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ANNUAL REPORT 2009

SIXTH FRAMEWORK PROGRAMME

LYCOCARD®

NOTE FROM THE CO-ORDINATOR
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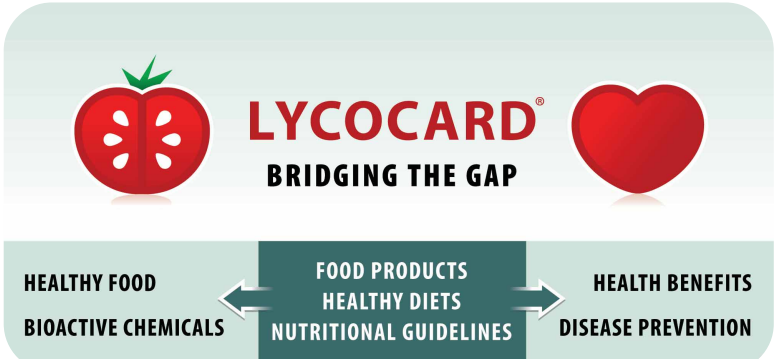
In June, we experienced the Hungarian hospitality during our General Assembly Meeting 2009 in Debrecen. Our third annual report was presented there to the reviewers and to our scientific officer. The third year of LYCOCARD yielded a lot of results and led to ten papers in scientific journals. For example, membrane transporters have been identified, being responsible for the uptake of carotenoids into human enterocytes. Further investigations will examine the influence of genetic polymorphisms of carotenoid transporters on the intestinal absorption of lycopene. Two papers from the third year of the project showed different possibilities how to deliver lycopene to cells. Different processing steps were investigated on their effects on bioactive compounds in tomato products. Homogenisation has the potential to improve the nutritional value of tomato puree. As was also done in the second year, many partners presented their results at scientific conferences or at industrial meetings. One important scientific conference in the field of carotenoids was the 15th International Symposium on Carotenoids in Okinawa, Japan, where some LYCOCARD scientists used the occasion to present and to discuss their project work with colleagues. Integration of our industrial partners was intensified and in autumn 2009 a possibility will be presented to the tomato processors to allow interactions also with external industrial partners to increase the visibility and usage of LYCOCARD's results. We are looking forward to the results of ongoing human intervention trials investigating cardio-protective effects of tomato products.

It was impressing to see a further increased number of exchanges of young scientists. These young people are the future of Europe and they like to use the boundless possibilities to improve their scientific capabilities. Some highlights from the third year are presented below.



LYCOPENE, (*E*)- AND (*Z*)-ISOMERS AND ITS METABOLITES: OBTAINING AND BIOLOGICAL ACTIVITIES IN *IN VITRO* EXPERIMENTAL MODELS.

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Participation of tomato consumption to the prevention of cardio-vascular diseases is now well-documented in the scientific literature, but mechanisms explaining these effects are not well known. (*all-E*)-Lycopene, the main pigment of tomato is thought to participate to the preventive effects, but it is also highly probable that its (*Z*)-isomers and/or its metabolites are also bioactive. The aim of the in vitro studies of LYCOCARD are to find out the mechanisms implied in the biological process of cardio-vascular diseases on which (*all-E*)-lycopene, (*Z*)-isomers of lycopene and its metabolites could have an effect. After significant progress during the first 2 years, new determinant scientific data were obtained during this third year.

First, the different molecules to be tested were prepared in significant quantity by different partners. (*all-E*)–lycopene was extracted from tomato and purified by one partner who provided it to the other partners of the LYCOCARD consortium, thus making available for all a homogenous and pure source of lycopene. Lycopene (*Z*)-isomers were obtained by isomerisation of (*all-E*)-lycopene and purified by preparative LC techniques. Lycopene metabolites were prepared in high amounts (up to 200 mg) and high purity (>95%) by organic synthesis and were distributed to partners for testing their biological activity.

Second, antioxidant activity of (*all-E*)-lycopene, (*Z*)-isomers of lycopene and its metabolites were evaluated by various test systems: L-ORAC, chemiluminescence, DPPH assays, LDL oxidation and lipid peroxidation in gastro-intestinal mimicking system. Tests are being finished and structure-relationship activity should be available during next year.

Other non-antioxidant activities of lycopene, its isomers and metabolites were evaluated concerning cell signalling systems, a main hypothesis which could explain the bioactivity of carotenoids in vivo. Lycopene metabolites were indeed shown to be potentially bioactive for the nuclear receptor activation in reporter cell line. One of the main achievements in this field was that (*5Z*)-lycopene and one lycopene metabolite were found to regulate the gene expression in adipocytes stronger than (*all-E*)-lycopene. To get more details of the molecular mechanism implied, analysis of transcriptome and proteome of adipocytes has begun and results should be available during the next period. Other biological activities implied in cardio-vascular diseases were tested in cells sytems using (*all-E*)-lycopene. If no effect of lycopene could be found on NO production in endothelial cells, (*all-E*)-lycopene was shown to modulate redox-sensitive proteins involved in cell cycle progression, in apopotosis induction and in cell survival.

Finally, as no effect of lycopene could be possible without the molecule being absorbed, the LYCOCARD consortium is interested in studying the processes of intestinal uptake of lycopene. After having found that SRB1, a cholesterol transporter is involved in the active lycopene transportation through the intestine, another receptor, CD36 was shown to be also involved.

During this third year, new research themes appeared concerning possible other bioactivation pathways of lycopene metabolites. Next year, efforts on the characterization of bioactivity will be continued as will the studies which aim to analyse lycopene metabolites potentially formed in cell systems, for example when lycopene is in presence of cigarette concentrate, or in vivo after human supplementation.

THE FOLLOWING ARTICLES WERE PUBLISHED DURING THE LAST YEAR:

Delivery of lycopene to physiologically relevant vascular cells.Lorenz M, Stangl V, Jacob C, Daemen K, Böhm V, Fröhlich K, Baumann G, Stangl K, Simone R and Palozza P, J. Food Lipids 2009, 16, 259–272

Lycopene prevents 7-ketocholesterol-induced oxidative stress, cell cycle arrest and apoptosis in human macrophages. Palozza P, Simone R, Catalano A, Boninsegna A, Böhm V, Fröhlich K, Mele MC, Monego G, Ranelletti FO. J. Nutr. Biochem. 2009 Jan 19

Effect of beta-carotene-rich tomato lycopene beta-cyclase (tlcy-b) on cell growth inhibition in HT-29 colon adenocarcinoma cells. Palozza P, Bellovino D,Simone R, Boninsegna A, Cellini F, Monastra G, Gaetani S., Br. J. Nutr. 2008, Dec 24,1-8

INVESTIGATION OF PHYSIOLOGICAL EFFECTS OF TOMATO PRODUCTS *IN VIVO*

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The transformation and validation of the *in vitro* results to the *in vivo* situation represents an important part of Lycocard. Therefore, animal experiments as well as clinical studies are considered a major component of the project. Since a number of epidemiological studies suggest protective cardiovascular effects of tomato and tomato products, the elucidation of underlying mechanisms *in vivo* remains a crucial challenge. Several partners within the project are engaged in the investigation of physiological effects of tomato products that could contribute to beneficial cardiovascular effects. Most importantly, the exchange of biological materials (blood samples, plasma, blood cells etc.) and data from all animal experiments and human intervention studies between all LYCOCARD partners has been organized. A data file containing all relevant information about details and outcomes of human studies within LYCOCARD was generated.

1. ANIMAL EXPERIMENTS

Using a reporter mouse model, the activation of several transcription factors by lycopene, lycopene isomers and metabolites are investigated. In the third year of the project, it could be shown that lycopene as well as lycopene metabolites are potent activators of transcription factors in these reporter animal models. The elucidation of transcription factors activated by tomato ingredients is a prerequisite for the induction of gene expression with potential benefits on the cardiovascular system.

Another study investigates the cardiovascular protective effects of lycopene in a New Zealand White rabbit (NZWR) animal model. Here, the potential impact of lycopene on the progression of atherosclerosis in rabbits is studied. During the third year, first results of the effects of lycopene on progression of atherosclerosis as well as on the extent of lesions in the aorta could be obtained in preliminary animal experiments. In addition, the appropriate time period and other conditions for the progression of fat-induced atherosclerosis in New Zealand White rabbits have been established, and will be used in the main experiment in the next year.

2. HUMAN INTERVENTION STUDIES

The design and preparation of human intervention studies takes a considerable time. Therefore, the first results of human studies were only beginning to emerge in the third year. Some partners faced problems with local ethic committees for permission of human studies. However, these issues are now resolved. Good progress has been achieved in the initiation and realization of human studies within the third year. Several aspects of physiological effects of tomato products are studied by different partners of the project.

The impact of a tomato-rich diet on redox status of mononuclear cells in chronic smokers and hypercholesterolemic patients is being investigated. The indication of *in vivo* markers of oxidative stress in mononuclear cells from these subjects is in progress. The analysis of lycopene in plasma and different lipoprotein fractions, including a survey for differences between smokers and non-smokers in lycopene levels as well as in lipoprotein and oxidation status has been started by another partner. A study of lycopene isomerisation and distribution in different lipoprotein fractions in humans using tomatoes with isotopic labelled lycopene is in preparation. The cultivation of tomatoes with isotopic labelled lycopene as a prerequisite for this study is finished.
The effects of tomato products on endothelial function as early marker for atherosclerosis have been investigated by another partner. A study with 19 postmenopausal women was finished within the third year. However, despite increases in lycopene plasma levels, no significant effects on endothelial function have been observed after supplementation with tomato products for one week. Next, the influence of tomato supplementation on endothelial function in smokers will be studied. Another human study investigating the health properties of traditional and lycopene-enriched tomato products in obese and normal weight subjects has started. In the third year, the supplementation on obese people was completed, while the analysis of the collected blood samples is still ongoing.

Since lycopene can exert its potential beneficial cardiovascular effects only after it is absorbed into the blood, the search for genetic variants involved in intestinal lycopene absorption represents another important issue within the project. In the third year, 182 subjects were genotyped for 58 single nucleotide polymorphisms in candidate proteins involved in lycopene absorption. The plan for the next year will be to investigate, which polymorphisms or combination of polymorphisms correlate with the extent of lycopene absorption.

Due to the high complexity of *in vivo* studies and the variety of objectives included in the project, a careful design of human intervention studies is necessary. Since the first completed clinical study within the project did not achieve the expected results on endothelial function, a systematic search for alternative markers/endpoints in human intervention studies is necessary and is extensively discussed between the project partners involved.

THE FOLLOWING ARTICLES OF THE *IN VIVO* STUDIES WERE PUBLISHED RECENTLY. SEVERAL ADDITIONAL MANUSCRIPTS HAVE BEEN SUBMITTED OR ARE IN PREPARATION.

Borel P, Moussa M, Reboul E, Lyan B, Defoort C, Vincent-Baudry S, Maillot M, Gastaldi M, Darmon M,

Portugal H, Lairon D, Planells R. Human fasting plasma concentrations of vitamin E and carotenoids, and their association with genetic variants in apo C-III, cholesteryl ester transfer protein, hepatic lipase, intestinal fatty acid binding protein and microsomal triacylglycerol transfer protein. Br. J. Nutr. 2009;101:680-687.

Jacob K, Periago MJ, Böhm V, Berruezo GR. Influence of lycopene and vitamin C from tomato juice on biomarkers of oxidative stress and inflammation. Br. J. Nutr. 2008;99:137-146.

Moussa M, Landrier JF, Reboul E, Ghiringhelli O, Coméra C, Collet X, Fröhlich K, Böhm V, Borel P. Lycopene absorption in human intestinal cells and in mice involves scavenger receptor class B type I but not Niemann-Pick C1-like 1. J. Nutr. 2008;138:1432-1436.

LYCOPENE AND OTHER BIOACTIVE COMPOUNDS IN TOMATO AND TOMATO PRODUCTS

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The main aims of the food product studies are to find out which factors determine the content, stability and bioavailability of lycopene and other bioactive compounds in tomato and tomato products. This will help the industrial partners to design new tomato products to be tested in in vivo studies.

During the last year, the research activities have focused on the following aspects:
Effect of cultivar, ripening stage, year of harvest and post-harvest ultraviolet light treatment on tomato bioactive compounds
Effect of processing and storage conditions on bioactive compounds
Contribution of bioactive compounds to antioxidant activity.
Study the in vivo metabolites of lycopene after the intake of tomato products.

Many of the objectives have been achieved during 2008-2009 and some conclusions can be drawn so far.

Tomato cultivars provided by CONESA were analyzed for total phenolic compounds, folates, vitamin C and antioxidant activity. Moreover, variations in those parameters were evaluated between years 2006-2008. Overall, the content of bioactive compounds was mainly affected by cultivar since no clear trend was detected between ripening stage (except for lycopene) and climatological conditions associated with the year of harvest. Several industrial cultivars were also analysed by AGRAZ to determine the total lycopene content.

A pilot study was done to evaluate the effect of a post-harvest treatment with ultraviolet light (UV). After UV exposure increases in lycopene, phenolic compounds and antioxidant activity were observed. These results are promising and raise the possibility of enhancing the content of bioactive compounds during post-harvest handling. However, the study will be performed again to confirm this effect.

During this period, a storage trial with tomato juices manufactured by JUVER was completed and it was shown that lycopene was substantially stable during storage of tomato juice for 1 year (losses < 20%). Phenolic compounds were also stable, whereas folates and vitamin C were markedly affected during storage. The room temperature during storage is appropriate to maintain the lycopene and phenolic compounds, while other bioactive compounds, especially folates and vitamin C, are less stable and could require special conditions.

CONSERVE ITALIA is still performing a storage trial lasting 3 years with different tomato products (passata and peeled tomato) and final results will be available at the end of the trial. The industrial partner CONESA carried out a study to evaluate lycopene stability during different steps of industrial processing of diced tomato, tomato paste and tomato powder. The samples are currently being analyzed.

THE RESULTS OBTAINED HAVE BEEN PUBLISHED IN THREE DIFFERENT SCIENTIFIC ARTICLES:

Perez-Conesa D., Garcia-Alonso F.J., Garcia-Valverde V., Iniesta M.D., Jacob K., Sanchez-Siles L.M., Ros G., Periago M.J. Changes in bioactive compounds and antioxidant activity during homogenization and thermal processing of tomato puree. Innovative Food Science and Emerging Technologies, 10 (2), 179-188, (2009).

Iniesta M.D., Perez-Conesa D., Garcia-Alonso F.J., Ros G., Periago M.J. Folate content in tomato (Lycopersicum sculentum). Influence of cultivar, ripeness, year of harvest, and pasteurization and storage temperatures. Journal of Agricultural and Food Chemistry, 57, 4739-4745, (2009).

Garcia-Alonso F.J., Bravo S., Casas J., Perez-Conesa D., Jacob K., Periago M.J. Changes in antioxidant compounds during the shelf life of commercial tomato juices in different packaging materials. Journal of Agricultural and Food Chemistry, 57(15): 6815-6822 (2009).

Antioxidant activity was evaluated by different in vitro tests (TEAC, FRAP, ORAC). The major contributors to total antioxidant activity were vitamin C and phenolic compounds, whilst the contribution of lycopene was low. However, lipophilic tomato extracts protected cultured cells from induced oxidative stress and DNA damage. Interestingly, protection was achieved at physiologically attainable lycopene concentrations.

In addition, a preliminary *in vivo* study was carried out in the University of Jena in collaboration with INRA Avignon to determine the *in vivo* metabolites of lycopene after the intake of tomato products.

The results obtained so far are being taken into consideration for the design and development different tomato based product for nutritional improvement addressed by the industrial partners JUVER, CONSERVE IITALIA and CONESA.

DISSEMINATION & TRAINING

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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 third year of the project, the main dissemination actions have been:
The presentation of the project's initial results at various scientific or trade meetings, with two major events in June 2008: the 15th International Symposium on Carotenoids in Okinawa (Japan) for the scientific community and the 8th World Processing Tomato Congress & 11th ISHS Symposium on the Processing Tomato held in Toronto (Canada), 8-11 June 2008, more targeted at the tomato processing industry.

Regular updates of the **www.lycocard.com** and **www.tomatoandhealth.com** websites, the latter being totally overhauled in June 2009 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).
The publication of many articles in peer-reviewed scientific publications, but also in the technical press and more general public media, including several interviews by **LYCOCARD** scientists on national and regional radio and television programmes.

Moreover, a number of different training courses for scientists and technical staff in the tomato processing industry were also organised during the year, and exchange of staff between partners enabled good communication and exchange of skills within the consortium

All these dissemination activities will continue during the fourth year with, in addition, the release of a series of project presentation videos with interviews by scientists by the end of 2009, and the forthcoming launch of the **LYCOCARD** Industrial Platform to involve more closely the industry in the activities of the project.

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

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