycopene. The resulting metabolites along with oxidation products of cholesterol may influence the activity of vascular cells and monocytes within the sub-intimal space of the artery. During the next twelve months it is hoped that the role of these lycopene metabolites will be more clearly defined.

EU states are witnessing a spiralling epidemic in cardiovascular disease and rocketing diabetes and chronic obesity with proportional burden of cost and increasing demands on health systems. Cardiovascular disease alone cost the EU 170 Billion € last year. Lowering the risk of disease has therefore become imperative.

“We are trying to persuade populations to adopt heart healthy eating habits by sending themselves a nutritional Valentine’s card” says Dr. Cristina Mele of NUTRIUNIT (project partner, Rome).

“Literally putting a little love in your heart by making small dietary changes can make massive and instant improvements to your health and quality of life”

LYCOCARD may be part of the solution; a 5-year integrated project within FP6, that started in April 2006 to identify exactly why lycopene, sourced mostly from processed tomato products, may play an important role in protecting EU citizens by preventing cardiovascular disease. Project partners, Deutsche Herzstiftung (German Heart Foundation) and NUTRIUNIT (Rome University) will transmit the results with obvious benefits for consumers, health care and EU tomato industry.

Studies now show consumers making informed dietary choices based on solid scientific conclusions and as the project progresses, LYCOCARD will develop new health-food products based on its research.

“Findings will lead to novel dietary guidelines helping consumers select specific diets to prevent and minimise disease risk” explains LYCOCARD project co-ordinator Dr. Volker Böhm of the University of Jena. “LYCOCARD will therefore improve the health of consumers in Europe (and worldwide). This helps reduce the growing cost of health care. In addition, the European food industry will be strengthened by increasing demand for health-related tomato products.”

“Effectively transmitting these dietary guidelines to the health-care community and general public is the greatest challenge. Dissemination activities revolve around the strong image of the project logo and the innovative way the project has branded itself to represent heart-healthy nutrition through personal self-caring,” says Dr. Böhm.”

The logo in reverse now brands our new, online magazine “Tomato+Health” published to provide a broad range of nutritional information resources for different interest groups and includes puzzles and games for children, classroom teaching materials, a strong press kit and dietary information for health professionals and researchers. The e-zine launched at the International Tomato Day, in Parma, Italy, on the 18th October 2007.”

While EU states ponder the wisdom of investing in preventative measures against the looming threat of overwhelmed health systems, they might stop and consider taking a closer look at this small part of the solution.
**IN VIVO INVESTIGATIONS OF THE BIOLOGICAL ACTIVITIES OF LYCOPENE**

**DR. RALPH RÜHL**

2007-2008 Project Pillar Leader of the *in vivo* studies within LYCOCARD

Laboratory of Nutritional Bioactivation and Bioanalysis and Apoptosis and Genomics Research Center of the Hungarian Academy of Sciences, Department of Biochemistry and Molecular Biology, University of Debrecen, Nagyerdői Krt. 98, 4012 Debrecen, Hungary.

Lycopene and various carotenoids in tomatoes like β-carotene, phytofluene and phytoene have been shown to be highly bioactive derivatives. Various mechanisms have been described in the literature and various pathways are still elusive. Carotenoids in general could act as anti-oxidants but this seems to be just one out of the various potential mechanisms of action. In our research projects from LYCOCARD we focus additionally on lycopene isomerisation and degradative lycopene metabolites as well as on metabolites from various other tomato carotenoids.

Additional to the metabolic activation of tomato carotenoids and the investigation of the antioxidant potential, our focus is on the involvement of transporter mechanisms of these carotenoids. Especially genetic polymorphisms of intestinal transporters for lycopene are currently studied.

**- What is new in 2008?**

First of all, our LYCOCARD partner from Avignon, Catherine Caris-Veryat and colleagues synthesised and purified various cleavage derivatives from *(all-E)*-lycopene. In addition, various new potential metabolites are now commercially available and/or have been synthesised by our contract partner. These derivatives have been delivered to various groups of the LYCOCARD project and will be examined in various *in vitro* and *in vivo* systems. The aim of these studies is to determine how lycopene metabolites modify gene regulation via nuclear hormone receptor pathways.

Additionally, we got from the group of our LYCOCARD coordinator various lycopene isomers and could examine their biological potential in various biological model systems. The aim of these studies will be to determine if there are biological effects preferentially or more efficiently mediated by lycopene isomers in comparison to the *(all-E)*-lycopene.

Using animal models for carotenoid research has proven to be quite difficult, due to significant differences in blood transport, metabolism and especially intestinal uptake in comparison to the human situation. In addition we want to study the effects of lycopene on atherosclerosis prevention. Our LYCOCARD partner from Berlin is working now with rabbits as an animal model and they will also focus on other animal models for their studies.

Secondly, the group of our LYCOCARD partner Patrick Borel now has identified selected target transporters in *in vitro* studies and they will study more deeply the influence of the genetic polymorphism of these target transporters in a randomised trial with human subjects on lycopene absorption.

Thirdly, we are also investigating the influence of a tomato-rich diet on the production of reactive oxygen species (ROS) production in isolated mononuclear cells, oxidative stress and the lipid status from volunteers before and after supplementation with a tomato-rich diet. These human supplementation studies are in progress now. New methodologies for these investigations have been established in LYCOCARD labs and various serum samples will be investigated with these technologies to determine the anti-oxidative potential and mechanism of tomato-diet and especially lycopene.

Three human supplementation studies have been performed additionally in the LYCOCARD groups of Verena Stangl from Berlin, Volker Böhm from Jena and Alvaro Morente from Rome. The aim of these three supplementation studies is to investigate: a) the effect of tomato products on endothelial function, b) the isomerisation of lycopene and c) if lycopene enriched tomato products may increase serum lycopene levels and other biomarkers more than non enriched tomato products as well as traditional products. All these human supplementations are still in progress or have been finished. Serum samples from these volunteers partly have been distributed in between the LYCOCARD partners and will be investigated using various state of the art technologies.

**IN-VIVO STUDIES HAVE BEEN ORGANISED INTO THE FOLLOWING WORK PACKAGES (WP):**

- **WP 15.** Determination of nuclear receptor activation by lycopene, lycopene *(Z)*-isomers/metabolites and tomato preparations in transgenic animals (Dr. R. Rühl, Debrecen University, Hungary).
- **WP 16.** Analysis of the redox status of mononuclear cells isolated from chronic smokers and hypercholesterolemic patients before and after supplementation of a tomato-rich diet (Dr. P. Palozza, Catholic University of Rome, Italy).
- **WP 17.** Effect of polymorphisms in intestinal lycopene transporter(s) on lycopene bioavailability (Dr. P. Borel, INRA/INSERM/Marseille University, France).
- **WP 18.** Evaluation of oxidative stress and lipid status in human plasma: influence of smoke exposure and lycopene supplementation (Dr. G. Lowe, Liverpool University, UK).
Finally, all our in vivo studies partly with laboratory animals and partly using human supplementation trials are in the progress. Several new methodologies have been established in LYCOCARD groups and will be used to determine the mechanism of action of lycopene, lycopene isomers, lycopene metabolites and tomato products. Our two main focuses are: a) The preventive mechanisms of lycopene / tomato products on cardiovascular functions and b) the involvement of lycopene isomers and lycopene degradative metabolites in lycopene nutritional / biological activity. During the next twelve months we will get more knowledge, how tomato derived compounds like lycopene, its isomers and its metabolites modify these various nutritional protective pathways.

WP 19. Study of lycopene isomerisation in human plasma by isotopically labelled lycopene (Dr. V. Böhm, Jena University, Germany).

WP 20 Evaluation of arteriosclerosis prevention by lycopene in an animal model and in humans (Dr. V. Stangl, Charité Berlin, Germany).

WP 21 WP 22 WP 23 In vivo effect of diets enriched with traditional and newly developed tomato products on obese and normal weight subjects (Dr. A. Mordente, Nutriunit Rome, Italy).
LYCOPENE AND OTHER ANTIOXIDANTS IN TOMATOES AND TOMATO PRODUCTS

DR. MARÍA JESÚS PERIAGO
2007-2008 Project Pillar Leader of food product studies within LYCOCARD

During the second year of the Project the research activities in Food Product Studies have been focused on the study of the effects of agronomical and post-harvest treatments on the content of lycopene and other bioactive compounds in tomatoes, in determining the antioxidant activity and evaluating the effects of industrial processing and storage on the contents of lycopene and other bioactive compounds in different tomato products.

The goal of these trials is to provide information to the European consumers about the beneficial effects of the consumption of tomatoes and tomato products related to their content of antioxidant carotenoids, folates, phenolic compounds and vitamin C. So, the results obtained during this period have provided answers to the following questions:

Which are the main factors that determine the lycopene content and other bioactive compounds such as phenolic compounds, vitamin C and folates? The content of these compounds depends on the cultivar and the maturity index, but agronomical factors during tomato production in the field also determine their content.

How can tomatoes be handled in the field and post-harvest to improve the content of lycopene and other bioactive compounds? Tomatoes ripened on-vine provided higher content of these compounds, since off-vine maturation during storage depend on the storage conditions. In addition, new technologies applied during the storage (such as UV light, controlled atmosphere etc.) of raw tomatoes could also affect the off-vine synthesis of bioactive compounds.

What is the contribution of different antioxidant compounds to the antioxidant capacity of tomatoes, considering the lipophilic and hydrophilic activity? The antioxidant capacity of tomato extracts depends on the antioxidant activities of their compounds. Hydrophilic activity is mainly associated with the content of vitamin C and phenolic compounds, which show a high scavenging activity, whereas the antioxidant activity of lycopene as scavenger of free radicals is poorer than hydrophilic compounds. In tomato products, antioxidant activity depends on the thermal treatment applied during processing.

Can tomatoes prevent the oxidation of macromolecules (lipid, protein and DNA) using an in vitro model? The hydrophilic extract of tomatoes shows a higher antioxidant activity in in vitro cell model than the lipophilic extract containing lycopene. More studies are being carried out to design a protocol to evaluate the prevention effects of tomato extracts against macromolecules oxidation.

How can industrial processing and storage affect the total content of lycopene as well as antioxidant compounds and lycopene isomerisation? Tomatoes products show a high content of lycopene and other bioactive compounds, mainly phenolic compounds. However, the industrial process leads to a slight reduction of other bioactive compounds with vitamin activity such as vitamin C and folates, which exhibit the activity of vitamin B9. These changes also affect to the antioxidant capacity of tomato products. The effect of storage on the contents of bioactive compound is also taken in consideration in order to provide the information about the best conditions of storage to preserve the beneficial effect of tomatoes.

FOOD PRODUCTS STUDIES HAVE BEEN ORGANISED INTO THE FOLLOWING WORK PACKAGES (WP):

WP 24. Analysis of bioactive compounds in tomatoes (Dr. M. J. Periago, Uni Murcia, Spain, and J. Fernández, AGRAZ, Spain).
WP 25. Evaluation of the antioxidant activity of tomatoes and tomato-based products (Dr. P. Palazza, Catholic University of Rome, Italy, Dr. M. J. Periago, Uni Murcia, Spain, and J. Fernández, AGRAZ, Spain).
WP 26. Search for in vivo metabolites of lycopene and human intervention studies with newly developed tomato products. (Dr. V. Böhm, Jena University, Germany).
WP 27. Accessibility and availability of bioactive compounds (Dr. C. Caris-Veyrat, INRA Avignon, France, and Dr. M. J. Periago, Uni Murcia, Spain).
WP 28. Evaluation of tomato components following processing/Evaluation of lycopene stability during storage. The effect of tomato product processing and storage on the content of lycopene and other bioactive compounds is being evaluated between Uni Murcia, Spain and two tomato industry processors. (F. Hermosilla, JUVER Alimentación S.L.U., Spain and R. Pérez, CONESA S.A., Spain).

During the two years of the project the work has been going on in WP 24, WP 25, WP 28 and WP 29, obtaining preliminary results according to the planned objectives. The activities related with the WP 26, WP 27 and WP 30 will be carried out in the following years.
Dissemination of the project’s aims and results is an integral part of LYCOCARD. Since the start of the project, major efforts have been made to make sure the scientific community, related fruits (especially tomato processing) industries, patient care organisations, and the general public are kept informed through a variety of activities: websites, conferences, press releases and publications.

In the second year of the project, the main dissemination actions have been:

- The presentation of the project’s first results at various scientific or trade meetings, with two particular events: one targeted at the research community was a specific session entitled “LYCOCARD: Investigating the role of lycopene in the prevention of cardiovascular diseases” which was held during the 10th European Nutrition Conference (Paris, France, 10-13 July 2007), while the Tomato Day Conference held in Parma (Italy) on 18 October 2007 was directed specifically towards the tomato processing industry, with a special emphasis on the collaboration between the scientists and the 4 SME industrial partners.

- The launch in October 2007 of the www.tomatoandhealth.com website, LYCOCARD’s latest communication platform to promote the health benefits of the Mediterranean diet (heart healthy food featuring tomato and other lycopene bearing fruits in a dietary context) to educate different target groups (adults, children, health community and the media). Viral communications materials were launched from this site in February 2008 including an online heart-health cooking show, i-cookTV, with free access and mp4 and mp3 downloads.

- The continuous update of the www.lycocard.com website, and the November launch of its German language version. The Extranet section gives information on the state-of-the-art and the consortium, and project results are added as the project progresses. The Intranet (member area) section provides consortium members a secure way to exchange information confidentially.

- The publication of the first LYCOCARD annual report in July 2007: this document has been widely distributed by consortium members and its contents translated into several languages. These versions are available freely from the project homepage.

- The publication of a one-page colour article presenting the project in the September and November 2007 issues of Parliament Magazine to inform MSP and Brussels officials of the existence and aims of the project.

- The creation of presentation posters in several languages, banners and a PowerPoint presentation which have been used by project partners to present the project at various scientific and industry events.

- A number of different training courses for scientists and technical staff in the tomato processing industry were also organised during the year.

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To find out more:
Over the next few months, there will be many new opportunities to learn more about the project and chances to meet with the LYCOCARD scientists at the following events:

8th World Processing Tomato Congress & 11th ISHS Symposium on the Processing Tomato (www.worldtomatocongress.com)
Toronto (Canada), 8-11 June 2008

15th International Symposium on Carotenoids (www.carotenoidssociety.org)
Okinawa (Japan), 22-27 June 2008

A series of project presentation videos with interviews by scientists will be filmed and made available on the project website and on DVD later in 2008.

Moreover, LYCOCARD scientists are currently working on a textbook for students and teachers of food science and nutritional studies with a focus on how nutrition relates to cardio-health. The book entitled “HEART-HEALTH NUTRITION” will be written by LYCOCARD scientists and other experts in their field and will be published in Spring 2009.

For more information and for regular updates about the LYCOCARD project, please consult www.tomatoandhealth.com or www.lycocard.com

For further details contact: claudia@lycocard.com
Integrated project funded by the European Commission within the Sixth Framework Programme.
Tomato+Health is the newest most exciting health +
nutrition magazine on the web. It’s an open access
resource providing impartial, unbiased dietary
advice completely free of advertising. The site
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