LYCOCARD®

www.lycocard.com

ANNUAL REPORT 2010

SIXTH FRAMEWORK PROGRAMME

NOTE FROM THE CO-ORDINATOR DR. VOLKER BÖHM

Institute of Nutrition, Friedrich Schiller University, Dornburger Str. 25-29, 07743 Jena, Germany.
Volker.Boehm@uni-jena.de

How time goes on! Already the fourth annual report for the European Commission was prepared and our General Assembly Meeting 2010 in Marseille is just ahead of us. The fourth year was also a scientifically very exciting one, as in fact all years were since the beginning of LYCOCARD in April 2006. Within our *in vitro* studies, isolation and characterization of lycopene isomers and oxidation products continued. A lot of experiments were done with these lycopene metabolites. Mice were used to get a deeper insight into *in vivo* metabolism of lycopene. Human saliva samples were used to analyse polymorphisms, being responsible for differences in absorption of lycopene. Food product studies extended storage experiments to get more information on the behaviour of lycopene and other tomato product ingredients. Processing conditions were optimised based on results from the last years. Resulting newly developed tomato products were produced by the industrial partners and used within an ongoing human intervention trial. A mixture of cardiovascular relevant markers (e.g. markers of inflammation, oxLDL, etc.) is being investigated in two human intervention trials to look for primary prevention effects of tomato products. Continuing from the last years, our dissemination work updated the website "Tomato+Health" using e.g. video presentations, interviews and recipes. This website is now available also in German: Italian and Spanish versions are being prepared. LYCOCARD was presented orally or as a poster 25 times at conferences. Two big steps were the launch of the LYCOCARD industrial platform, based on the collaboration with the EU project HEALTHGRAIN, and establishment of the collaboration with the well known Framingham Heart Study in Boston, USA.

Thus, European and global networks were strengthened during the fourth year of LYCOCARD. In addition, industrially relevant discussions were intensified to be prepared for our final tasks and to present final results within less than 10 months from now on. Some highlights from the fourth year are presented below.

LYCOPENE, ITS METABOLITES AND ITS OXIDATIVE/DEGRADATIVE PRODUCTS: BIOLOGICAL ACTIVITIES IN *IN VITRO* MODELS

DR. RALPH RÜHL

2009-2010 Project Pillar Leader of the *in vitro* 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, Nagyerdei Krt. 98, 4012 Debrecen, Hungary.
rruehl@indi.biochem.dote.hu

Tomato consumption and especially the molecule lycopene, the red pigment of tomatoes, seem to be associated with the prevention of various cardiovascular diseases. The mechanisms how lycopene and other carotenoids in tomato preparations might transmit this activity is still unknown.

The aim of the *in vitro* studies project of the LYCOCARD EU project was and is still to find out the mechanisms of preventive effects of tomatoes and tomato products on cardiovascular diseases with a special focus on lycopene.

LYCOPENE UPTAKE AND ISOMERISATION

In tomatoes and especially in tomato preparations like tomato sauce, tomato ketchup and tomato purée the concentrations of lycopene and various other tomato carotenoids are much higher than in fresh tomatoes. These tomato preparations are also consumed in much higher amounts in the diet in Western countries.

Lycopene in the (*all-E*)-form is present mainly in the human diet, while lycopene mainly occurs in various isomers ranging from (*5Z*)-, (*9Z*)-, (*13Z*)-lycopene as well as various other isomeric configurations in smaller amounts in the human organism. Our partners in the *in vitro* project recently developed a new HPLC method to separate these various lycopene isomers as well as a way to isolate them for usage in our *in vitro* experiments.

One of the main achievements by our *in vitro* work task was that (*5Z*)-lycopene was found to have a stronger effect in the regulation of the gene expression in adipocytes than (*all-E*)-lycopene. Transcriptome and proteome analysis of human and mouse adipocytes confirmed these effects.

Lycopene uptake and lycopene isomerisation in the human organism seem to be the initial steps to understand lycopene mediated bioactivity. Our partners from the *in vitro* work task found that SR-BI and CD36 are the key proteins which are responsible for lycopene uptake by the enterocytes in the intestine. In addition, it seems that these two proteins are not involved in lycopene isomerisation.

RELEVANT AND BIOACTIVE LYCOPENE METABOLITES

In addition to lycopene isomers, our partners in the *in vitro* work task prepared various potential lycopene metabolites like apo-lycopenoids, apo-lycopenoic acids, apo-lycopenols and diapo-derivatives either via oxidation and HPLC separation or via targeted organic synthesis.

Lycopene metabolites like apo-14´-lycopenal, apo-12´-lycopenal, apo-10´-lycopenal were detected in human serum after tomato product supplementation and in mice treated with lycopene. In addition to these lycopene-aldehydes, we were also able to detect apo-10´-lycopenoic acid in treated mice.

Unfortunately, the initially proposed direct pathway of lycopene metabolism to apo-10´-, apo-12´- and apo-14´-lycopenoids via carotene oxygenases 1 and 2 does not seem to be the physiological relevant pathway, because the detected derivatives were just present in very low concentrations. In addition, in cellular reporter assays these derivatives were also just minor active. But our *in vitro* team is still trying to find out the relevant pathways of lycopene metabolism to bioactive derivatives and we generated a lot of knowledge to find out the nutritionally relevant pathways of lycopene mediated bioactivity.

NUCLEAR HORMONE RECEPTOR ACTIVATION PATHWAYS OF LYCOPENE METABOLITES

We found out that especially nuclear hormone receptor (retinoic acid receptor / RAR, retinoid-X receptor / RXR and peroxisome proliferator-activated receptor PPARs) pathways, which are important hormonal regulator pathways for differentiation, proliferation, cell cycle control, inflammation, regulation of metabolism and especially lipid homeostasis, seem to be regulated by lycopene indirectly via bioactive lycopene metabolites.

ANTIOXIDANT ACTIVITY OF LYCOPENE AND LYCOPENE ISOMERS

Various lycopene isomers as well as lycopene metabolites have been tested in various test systems used by our *in vitro* partners. As it was expected, lycopene, lycopene isomers, other tomato carotenoids and lycopene metabolites were active antioxidants in *in vitro* assays.

A recent review article by an US group as well as our data let us speculate that simple radical scavenging effects are not able to explain these antioxidant effects. Lycopene metabolite mediated effects via receptor activation mechanisms which transmit anti- or pro-inflammatory signalling as well as anti- and pro-oxidative signalling seem to be more relevant.

ONGOING RESEARCH

Various important steps have been found to explain lycopene bioactivity by our *in vitro* group members, but there are still some main questions to elucidate:

- How relevant are these experiments and results for the human situation? Especially the lycopene concentrations present in the human organisms are much lower than the concentrations

necessary to enable anti-oxidant effects. Possibly, lycopene metabolism and higher activity of lycopene metabolites are also the key steps for lycopene mediated bioactivity.

- Which are the involved enzymes and which lycopene metabolites are present physiologically and after lycopene ingestion in the human organism? The main pathway of metabolic activation of lycopene is still unknown.
- Concentration dependent effects of tomato products and lycopene: We still do not know if after ingestion of higher amounts of tomato products and higher amount of lycopene we can observe better effects in respect for the human health.

RECENT PUBLICATIONS:

Müller L, Theile K, Böhm V. In vitro antioxidant activity of tocopherols and tocotrienols and comparison of vitamin E concentration and lipophilic antioxidant capacity in human plasma. Mol. Nutr. Food Res. 2010:54: 731-742.

Palozza P, Bellovino D, Simone R, Boninsegna A, Cellini F, Monastra G, Gaetani S. Effect of ß-carotene-rich tomato lycopene ß-cyclase (tlyc-b) on cell growth inhibition in HT-29 colon adenocarcinoma cells. Br. J. Nutr. 2009:102:207-214.

Palozza, P, Simone R, Catalano A, Boninsegna A, Böhm V, Fröhlich K, Mele MC, Monego, G, Ranelletti FO. Lycopene prevents 7-keto-cholesterol induced oxidative stress, cell cycle arrest and apoptosis in human macrophages. J. Nutr. Biochem. 2010:21:34-46.

INVESTIGATION OF PHYSIOLOGICAL EFFECTS OF TOMATO PRODUCTS *IN VIVO*

DR. GORDON M. LOWE

2009-2010 Project Pillar Leader of the *in vivo* studies within LYCOCARD

School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK
G.M.Lowe@ljmu.ac.uk

Atherosclerosis and coronary heart disease are principal causes of death within the Western world. Atherosclerosis is characterised by two lesions, fatty streaks and plaque development that results in restricted blood flow. The disease begins in the teenage years and subsequently develops over a number of years. The events that lead to atherosclerosis are very complex but involve the oxidation of low density lipoproteins and an inflammatory response. There are many risk factors including genetic susceptibility, lack of exercise, high lipid consumption, cigarette smoking and diabetes. The consumption of tomato products including lycopene has been linked with reducing the risk of atherosclerosis. The *in vivo* section of LYCOCARD is focused on examining uptake of lycopene from the diet, its role as an antioxidant, its role in protecting against inflammatory responses. The following section shows some of the important findings for year 4 of the project.

DIETARY UPTAKE

Dietary transporters have been identified that lead to lycopene uptake from the diet. One such transporter is called SR-BI. One study is currently investigating how changes in genetic make-up of individuals may affect the expression and activity of these transporters. Further to this, a non-toxic isotopically-labelled lycopene was fed to two volunteers and within 4-5 h of the meal both (*all E*)-lycopene and its associated isomer could be detected in the plasma. These are very important experiments which will give a greater understanding of mechanics and kinetics of lycopene uptake in the human gut.

INFLAMMATORY RESPONSES

Inflammatory responses are associated with progression of atherosclerosis, and within LYCOCARD obese and normal volunteers were given hypocarloric or normocaloric diets, that in some cases were supplemented with a tomato enriched diet. In all cases the inclusion of the enriched tomato diet had noticeable effects in reducing two important inflammatory biomarkers. The tomato enriched diet also increased the circulating plasma concentrations of lycopene and ß-carotene, two important carotenoids derived from tomato products. It also augmented the effects of hypocaloric diets on obese volunteers, helping further to reduce triglycerides and the Body Mass Index (BMI) of these individuals. The carotenoids were also productive in reducing biomarkers that indicate increased risk of chronic diseases

SMOKING

It has been reported that smokers have lower circulating concentrations of lycopene than non-smokers. Studies are underway in LYCOCARD to determine if this is still the case or if improvements in diet or changes in smoking habits have reversed this. Smoking also affects the blood vessels, and a study within LYCOCARD indicated that an increase in tomato products did not improve endothelial function nor alter the lipid profile to any extent. However, the supplementation to diet was only for a relatively short period in time. Further studies on smokers are underway, the initial part of this will determine if cigarette smoke can change the profiles of lycopene in smokers. It is thought that the chemicals in the smoke can convert some of the (*all E*)-lycopene to its (Z)-isomers and that smokers will have a different profile of lycopene in their blood and the results from this will be known early in 2011. Studies will also focus on whether processed tomato products can reduce some of the risk factors present in smokers. Studies will examine if an increased consumption of processed tomatoes can reduce circulating oxidised LDL, a marker for increased risk of developing atherosclerosis.

GENETIC EFFECTS

Work in this area is very complex, but research in this area suggests that degradation products of lycopene may activate certain genes in the body. Work on mice has indicated that tomato products indeed do activate genes with some beneficial effects.

ONGOING RESEARCH

Many studies are underway. One of them includes a repeat study increasing the amount of processed tomatoes fed to the individuals, to see if this offers even more protection against oxidative damage. As we approach the final year of the LYCOCARD study it is hoped that upon completion we will understand more about the uptake of lycopene, have a better idea of the length of time needed for products of processed tomatoes to exert an effect, the role of lycopene as an antioxidant within the human body and as an agent that can initiate the expression of protective genes within the body.

RECENT PUBLICATIONS:

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

DR. MARÍA JESÚS PERIAGO

2009-2010 Project Pillar Leader of food product studies within LYCOCARD

Dept. of Food Technology, Food Science and Nutrition -
University of Murcia, Campus Universitario Espinardo - Murcia, Spain.
mjperi@um.es

Tomatoes provide an optimal mix of dietary antioxidants that may be responsible for the reported health benefits of tomato consumption on cardiovascular diseases. However, technological processing, packaging materials and storage conditions have an impact on the nutritional quality of tomato products by affecting the stability of antioxidant nutrients to different extents. The research activities of Food Products Studies are focused on the study of the content of lycopene and other bioactive compounds in tomato and tomato products along the food chain, with the objective to design a new tomato product with beneficial effects for human health.

This pillar includes the analysis of raw materials (different tomato cultivars) and tomato products (tomato juice, passata, tomato paste, diced tomato and tomato powder) with the objective to ascertain the influence of the postharvest handling, processing and storage on the content of lycopene, phenolic compound, folates and ascorbic acid. The partners involved in the Food Product Studies are: University of Murcia (Murcia, Spain), University of Jena (Jena, Germany) and the industrial partners Juver Alimentación-Conserve Italia (Murcia, Spain), Conesa (Badajoz, Spain) and Agraz (Badajoz, Spain).

During the fourth years of the project, the research activities have achieved the following goals:

LYCOPENE AND BIOACTIVE COMPOUNDS IN TOMATO

The effects of cultivar, on-vine ripening and year of harvest on the content of lycopene, phenolic compounds, folate and ascorbic acid in raw tomatoes were studied. Among all the bioactive compounds only lycopene is clearly affected by the maturity stage. The content of ascorbic acid, phenolic compounds and folates were mainly determined by the tomato cultivar. The main phenolic compounds of tomatoes are chlorogenic acid, caffeic acid, ferulic acid, rutin, quercetin and naringenin. The 5-methyl-tetrahydrofolate (5-MTHF) was the only compound identified in raw tomato.

EFFECT OF INDUSTRIAL PROCESSING

The effect of industrial processing has been studied in tomato juice and in tomato products. The homogenization of tomato purée during tomato juice processing increases the extractability of

folates, but thermal treatment with temperatures higher than 108 ºC could cause losses of folates and ascorbic acid. In general, thermal processing of tomato to obtain tomato products leads to an enhancement of the nutritional value by increasing mainly the lycopene and phenolic antioxidants. Labile compounds (mainly vitamins), however, are degraded.

STABILITY OF LYCOPENE AND OTHER BIOACTIVE COMPOUNDS DURING STORAGE OF TOMATO PRODUCTS

The stability of the antioxidant compounds of tomato juices during storage for 12 months at different temperatures (8 °C, 22 °C, and 37 °C) and using two different packaging materials (Tetra pack and glass bottles) has been analyzed. In tomato juices, total lycopene, total phenolic compounds and total flavonoids remained almost stable during storage for 12 months, regardless of the packaging material used. However, ascorbic acid and folate contents were markedly affected by storage conditions decreasing parallel the hydrophilic antioxidant activity of the juices. The effect of storage on the bioactive compounds of tomato products shows a similar behaviour, with slightly changes in the lycopene and phenolic compounds contents (even during 36 months of the storage), but decreasing significantly the content of ascorbic acid.

EVALUATION OF ANTIOXIDANT ACTIVITY

The antioxidant activity of tomato extract has been analysed in raw materials and in tomato products. The hydrophilic and lipophilic antioxidant activity has been quantified and the relationship with the main antioxidant compounds has been determined. In addition, the antioxidant properties of tomato extracts have been evaluated using cell culture models. These approaches involve the pre-incubation or co-incubation of cells with lipophilic, hydrophilic or combined tomato extracts, followed by the exposure to different concentrations of the oxidant tert-butyl hydroperoxide (tBOOH). The ROS generation, the lipid oxidation compounds and DNA damage in cell cultures was measured to determine the protective effect of tomato extracts at physiological concentrations.

ONGOING RESEARCH

- Development of new tomato functional products: with the scientific information obtained in previous activities two tomato products with an improvement of its nutritional properties have been designed by the industrial partners Juver Alimentación and Conesa.
- Human intervention studies: with the new tomato products an intervention study is ongoing at the University of Jena (Germany) to ascertain the beneficial effect of their consumption for the prevention of cardiovascular diseases.
- *In vitro* accessibility and availability of lycopene: in vitro digestion assay is applied in tomato product to evaluate the effect of the several critical factors that could determine the accessibility and availability of lycopene during the gastric and intestinal digestion.

RECENT PUBLICATIONS

García-Alonso F.J., Bravo S., Casas J., Pérez-Conesa D., Jacob K., Periago M.J. Changes in antioxidant compounds during the shelf life of commercial tomato juices in different packaging materials. J. Agr. Food Chem. 2009: 57:6815-6822.

Iniesta M.D., Pérez-Conesa D., García-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. J. Agr. Food Chem. 2009:57:4739-4745.

Pérez-Conesa D., García-Alonso F.J., García-Valverde V., Iniesta M.D., Jacob K., Sánchez-Siles L.M., Ros G., Periago M.J. Changes in bioactive compounds and antioxidant activity during homogenization and thermal processing of tomato puree. Innov. Food Sci. Emerg. Technol. 2009:10:179-188

DISSEMINATION & TRAINING

SOPHIE COLVINE

2009-2010 Project Pillar Leader of the dissemination and training packages within LYCOCARD

Mediterranean International Association of the Processing Tomato (AMITOM), 37 lot les Valérianes, 84700 Sorgues, France.
colvine@tomate.org

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, the industry (and especially the tomato processing industry), the medical profession, the patient care organisations, and the general public are kept informed of progress through a variety of activities.

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

- 25 presentations of the project's results and ongoing research at scientific or trade events
- 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.
- The publication of newsletters and an annual report
- 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 German version of the website (www.tomateundgesundheit.de) was launched in March 2010. The Italian (www.pomodoroesalute.it) and Spanish versions (www.tomateysalud.es) will be online soon, with more languages in the pipeline (French, Turkish, …).

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 researchers between project partners enabled good communication and exchange of skills within the consortium

All these dissemination and training activities will continue during the fifth and final year with a closing conference planned in the spring 2011 to present the final results of the project.

THE LYCOCARD INDUSTRIAL PLATFORM

The LYCOCARD Industrial Platform is designed as a forum for dialogue between the industry and scientists to discuss new advances in research and to effectively communicate health messages in an accessible and generic manner from its Internet base. It was launched in October 2009 and already includes a dozen companies, of which many are SMEs.

These companies pay a small fee to become a partner in the project, and notably to:

- Extend the reach of the project through funding additional dissemination activities and notably the development of the different language versions of the www.tomatoandhealth.com website
- Be able to use the project logo on packaging, point of sale materials and company
- Communication material (including websites) to communicate on their contribution to the project and further extend its reach
- Be part of a forum to discuss research carried out as part of the LYOCARD project and potential further research work.

The platform will ensure a legacy to the project through pursuing collaboration after its official end in March 2011. Membership is still open to any company or organization interested and they should contact Sophie Colvine with any query.

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

	seit 1558 www2.uni-jena.de/biologie/ieu/ew-eng/	www.inserm.fr	www.amitom.org
www.um.es/dp-tecnologia-alimentos	www.rm.unicatt.it	www.biochem.dote.hu	www.ljmu.ac.uk/bml/
www.charite.de/herz/	www.herzstiftung.de	www.avignon.inra.fr	www.inran.it
www.juver.com	www.e-conesa.com	www.caledoniansciencepress.com	www.agraz.com

Tomato+Health

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 broadcasts the latest news from its pressroom and gives science updates for health-professionals and patient groups. There are loads of fun recipes, games and puzzles for kids and tasty Mediterranean recipes available as mp3 and mp4 download from the super fast i-cookTV video show.

www.tomatoandhealth.com

www.tomateysalud.es

www.pomodoroesalute.it

www.tomateundgesundheit.de

www.tomateetsante.com