INFORME TÉCNICO

PROGRAMA DE FORMACIÓN PARA LA INNOVACIÓN AGRARIA

GOBIERNO DE CHILE
FUNDACIÓN PARA LA
INNOVACIÓN AGRARIA

CONTENIDO DEL INFORME TÉCNICO

PROGRAMA DE FORMACIÓN PARA LA INNOVACIÓN AGRARIA

1. Antecedentes Generales de la Propuesta

Nombre: Gustavo E. Zúñiga Navarro

Código:

Nombre Postulante Individual: Gustavo E. Zúñiga Navarro

Lugar de Formación (País, Región, Ciudad, Localidad): Alemania: Berlin Hannover y Marburg.

Fecha de realización: 4-10-01 al 11-10-03

Objetivos de su participación en la actividad.

- Conocer realidades biotecnológicas en países desarrollados
- Realizar contactos con instituciones alemanas que realicen proyectos afines
- Establecer contactos para comercializar productos bioactivos de plantas chilenas. Antioxidantes y aleloquímicos y productos para biofarmacia.

2. Antecedentes Generales: describir si se lograron adquirir los conocimientos y/o experiencias en la actividad en la cual se participó (no más de 2 páginas).

El objetivo principal de esta actividad fue la visita a la feria Biotécnica 2003. Esta es la principal feria biotecnológica en el mundo. En ella se muestran los últimos desarrollos alcanzados en el campo biotecnológico ya sea a nivel de técnicas avanzadas como en equipos de ultima tecnología.

La importancia de esta feria se traduce en que durante su ejecución fue visitada por 12,000 biotecnólogos provenientes de 30 países. En Biotécnica 2003 se presentaron 947 expositores, de los cuales 282 fueron extranjeros.

Durante los días de la feria pudimos conocer de manera grupal las presentaciones hechas por algunas entidades biotecnológicas, como Bio Con Valley,

Las charlas individuales sostenidas con algunos expositores de la feria, fueron muy estimulantes. Con el profesor Manfred Séllner de la University of Technology-Wismar, se han iniciado conversaciones a fin de establecer una colaboración en el campo de la inmersión temporal para aumentar la biomasa en la técnica del cultivo de tejidos vegetales.

El Dr. Matthias Prucha de Affymetrix, nos ha asesorado en las nuevas técnicas de aislamiento y caracterización de genes enviándonos material bibliografico de apoyo de apoyo. Con el Dr. Bernhard Nüßlein de Nadicon se han iniciado también contactos para caracterizar bacterias del suelo que presentan propiedades biotecnológicas interesantes.

Además, de la visita a Biotécnica 2003 se realizaron una serie de otras actividades cuyo detalle se muestra en el punto 3. Resulta innegable que en cada actividad realizada se adquirieron conocimientos diversos. La visita a centros de investigación en la cuidad de Postdam, nos permitió conocer la importancia que tienes en Alemania tanto la investigación básica como aplicada. Se pudo conocer un centro de estudios con animales, en el cual se cumplen todas las normas de manejo y seguridad. La visita al Instituto para el procesamiento de cereales, resulto de gran interés pues este es un gran ejemplo de cómo se puede realizar la transferencia tecnológica desde un centro de investigación al sector productivo. Por tratarse este de un centro privado, muestra un gran nivel de eficiencia en la obtención de recursos, especialmente aquellos provenientes de empresas con las cuales establece convenios de desarrollo.

La visita al Instituto Max Planck en Marburg, constituyó una experiencia muy estimulante, pues nos permitió conocer un centro de investigación avanzado con equipos de ultima generación. En este instituto la optimización por los recursos queda de manera evidente al recorrer sus dependencias y ver como los espacios individuales son pequeños comparados con aquellos de uso común en donde se ubican los equipos avanzados.

3. Itinerario Realizado: entregar una relación de actividades de acuerdo al siguiente cuadro:

Fecha	Actividad	Objetivo	Lugar
6/10/03	Visita al German Institute Of Human Nutrition	Conocer la investigación realizada en en campo de la nutrición humana	Postdam
	Visita al Max-Rubner-Laboratorium	Conocer el funcionamiento de un laboratorio de manipulación de animales nivel de seguridad 3	Postdam
	Visita al Instituto para el procesamiento de cereales	Conocer el nivel de desarrollo alcanzado por una institución de I&D en el procesamiento de cereales	Postdam
	Charla técnica empresa Biopract	Identificar las formas de gestionar una empresa biotecnológica ambiental	Postdam

7/10/03	Visita Biotécnica 2003	Participar en calidad de observador en la Feria Biotécnica 2003. Charla con Ministerio Federal de Educación e investigación sobre programa alemán de biotecnología Contacto: Dr. Hans-Michael Biehl *Recepción de parte de feria Biotéchnica* Contacto: Andreas Grüber Recepción y presentación por Biocon Valley MVP Contacto: Dr. Heinrich Cuypers Reuniones con empresas expositoras de Biocon Valley MVP Visitas individuales a Stands y presentaciones técnicas.	Hannover
10/10/03	Visita Max Planck Institute for Terrestrial Microbiology-Marburg	Conocer algunos aspectos de la investigación y funcionamiento del MPI Presentación de Instituto Max-Planck Y las investigaciones en el área de analices funcional de interacciónes fitopatogenicas Dr. Bernhard Nüsslein Gerente General Nadicom GMBH Visita a laboratorios	Marburg

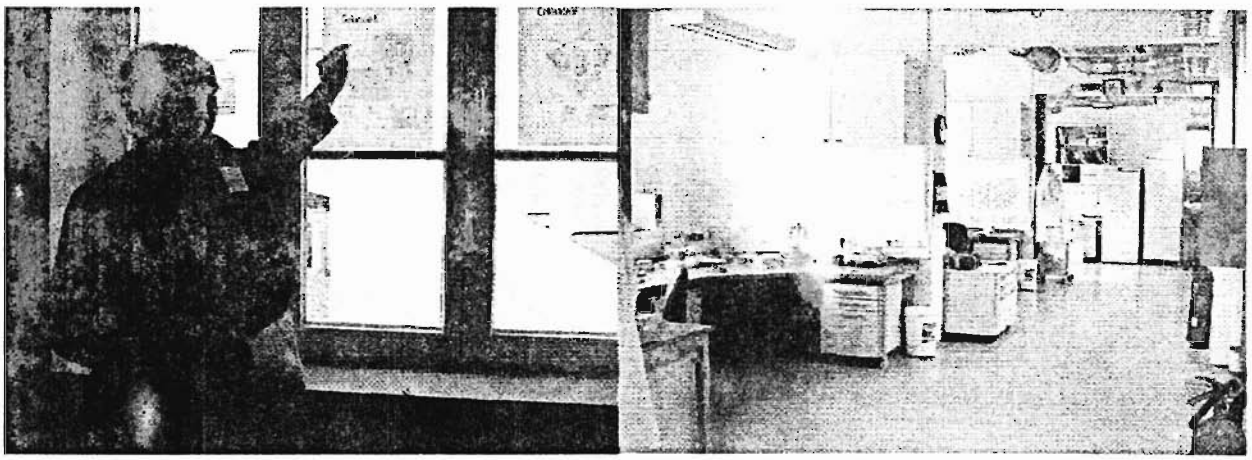
Señalar las razones por las cuales algunas de las actividades programadas no se realizaron o se modificaron.

4. Resultados Obtenidos: descripción detallada de los conocimientos adquiridos. Explicar el grado de cumplimiento de los objetivos propuestos, de acuerdo a los resultados obtenidos. Incorporar en este punto fotografías relevantes que contribuyan a describir las actividades realizadas.

En primer lugar se debe enfatizar el que se cumplieron todos los objetivos propuestos para esta actividad. A continuación se detallan e ilustran las actividades realizadas.

Visita al German Institute Of Human Nutrition (Dife). En este lugar pudimos conocer a través de una presentación oral. Este instituto de investigación cumple varios objetivos:

- Mejorar la salud humana a través de investigación básica y clínica en el campo de la nutrición.
- Caracterizar los mecanismos moleculares y patofisiológicos de las enfermedades moleculares.
- Identificar nuevos desarrollos terapéuticos.



Presentación del Instituto

Dependencias DIFE.

German Institute of Human Nutrition

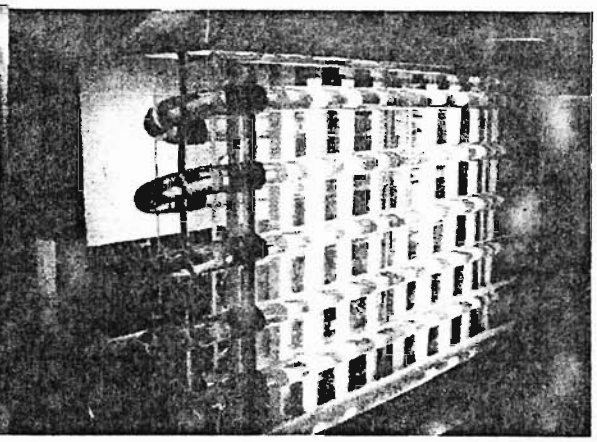
Visita al Max-Rubner-Laboratorium. En este lugar se realiza trabajo experimental con animales, bajo condiciones optimas en jaulas controladas. Por tratarse de un lugar con seguridad nivel 3 no fue posible tomas fotos dentro del laboratorio.

Dra Christa Thöne-Reineke encargada MRL

Visita al Instituto para el procesamiento de Cereales (IGV-GMBH). Este instituto fue fundado en 1960, convirtiéndose en 1990 en una Sociedad Limitada. En 1994 esta sociedad fue adquirida por 3 investigadores. Se dedica a 3 áreas de desarrollo: procesamiento de alimentos, agricultura y biotecnología. Los unidades que componen en IGV son

- Laboratorio de análisis acreditado
- Procesamiento de alimentos
- Biotecnología
- Análisis y caracterización de materias primas
- Transferencia tecnológica
- Capacitación y educación

Jóvenes capacitándose



Birreactor para microalgas.

Charla Dr.Matthias Gerhardt Biopract-GmbH. Esta empresa se dedica al desarrollo de productos y procesos biotecnológicos para la industria. Cuenta con un presupuesto de 1,5 millones de Euros. Los productos que Biopract ofrece son:

- Enzimas. Desarrollo y producción de preparaciones enzimáticas para uso en la agricultura.
- Análisis. Servicios analíticos relacionados con celulasas, amilasas y fitasas presentes en alimentos para animales.
- Biotecnología ambiental. Uso de microorganismos en procesos de remediación de suelos.
- Investigación y desarrollo. Desarrollo de procesos biotecnológicos, investigación aplicada, productos biotecnológicos, métodos analíticos, investigación por contrato.

Visita a la Feria Biotécnica. Nuestro principal interés en esta feria estuvo en el conocimiento de actividades relacionadas con la biotecnología vegetal, sin embargo se visitó stands relacionados con bioinformática, farmacia y equipos de laboratorio. Entre los Stands visitados destacan.

Ministerio de Educación. Se nos mostraron algunos aspectos del desarrollo y financiamiento de la biotecnología en Alemania.

Grupo junto al encargado de Ministerio de Educación.

BioCon Valley. El Dr. Einrich Cuypers nos brindo una charla explicativa sobre este polo biotecnológico. Las universidades de Greifswald y Rostock han creado institutos de investigaciones productivos que trabajan en la aplicación de la biotecnología moderna en diferentes áreas: mejoramiento animal y vegetal; medicina humana y veterinaria; y tecnología medica.

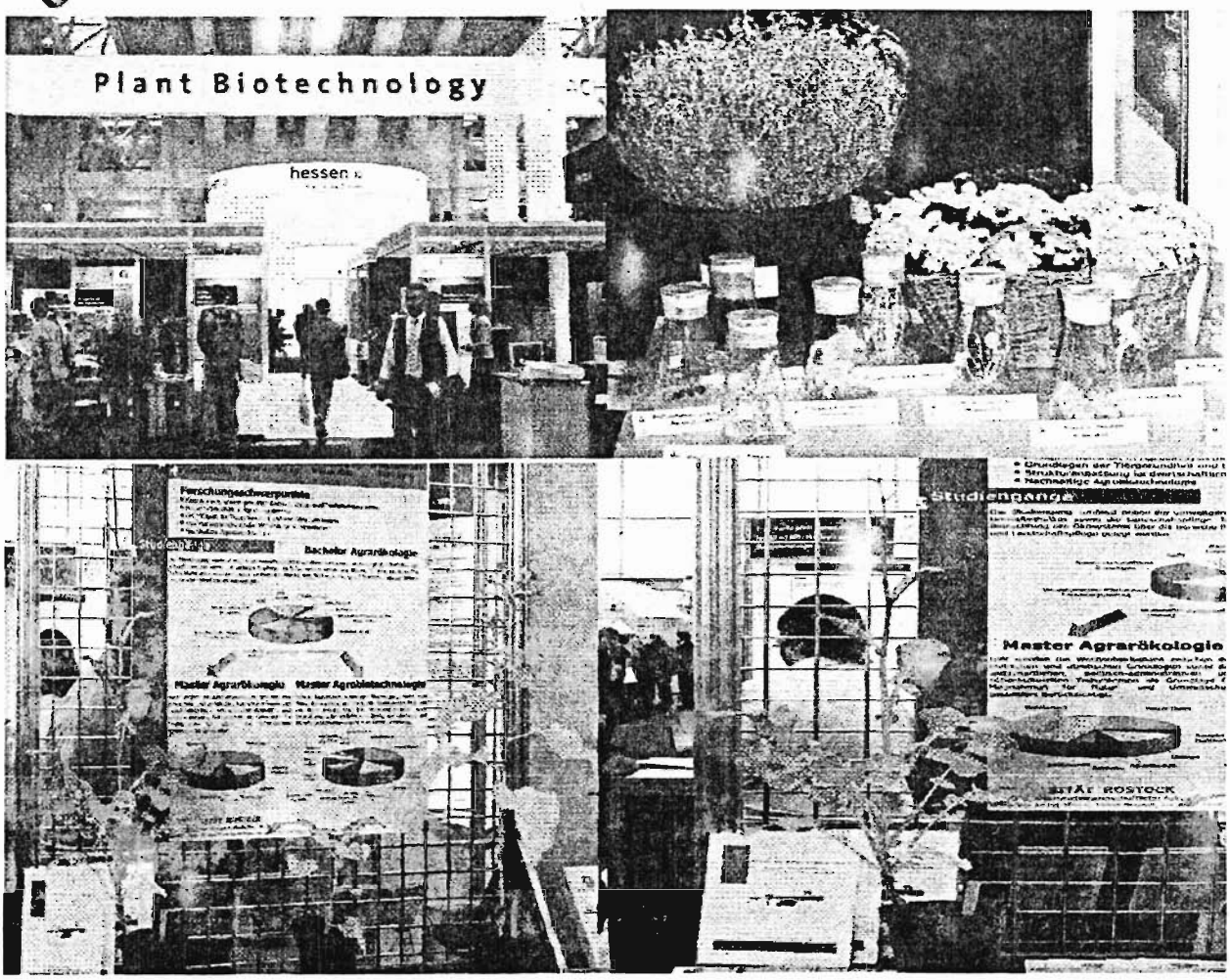
Grupo junto al Dr. Heinrich Cuypers en la presentación de BioCon Valley.

Las siguientes fotografías muestran distintas vistas de la feria.

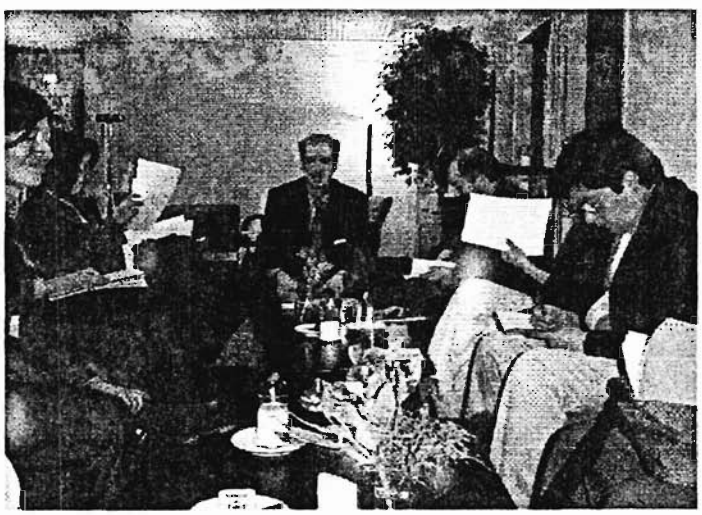
Vista General de la Feria

Grupo al ingreso de la Feria.

Charla con el Dr. Nüßlein de Nadicom

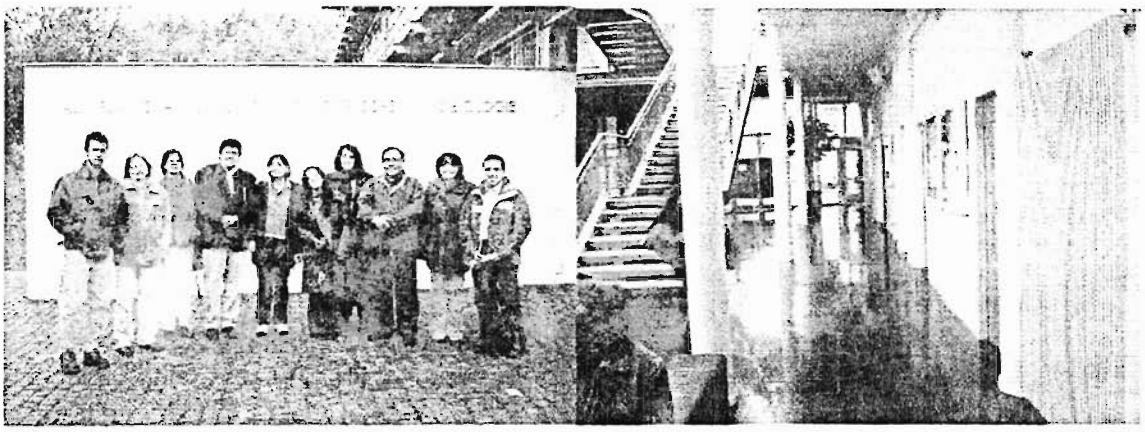


Área dedicada a la biotecnología vegetal en Biotécnica 2003

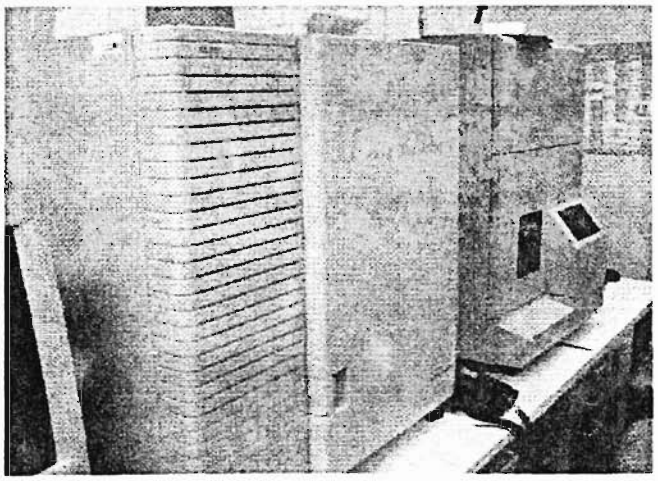


Grupo junto a Andreas Grüber Project Manager de Biotechnica 2003.

Visita al Max Planck Institute para la Microbiología Terrestre- Marburg. En este instituto se nos ofrecieron dos charlas a cargo de los doctores Jörg Kämper y Dr. Bernhard Nuβllein en las cuales pudimos conocer algunos aspectos de la investigación que allí se realiza. Cabe destacar un concepto nuevo que se aplica en Alemania relacionado con la creación de empresas asociadas a centros de investigación avanzados. Este es el caso de la empresa Nadicom, que realiza sus actividades de I&D asociadas a este instituto



Max Planck Institute-Marburg.



Equipos para análisis geonómicos

5. Aplicabilidad: explicar la situación actual de los temas en Chile (región), compararla con la tendencias y perspectivas en el país (región) y feria visitados y explicar la posible incorporación de los conocimientos adquiridos, en el corto, mediano o largo plazo, los

procesos de adaptación necesarios, las zonas potenciales y los apoyos tanto técnicos como financieros necesarios para hacer posible su incorporación en nuestro país (región).

Nuestro país posee un gran potencial biotecnológico, para poder reproducir los que vimos en esta feria se necesita generar una política biotecnológica clara, que los empresarios asuman riesgos que permitan la generación de nuevas empresas. Se requiere además, de una mayor masa critica en todas las áreas de la biotecnología, para lo cual se hace necesario el desarrollo de programas de postgrado modernos que apuntes a la generación de productos biotecnológicos.

6. Contactos Establecidos: entregar una relación de contactos establecidos de acuerdo al siguiente cuadro:

Institución/Empresa	Persona de Contacto	Cargo/Actividad	Fono/Fax	Dirección	E-mail
BVT. Bioprocess Institute	Holger Hübner	Investigador	091318523006		holger.huebner@bvt.cbi.uni-erlangen.de
IGV, GmbH	Ralph Thomann	Chemist, section manager	49332008920 1		r_thomann@igv-gmbh.de
Prophyta	Matthias von Erffa	Marketing and Technical support	4938425230		mverffa@prophyta.com
Affymetrix	Matthias Prucha	Field Application Specialist	49893308950 1		Mathias_prucha@affymetrix.co.uk
BioCon Valley	Heinrich Cuypers	Señor Project Manager	49383451510 8		hc@bcv.org
ICon Genetics	Yuri Y. Gleba	Director	49 3455559887		gleba@icongenetics.de
Biopract GmbH	Matthias Gerhardt	Managing Director	030 63926502		sales@biopract.de
NTB Newbiotechnic	Rafael Camacho	Manager	34954081031		funamal@newbiotechnic.com
Puleva Biotech	Miguel Moreno	Development Manager	34958240283		ma.moreno@pulevabiotech.es
Protedyne	Heins Steiner	Sales Manager	49898565340 0		heinz@protedyne.com
Nadicom	Bernhard Nußllein	Investigador	0642113175		nuesslein@nadicom.com
CSIC-España	Irene Herrera	OTT.CSIC	34915616800		i.herrera@orgc.csic.es

7. Detección de nuevas oportunidades y aspectos que quedan por abordar: señalar aquellas iniciativas detectadas en la actividad de formación, que significan un aporte para el rubro en el marco de los objetivos de la propuesta, como por ejemplo la posibilidad de realizar nuevos cursos, participar en otras ferias y establecer posibles contactos o convenios. Indicar

además, en función de los resultados obtenidos, los aspectos y vacíos tecnológicos que, a la luz de los conocimientos adquiridos en esta actividad, aún quedan por abordar para la modernización del tema en el país.

En este aspecto se pueden mencionar varias oportunidades relacionadas con nuestro trabajo. El desarrollo de investigaciones que permitan aumentar el valor agregado de nuestro recursos naturales representa a mi modo de ver una gran oportunidad. Debemos dejar de ser exportadores de materia prima si elaborar, para convertirnos en exportadores de productos terminados con un alto valor agregado.

8. Resultados adicionales: capacidades adquiridas por el participante individual y/o el grupo, como por ejemplo, formación de una organización, incorporación (compra) de alguna maquinaria, desarrollo de un proyecto, firma de un convenio, etc.

Se establecieron contactos con representantes de la empresa española Puleva, quienes se mostraron muy interesados en antioxidantes de origen vegetal, para ser incorporados en los productos lácteos que Puleva produce. Con la empresa Nadicom se trabaja en un protocolo de colaboración.

9. Material Recopilado: junto con el informe técnico se debe entregar un set de todo el material recopilado durante la actividad de formación (escrito y audiovisual) ordenado de acuerdo al cuadro que se presenta a continuación (deben señalarse aquí las fotografías incorporadas en el punto 4):

Tipo de Material	Nº Correlativo (si es necesario)	Caracterización (título)
Ej.:		
Annual Report.	1	Dife. Annual Report.
Poster	2	Biopract/metanol
Paper	3	NTB NewBiotechnic
Paper	4	Understanding *Trichoderma*
Reporte	5	Biotecnology at BASF/Sungene
Reportes varios	6-8	Informes-CSIC
Foto1	Tapa	Ingreso a la Feria
Fotos	2-5	Dife
Fotos	6	MR Laboratorium
Fotos	7-10	IGV-
Fotos	11	Grupo junto encargado Ministerio de Educación.
Fotos	12-13	BioCon Valley
Fotos	14-21	Feria Biotécnica 2003
Fotos	22-24	Max Planck

10. Aspectos Administrativos

10.1. Organización previa a la actividad de formación

a. Apoyo de la Entidad a cargo de la organización del viaje (Camchal)

__X__ bueno _____ regular _____ malo

El apoyo brindado por la Camchal a través de Antje Wandelt, resulto excelente en término de los contactos previos establecidos, hoteles seleccionados y transporte. Esto permitió además que el grupo funcionara sin ninguna dificultad (Justificar)

b. Información recibida durante la actividad de formación

__X__ amplia y detallada _____ aceptable _____ deficiente

c. Trámites de viaje (visa, pasajes, otros)

_X__ bueno _____ regular _____ malo

d. Recomendaciones (señalar aquellas recomendaciones que puedan aportar a mejorar los aspectos administrativos antes indicados)

10.2. Organización durante la actividad (indicar con cruces)

Ítem	Bueno	Regular	Malo
Recepción en país o región de destino	X		
Transporte aeropuerto/hotel y viceversa	X		
Reserva en hoteles	X		
Cumplimiento del programa y horarios	X		

En caso de existir un ítem Malo o Regular, señalar los problemas enfrentados durante el desarrollo de la actividad de formación, la forma como fueron abordados y las sugerencias que puedan aportar a mejorar los aspectos organizacionales de las actividades de formación a futuro.

11. Conclusiones Finales: entregar las conclusiones finales del participante de la actividad de formación, incluyendo el nivel de satisfacción de los objetivos personales.

La actividad realizada constituye una experiencia muy interesante pues nos permitió entre otros aspectos:

- Conocer centros de investigación con un alto desarrollo tecnológico
- Conocer la realidad biotecnológica de países desarrollados
- Participar de charlas técnicas en temas de interés, en las cuales se mostraron los últimos desarrollos.
- Establecer vínculos con investigadores de centros biotecnológicos en el campo vegetal.

Fecha: 12/11/03

Nombre y Firma beneficiario de la beca: Gustavo E Zúñiga

AÑO 2003

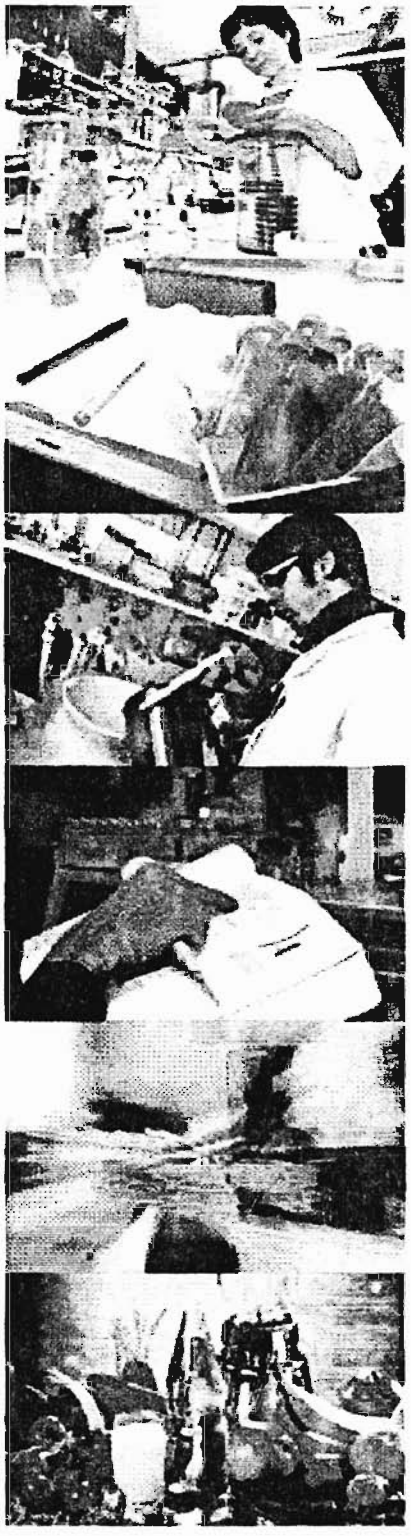
DIfE

Deutsches Institut für Ernährungsforschung Potsdam-Rehbrücke

Jahresbericht 2001 - 2002

German Institute of Human Nutrition

Annual Report 2001 - 2002



Leibniz Gemeinschaft

Impressum / Imprint

Herausgeber / Publisher
Deutsches Institut für Ernährungsforschung
Potsdam-Rehbrücke
Mitglied der Wissenschaftsgemeinschaft
Gottfried Wilhelm Leibniz (WGL)
Arthur-Scheunert-Allee 114-116
14558 Bergholz-Rehbrücke
http://www.dife.de

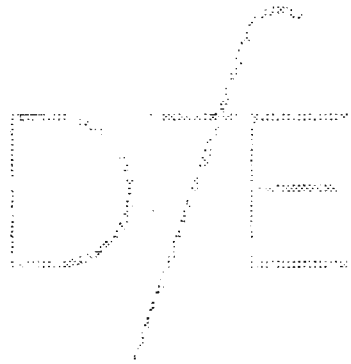
Redaktion / Editors:
Prof. Dr. Dr. Hans-Georg Joost
Dr. Susanne Schelosky
Dr. Gunda Backes
Dr. Lynne Rogers

Gesamtherstellung / Production:
MildeMarketing
Medienhaus/ZFF
August-Bebel-Str. 26-53
14482 Potsdam
Tel. + (49) - 331 - 72 15 370
Fax + (49) - 331 - 72 15 380
milde@mildemarketing.de
www.mildemarketing.de

Grafik / Graphic:
focus werbeagentur
www.focuspotsdam.de

Druck:
Brandenburgische Universitätsdruckerei
und Verlagsgesellschaft Potsdam mbH

Bildnachweis / Photocredits
Cover: DIfE, Bernd Lammel,
Informationszentrale
Deutsche Mineralwasserwirtschaft.
Innenteil: S. 2, 18, 21, 31, 33, 41, 58, 59, 67 DIfE,
S. 5, 6, 7, 8, 43 Bernd Lammel



Deutsches Institut für Ernährungsforschung Potsdam-Rehbrücke

Jahresbericht 2001 - 2002

German Institute of Human Nutrition

Annual Report 2001 - 2002

DIfE kurzgefasst

Das Deutsche Institut für Ernährungsforschung Potsdam-Rehbrücke (DIfE) hat die Aufgabe, experimentelle und angewandte Forschung auf dem Gebiet Ernährung und Gesundheit zu betreiben. Das Ziel ist, die molekularen Ursachen ernährungsbedingter Erkrankungen zu erforschen und neue Strategien für Prävention, Therapie und Ernährungsempfehlungen zu entwickeln. Die Grundlagen dafür werden von den am DIfE tätigen Wissenschaftlern in interdisziplinärer Zusammenarbeit mit einem breiten naturwissenschaftlichen, medizinischen und epidemiologischen Methodenspektrum erarbeitet. Dabei konzentriert sich das Institut besonders auf die zur Zeit wichtigsten Erkrankungen, an deren Entstehung ernährungsbedingte Faktoren beteiligt sein können: Adipositas, Diabetes und Krebs.

Die Ernährungsforschung in Rehbrücke begann 1946 mit dem Institut für Ernährungs- und Verpflegungswissenschaften und wurde von 1959 bis 1991 im Zentralinstitut für Ernährung der Akademie der Wissenschaften der DDR fortgesetzt.
Das Deutsche Institut für Ernährungsforschung wurde 1992 von der Bundesrepublik Deutschland und dem Land Brandenburg als selbstständige Stiftung des öffentlichen Rechts gegründet. Es ist Mitglied der Wissenschaftsgemeinschaft Gottfried Wilhelm Leibniz. Mitarbeiter des DIfE übernehmen Lehrverpflichtungen im Studiengang Ernährungswissenschaften an der Universität Potsdam und im Studiengang Medizin an der Freien Universität Berlin.

DIfE in brief

The mission of the German Institute of Human Nutrition (DIfE) is to conduct experimental and clinical research in the field of nutrition and health, with the aim of understanding the molecular basis of nutrition dependent diseases, and of developing new strategies for prevention, treatment, and nutritional recommendations.

Scientists at the DIfE pursue these scientific goals by interdisciplinary cooperation comprising a broad spectrum of experimental and epidemiological methods. A particular focus of the institute is research on the most important diseases at present, i.e., obesity, diabetes, and cancer, whose development may involve nutrition dependent factors.

Nutritional research in Rehbrücke began in 1946 at the Institute of Nutritional Sciences and Food Provision and continued at the Central Institute of Nutrition of the Academy of Science of the German Democratic Republic from 1969 until 1991.
The German Institute of Human Nutrition (DIfE) was founded in 1992 by the Federal Republic of Germany and the State of Brandenburg as an independent foundation. It is a member of the Wissenschaftsgemeinschaft Gottfried Wilhelm Leibniz, an alliance of scientific institutions.
The academic members of the DIfE have teaching obligations in nutritional sciences at the Universität Potsdam and in medicine at the Freie Universität Berlin.

Vorwort

Preface

The German Institute of Human Nutrition (DIfE) commemorated in 2002 the tenth anniversary of its reestablishment. Although ten years is a short time in the life of a research institute, the DIfE already has gained national and international attention within this period of time.

The departments of the institute are involved in numerous joint projects with regional partners (Bioregio Nutrigenomik) and with those in Germany (DFG) and in Europe (European Union projects). As member of an alliance of scientific institutions (Wissenschaftsgemeinschaft Gottfried Wilhelm Leibniz), the DIfE makes an important contribution to nutritional research in Germany, especially by its unique combination of molecular research, clinical research, and epidemiology. This position is to be maintained and broadened in the coming years.

In January of 2002, Prof. Dr. Dr. Hans Georg Joost became scientific director of the DIfE following the retirement of Prof. Dr. Christian Barth. Prof. Barth, who had taken over the DIfE in 1992 when it was reestablished, successfully implemented the recommendations of the Wissenschaftsrat to organize the institute along the lines of "nutrition and health," for which we wish to sincerely thank him again at this point. This change in directorship of the institute ensures continuity in DIfE's scientific alignment. Important areas of competence - such as causes and effects of the metabolic syndrome and the relation of nutrition to cancer - were strengthened and will be worked upon more intensivly in the future.

In January of 2001, the reconstruction of the buildings of the institute was completed. Renovation of not yet refurbished buildings, nearly half of the institute, was made possible by 50% financing by the European Union. Thus, the infrastructure of the institute has been completely modernized following a construction period of eight years in all. We wish to thank all of those - within and outside of the institute - who made this important improvement in the working conditions possible.

We would like to sincerely thank the DIfE personnel for their work in 2001 - 2002. In addition we would like to thank all of those in science, politics, and industry who have supported the work of the institute. We hope to have their continuous sympathy and support for the future development of the DIfE.

Prof. Dr. Dr. H.-G. Joost
Scientific Director

Dr. H. Schulz
Administrative Director

Im Jahr 2002 beging das Deutsche Institut für Ernährungsforschung den 10. Jahrestag seiner Neugründung. Zwar sind zehn Jahre eine kurze Zeit im Leben eines Forschungsinstitutes, doch hat das DIfE bereits jetzt eine hohe nationale und internationale Sichtbarkeit erlangt.

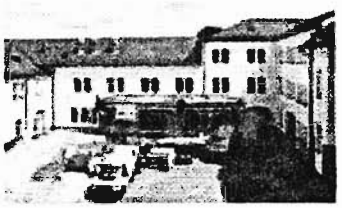
Die Abteilungen des Institutes sind an zahlreichen Verbundprojekten mit Partnern aus der Region (Bioregio Nutrigenomik), aus Deutschland (DFG) und aus Europa (EU-Projekte) beteiligt. Als Mitglied der Wissenschaftsgemeinschaft Gottfried Wilhelm Leibniz liefert das Institut einen wichtigen Beitrag zur deutschen Ernährungsforschung – insbesondere durch die Kombination von molekularer Forschung, klinischer Forschung und Epidemiologie. Diese Stellung gilt es in den nächsten Jahren zu bewahren und auszubauen.

Prof. Dr. Dr. H.-G. Joost
Prof. Dr. Johanna Wanka
Ministerin für Wissenschaft, Forschung und Kultur
Dr. H. Schulz

Im Januar 2002 loste Prof. Dr. Dr. Hans-Georg Joost den in den Ruhestand eintretenden Prof. Dr. Christian Barth als Wissenschaftlicher Direktor des DIfE ab. Prof. Barth hatte das DIfE 1992 nach seiner Neugründung übernommen und die Empfehlungen des Wissenschaftsrates zur Ausrichtung des Instituts auf das Gebiet "Ernährung und Gesundheit" erfolgreich umgesetzt. Dafür sei ihm auch an dieser Stelle nochmals herzlich gedankt. Der Wechsel in der Institutsleitung sichert die Kontinuität in der wissenschaftlichen Ausrichtung des DIfE. Wichtige Kompetenzfelder des DIfE – wie die Ursachen und Folgen des Metabolischen Syndroms und der Zusammenhang von Ernährung und Krebs – wurden verstärkt und werden in Zukunft noch konzentrierter bearbeitet werden.

Verabschiedung von
Prof. Dr. C. Barth (r.)

Im Jahr 2001 ist die letzte, größte Bauaktivität des DIfE erfolgreich abgeschlossen worden. Mit 50%iger Kofinanzierung durch die Europäische Union konnte der bis dahin noch nicht sanierte Teil (ca. 50 %) der Baulichkeiten des DIfE erneuert werden. Nach insgesamt acht Jahren Bauzeit ist nun die Infrastruktur des Institutes vollständig modernisiert. Wir danken allen – innerhalb und außerhalb des Institutes – die diese wichtige Verbesserung der Arbeitsbedingungen ermöglicht haben.



Letzte Bauaktivitäten

Für die bisher geleistete Arbeit möchten wir allen Mitarbeiterinnen und Mitarbeitern des DIfE herzlich danken. Unser Dank gilt ebenso allen Förderern des DIfE aus Wissenschaft, Politik und Wirtschaft. Wir hoffen sehr auf Ihre weitere Unterstützung bei der zukünftigen Entwicklung des Institutes.

Prof. Dr. Dr. Hans-Georg Joost
Wissenschaftlicher Direktor

Dr. Hartmut Schulz
Administrativer Direktor

Inhaltsverzeichnis

Prof. Dr. Dr. Hans-Georg Joost

Nutritional Research at the German Institute of Human Nutrition, 2001-2002

Prof. Dr. Dr. Hans-Georg Joost

Throughout life, humans ingest a variety of quite different substances with their diet. The nutrients, vitamins, minerals, and chemical compounds, in addition to supplying and catalyzing the metabolic pathways, have numerous other biological effects. Our diet has thus many desirable as well as undesirable effects on the organism and is equivalent to a lifelong pharmacotherapy. It is difficult to gain an accurate understanding of individual effects because of the complex mixture of substances involved and because results may become apparent only after years or even decades. Nevertheless, nutritional research is of utmost importance because of its significant potential in preventive medicine.

The mission of the German Institute of Human Nutrition is to improve human health through experimental and clinical research on the effects of nutrition, and through the nutritional recommendations resulting from research. Within this goal, the institute has focused on two diseases in which nutrition may play a preventive role: the metabolic syndrome and cancerous diseases.

At present, the metabolic syndrome, including obesity, hypertension, dyslipoproteinemia, and insulin resistance, is the most important nutrition-related disease. The incidence and severity of the metabolic syndrome have increased in all countries with a "Western" lifestyle and its corresponding nutrition. This development is paralleled by the increased incidence of type-2 diabetes, the major secondary complication of the metabolic syndrome. This trend has also been observed in the EPIC-Potsdam Study carried out by the Department of Epidemiology of the DIfE. Public health insurance in Germany has paid approximately four billion Euros for the pharmacotherapy of the metabolic syndrome and of its secondary complications. Conservative estimates assume that this figure will double within the next five years. If this trend cannot be reversed or at least brought to a halt, it will result in a lower average life expectancy and in the financial collapse of the public health-insurance system.

During the past two years, the Departments of Clinical Nutrition and Epidemiology have cooperated in identifying two important serum proteins, so-called cytokines, as risk factors in the development of type-2 diabetes, interleukin-6 (IL-6, Fig.1) and adiponectin. IL-6 is a mediator of the normal inflammatory response; increased IL-6 levels in serum are associated with the later appearance of diabetes. These findings suggest an involvement

Figure 1
Deduced from x-ray analysis, the three-dimensional protein structure of interleukin-6, an inflammatory-response mediator. Increased IL-6 concentrations in serum are associated with the later onset of type-2 diabetes.

Der menschliche Organismus nimmt durch die Ernährung Zeit seines Lebens eine Vielfalt sehr unterschiedlicher Stoffe auf. Die Nahrstoffe, Vitamine, Mineralien und chemischen Verbindungen dienen der Energiezufuhr und haben darüber hinaus zahlreiche biologische Wirkungen. Durch unsere Ernährung erzielen wir viele erwünschte, aber auch unerwünschte Wirkungen auf den Organismus, sie entspricht somit einer lebenslangen Pharmakotherapie. Exakte Kenntnisse über die einzelnen Effekte sind schwer zu erlangen – zum einen, weil sie durch ein komplexes Wirkstoffgemisch bewirkt werden, zum anderen, weil sich die Folgen oft erst nach Jahren bis Jahrzehnten zeigen. Dennoch ist dieser Forschungsgegenstand von größter Bedeutung, da der Ernährung eine überragende präventivmedizinische Bedeutung zukommt.

Das Metabolische Syndrom mit Adipositas, Hypertonie, Cholesterin-Stoffwechselstörung und Insulinresistenz ist die wichtigste ernährungsbedingte Erkrankung. In allen Ländern mit „westlichem" Lebensstil und entsprechender Ernährung nimmt die Häufigkeit und der Schweregrad des Metabolischen Syndroms zu. Auch die wichtigste Folge-Komplikation des Syndroms, der Typ-2-Diabetes, tritt parallel zu dieser Entwicklung häufiger auf. Dieser Trend lässt sich in der von der Abteilung Epidemiologie des DIfE durchgeführten EPIC (European Prospective Investigation into Cancer and Nutrition)-Potsdam-Studie beobachten. Für die Pharmakotherapie des gesamten Metabolischen Syndroms und seiner Folge-Komplikationen werden von den gesetzlichen Krankenkassen etwa 6 Milliarden Euro aufgewendet.

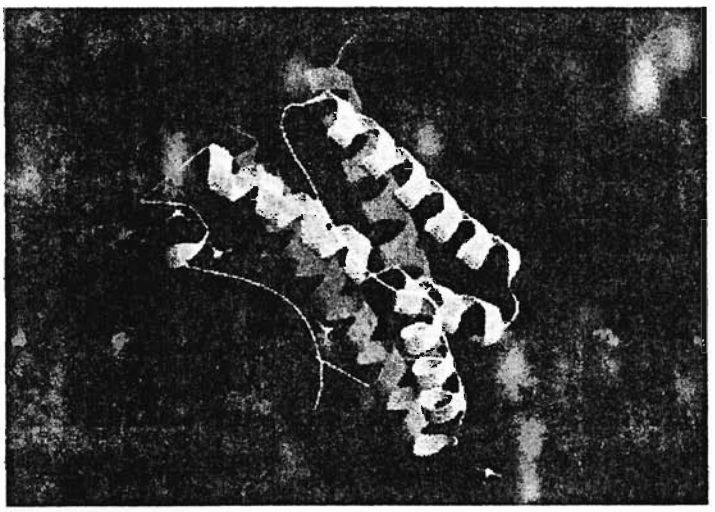


Abbildung 1
Mit Röntgenstrukturanalyse ermittelte Proteinstruktur von Interleukin 6, einem Entzündungsmediator. Erhöhte IL-6-Serumkonzentrationen sind mit dem späteren Auftreten des Typ-2-Diabetes assoziiert.
Quelle: PDB (Protein datenbank), www.rscb.org

Aufgabe des Deutschen Instituts für Ernährungsforschung ist es, durch experimentelle und klinische Forschung zur Wirkung der Ernährung und durch daraus abgeleitete Empfehlungen die Gesundheit des Menschen zu verbessern. Innerhalb dieses Ziels konzentriert sich das Institut besonders auf die beiden zur Zeit wichtigsten Erkrankungen, für die eine präventive Wirkung der Ernährung vermutet wird.

- das Metabolische Syndrom
- und die Krebserkrankungen

Konservative Schätzungen gehen von einer Verdopplung dieses Betrags in den nächsten 5 Jahren aus. Wenn es nicht gelingt, diesen Trend umzukehren oder wenigstens zu stoppen, wird es deshalb zur Senkung der durchschnittlichen Lebenserwartung und zur finanziellen Dekompensation der Arzneimittelausgaben kommen. In den beiden letzten Jahren wurden in einer Zusammenarbeit der Abteilungen Klinische Ernährung und Epidemiologie des DIfE zwei wichtige Serumproteine, sogenannte Zytokine, als Risikofaktoren für die Entstehung des Typ-2-Diabetes identifiziert, das Interleukin 6 (IL-6) und das Adiponectin.

- IL-6 ist ein Entzündungsmediator (Abb. 1), erhöhte IL-6-Serumkonzentrationen sind mit dem späterem Auftreten des Diabetes mellitus assoziiert. Dieser Befund spricht für eine Beteiligung des Immunsystems und entzündlicher Vorgänge an der Degeneration der insulinproduzierenden Zellen der Bauchspeicheldrüse.

- Adiponectin ist ein vom Fettgewebe produziertes Protein; niedrige Serumkonzentrationen sind ein Risikofaktor für die spätere Entstehung des Diabetes mellitus. Hierin zeigt sich die wichtige Rolle des Fettgewebes bei der Diabetesentstehung. Zukünftige präventive Maßnahmen können daran gemessen werden, inwieweit sie die Reduktion der Adiponectinspiegel normalisieren können.

Diabetesgenen zum Diabetes mellitus führt, und dass die Wirkung der Diabetesgene durch Gewichtskontrolle verhindert werden kann. Zudem ist nicht nur das absolute Körpergewicht, sondern auch die Ernährungsweise, die zu dem Gewicht geführt hat, bestimmend für die Entwicklung des Diabetes.

Übergewicht wird in unserer Gesellschaft häufig als Ausdruck einer Verhaltensstörung oder sogar als Willensschwäche stigmatisiert. Diesem Vorurteil wirkt die Forschung am DIfE entgegen. Sie zeigt, dass Übergewicht auch durch Störungen in den Mechanismen von Hungergefühl und Energiestoffwechsel bedingt sein kann. Personen mit Übergewicht essen mehr, als zum Erhalt des normalen Körpergewichtes nötig ist, weil sie ein inadäquates Hungergefühl und zudem einen niedrigeren Energieverbrauch als Normalgewichtige haben.

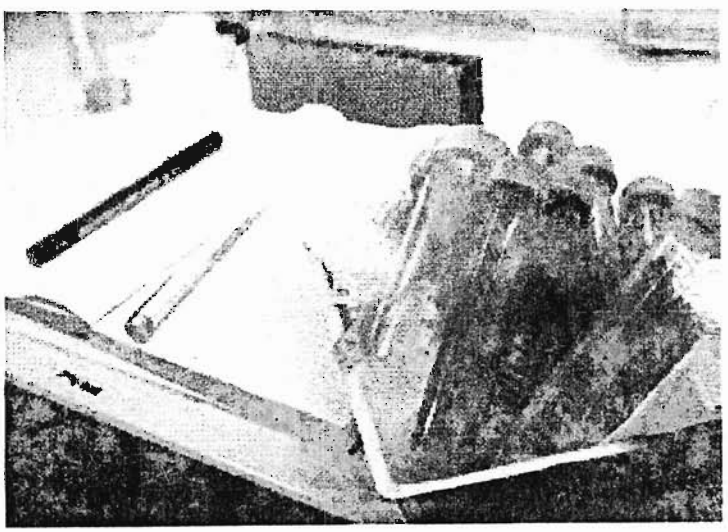


Abbildung 2
Laboralltag: Biologische Proben für biochemische und molekulargenetische Untersuchungen

Wichtige Fortschritte auf dem Gebiet des Metabolischen Syndroms wurden auch in experimentellen Untersuchungen der Abteilung Pharmakologie gemacht. In einem Mausmodell wurden Gene lokalisiert, die für Übergewicht oder Diabetes verantwortlich sind. Die Diabetesgene sind nicht für Übergewicht verantwortlich, werden aber erst durch Übergewicht wirksam. Interessanterweise wird zudem die Wirkung der Diabetesgene besonders durch fettreiche Ernährung verstärkt. Auf den Menschen übertragen bedeuten diese Daten, dass Übergewicht erst zusammen mit

Die gegenwärtige Forschung konzentriert sich daher auf die neuroendokrinologische Kontrolle des Hungergefühls (z.B. durch das im Magen gebildete Hormon Ghrelin) und die lange unterschätzte Rolle der Thermogenese (Arbeitsgruppe Energiestoffwechsel).
Die Ergebnisse der Grundlagenforschung und der klinischen Forschung müssen unverzüglich in die Praxis umgesetzt werden. Aus den Forschungsergebnissen müssen wirksame Strategien zur Diabetesprävention durch Gewichtsabnahme entwickelt werden; die Erkenntnisse zu den Effekten der einzelnen

of the immune system and of inflammatory processes in the degeneration of insulin producing cells of the pancreas.

Adiponectin is a protein produced in adipose tissue; lowered concentrations in serum are a risk factor for the later development of diabetes.

These data demonstrate the importance of adipose tissue for glucose metabolism and the development of diabetes. Furthermore, future preventive measures could be assessed as to which extent they are able to normalize adiponectin levels.

Experimental research in the Department of Pharmacology has led to significant progress in the area of the metabolic syndrome. In a mouse model, genes that are responsible for overweight or diabetes were localized. The diabetes genes are not responsible for overweight, but they are rendered effective by overweight. It is interesting that the malignancy of diabetes genes is increased by a high fat diet. Applying these data to humans would mean that obesity produces diabetes mellitus only when diabetes genes are present, and that the effects of diabetes genes can be prevented by weight control. In addition, not only the absolute body weight but also the composition of the diet is decisive for the onset of diabetes.

In our society, overweight is often considered to indicate a behavioral disorder or is even stigmatized as a lack of willpower. Research at the DIfE aims to disprove this assumption by showing that overweight can also be caused by disorders involving the mechanisms of hunger perception and of energy metabolism. Individuals with overweight appear to have an enhanced perception of hunger and, in addition, a lower energy expenditure than normal weight individuals. Present research thus focuses on the neuroendocrinological control of hunger perception (for example, by the hormone ghrelin produced in the stomach) and the long underestimated area of regulation of thermogenesis (Laboratory of Energy Metabolism).

The results of basic and clinical research have to be put into practice without delay. Based on the results of research, effective strategies to prevent diabetes by weight reduction must be developed; findings on the effects of individual dietary components must be applied accordingly. The DIfE does this at its Center of Nutritional Counseling, which offers practical courses in weight reduction.

Figure 2
Day-to-day business in laboratory: biological samples for biochemical and molecular genetic study

as well as individual consultations. It is very difficult to change eating habits; it is possible that, in some cases, overweight individuals can only be helped by pharmacotherapy. The DIfE contributes to this area by searching for new target molecules for anti obesity drugs.

One of the particularly successful areas of research at the DIfE in the years 2000 2002 involves the mechanisms of smell and taste perception (Fig 3). A number of receptors for qualities of sour and bitter taste have been identified and characterized. Thus, our knowledge about the sensory mechanisms with which we assess our food has been broadened considerably. After all, an important question is why certain foods and taste qualities are preferred and why an unhealthy diet often tastes so good. Moreover, why do tastes vary, and why is it so difficult to change the eating habits of some individuals? It has been known for a long time that the good taste but not the health-promoting effects are decisive for the food choice. How can these preferences be changed?

Like type 2 diabetes, cancer develops as the result of interaction of a genetic susceptibility with extrinsic factors. Reactive substances, natural as well as chemically synthesized compounds, cause mutations of the DNA, which may interfere with the control of cell growth. Numerous data support the assumption that nutrition plays a key role in the development of cancer. Especially in the case of carcinomas of the digestive tract, e.g., stomach and colon carcinomas, it has to be assumed that nutrition contributes to these by yet unknown agents or that other agents in nutrients have a protective effect. At present it is assumed that the risk of carcinomas of the digestive tract is increased by consumption of a high fat, high protein, and low-fiber diet, and that fruits, vegetables, and fiber have a protective effect (Fig.4).

The DIfE is involved in answering these questions by participation in the multi center, European wide EPIC Study. This study will identify eating habits that are associated with an increased risk of cancer, and quantify the role of nutrition in this risk. Because of the long latency period for development of cancer, it will take years before the study is able to provide final results. However, the preliminary results up to now support the assumption that a low consumption of fruits and vegetables is associated with a high cancer risk, whereas a high consumption of fiber is associated with a low cancer risk.

Figure 3
The senses of smell and taste determine if and why something tastes good.

Nahrungsbestandteile müssen entsprechend umgesetzt werden. Das DIfE tut dies in seinem Zentrum für Ernährungsberatung, das praktische Kurse zur Gewichtsabnahme, aber auch individuelle Beratung anbietet. Die Änderung von Ernährungsgewohnheiten ist sehr schwer und manchem Übergewichtigen ist möglicherweise nur mit medikamentöser Unterstützung zu helfen. Hierzu leistet das DIfE einen Beitrag, indem es an der Identifikation neuer Zielmoleküle für Arzneimittel arbeitet.

Ein in den Jahren 2000-2002 besonders erfolgreich bearbeitetes Forschungsfeld des DIfE sind die Mechanismen von Geruchs- und Geschmackserkennung (Abb.3). Von der Abteilung Molekulare Genetik wurden mehrere Rezeptoren für saure und bittere Geschmacksqualitäten identifiziert und charakterisiert.

Abbildung 3
Geruchs- und Geschmackssinn entscheiden, warum und wie gut etwas schmeckt.

Damit wurde unser Wissen über die sensorischen Mechanismen, mit denen wir Nahrung beurteilen, erheblich erweitert. Eine wichtige Frage ist ja, warum bestimmte Nahrungsmittel und Geschmacksqualitäten bevorzugt werden und warum oft eine ungesunde Nahrung so gut schmeckt. Und: warum sind die Geschmäcker verschieden, und warum lassen sich Ernährungsgewohnheiten bei manchen Menschen so schwer umstellen? Es ist seit langem bekannt, dass für den Kauf von Nahrungsmitteln in erster Linie der gute Geschmack, nicht eine gesundheitsfördernde Wirkung entscheidend ist. Wie kann man diese Präferenzen ändern?
Auch Krebs entsteht durch das Zusammenwirken einer genetischen Anlage mit exogenen Faktoren. Reaktive Substanzen – Naturstoffe ebenso wie chemisch synthetisierte Stoffe – bewirken Mutationen der DNS, die im ungünstigen Fall die Kontrolle des Zellwachstums verhindern. Die genetische Anlage modifiziert die Krebsentstehung über den Metabolismus der Fremdstoffe, die DNS-Reparatur, oder den programmierten Zelltod (Apoptose). Eine Fülle von Daten belegt die Annahme, dass die Ernährung eine wesentliche Rolle in der Krebsentstehung spielt. Vor allem für Karzinome des Verdauungstrakts wie Magen- und Kolonkarzinom muss angenommen werden, dass sie durch noch unbekannte Faktoren in der Ernährung entstehen können – oder dass andere Faktoren schützend wirken. Diese Faktoren sind bislang nicht sicher identifiziert. Begründete Annahmen sind, dass Karzinome des Verdauungstrakts mit dem Fleischverzehr assoziiert sind, und dass Obst, Gemüse und Ballaststoffe einen schützenden Effekt ausüben (Abb 4).

Das DIfE trägt zur Klärung dieser Fragen durch Teilnahme an der multizentrischen, europaweiten EPIC-Studie bei. Diese Studie wird Ernährungsgewohnheiten identifizieren,

die mit einem erhöhten Krebsrisiko einhergehen, und den Beitrag der Ernährung zum Krebsrisiko quantifizieren. Wegen der langen Latenz der Krebsentstehung wird es noch Jahre dauern, bis die Studie endgültige Ergebnisse liefern kann.

Die bisherigen, vorläufigen Daten sprechen jedoch dafür, dass ein geringer Verzehr von Obst und Gemüse mit einem höheren, ein hoher Verzehr von Ballaststoffen dagegen mit einem geringeren Krebsrisiko assoziiert ist.

Stevens DR, Seifert R, Bufe B, Muller F, Kremmer E, Gauss R, Meyerhof W, Kaupp UB, Lindemann B. Hyperpolarization-activated channels HCN1 and HCN4 mediate responses to sour stimuli. Nature 413:631-5 (2001).

Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF. Adiponectin and protection against type 2 diabetes mellitus. Lancet 361:226-8 (2003).

Plum, L., Giesen, K., Kluge, R., Junger, E., Linnartz, K., Schürmann, A., Becker, W., Joost, H.-G.: Characterization of the diabetes susceptibility locus Nidd/SJL in the New Zealand obese (NZO) mouse: Islet cell destruction, interaction with the obesity QTL Nob1, and effect of dietary fat. Diabetologia 45: 823-830 (2002).

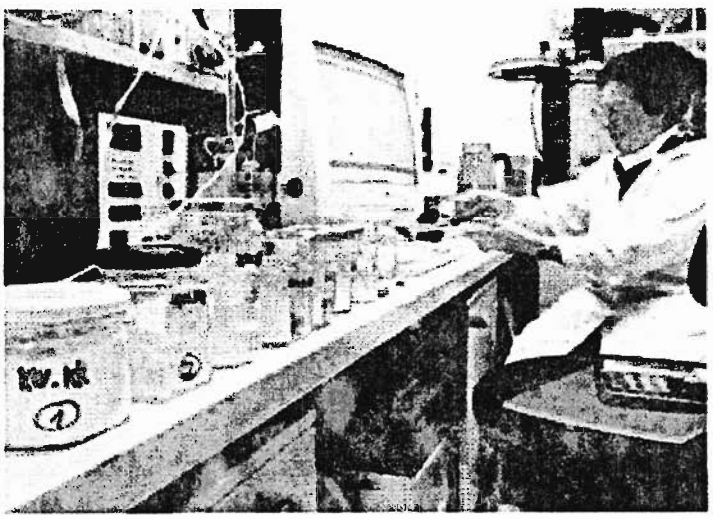


Abbildung 4
Probenvorbereitung für eine Testreihe über die Verstoffwechselung von Ballaststoffen im Darm.

Ausgewählte Veröffentlichungen
Selected Publications

Bufe B, Hofmann T, Krautwurst D, Raguse JD, Meyerhof W. The human TAS2R16 receptor mediates bitter taste in response to beta-glucopyranosides. Nature Genetics 32:397-401 (2002).

Schulz M, Kroke A, Liese AD, Hoffmann K, Bergmann MM, Boeing H. Food groups as predictors for short-term weight changes in men and women of the EPIC-Potsdam cohort. J. Nutr. 132:1335-40 (2002).

Bingham S A, Day N E, Luben R, Ferrari P, Slimani N, Norat T, Clavel-Chapelon F, Kesse E, Nieters A, Boeing H, Tjonneland A, Overvad K, Martinez C, Dorronsoro M, Gonzalez C A, Key T J, Trichopoulou A, Naska A, Vineis P, Tumino R, Krogh V, Bueno-de-Mesquita H B, Peeters P HM, Berglund G, Hallmans G, Lund E, Skeie G, Kaaks R, Riboli E.
Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. Lancet 361: 1496-1501 (2003).

Figure 4
Preparation of samples for a series of tests on the digestion of fiber in the intestine

Leitung: Prof. Dr. Wolfgang Meyerhof

Department of Molecular Genetics

Head: Prof. Dr. Wolfgang Meyerhof

What actually happens when something is tasted in the mouth and on the tongue? How is the brain informed? How is food consumption controlled, appetite regulated, and body weight maintained? Which extrinsic and intrinsic signals affect the orectic network? The Department of Molecular Genetics is looking for answers to these questions.

Molecular biology of taste

Increasing the content of chemoprotective compounds in food of plant origin is a potent dietary option for the prevention of diseases. However, consumers reject food rich in these bitter tasting phytonutrients, thereby promoting their removal during food processing (Fig.1). Bitter masking agents could circumvent this problem, but require a detailed understanding of the interaction of bitter compounds with their receptors on the human tongue. To identify human bitter taste receptors, we inserted the coding sequences of all candidate TAS2R genes into an expression cassette that enabled immunocytochemical detection and facilitated cell surface expression of the recombinant receptors in transfected cells (Fig.2). The cell line, which expressed the construct encoding TAS2R16, selectively responded to bitter-tasting β-glucopyranosides by increase in $[Ca^{2+}]i$. The concentration response relations of TAS2R16 for all of these chemicals closely resembled those obtained in human taste studies, suggesting that TAS2R16 represents a cognate human receptor for β-glucopyranosides (Fig. 3). The well known compounds amygdalin, the cyanogenic and bitter main component of almonds, and salicin of willow bark, with a 3500 year old history of proven antipyretic and analgesic action, belong to this class of chemicals. Adaptation frequently occurs in sensory systems upon prolonged or repeated presentation of a stimulus. Repeated stimulation of TAS2R16-expressing cells with phenyl β-D-glucopyranoside caused diminished responses to this compound and to all other tested β-pyranosides as well. Homologous desensitization of agonist occupied G protein coupled receptors likely accounts for this result. We made similar observations in human subjects. Shortly after the test solutions were taken up in the mouth, the panel members perceived the various β-D-glucopyranosides equally bitter. During prolonged exposure to one TAS2R16-agonist the subjects showed adapted bitter perception not only to that stimulus, but also to the other β-D-glucopyranosides. This cross-adaptation occurred among all tested β-D-glucopyranosides, but was not seen in the case of the unrelated bitter substance denatonium benzoate.

Figure 1
In the modernly equipped Olfactory and Gustatory Laboratory of the DIfE, up to ten probands can undergo computer-aided examinations simultaneously

* during part of the reporting period

Was geschieht eigentlich beim Schmecken im Mund und auf der Zunge und wie erfährt das Gehirn davon? Wie wird die Nahrungsaufnahme kontrolliert, der Appetit reguliert, das Körpergewicht gehalten? Welche externen und internen Signale beeinflussen das orexische Netzwerk? Die Abteilung Molekulare Genetik sucht Antworten auf diese Fragen.

Molekularbiologie der Geschmackswahrnehmung

Maik Behrens, Bernd Bufe, Stephanie Demgensky, Ulrike Lerner*, Ellen Schöley-Pohl*

Wegen ihres bitteren Geschmacks wird ein hoher Gehalt an gesundheitsfördernden pflanzlichen Inhaltsstoffen in Nahrungsmitteln vom Verbraucher nicht akzeptiert (Abb 1).

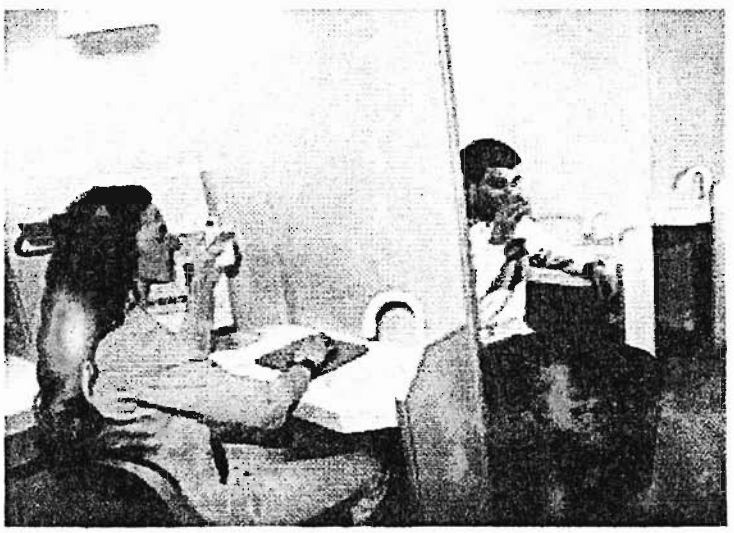


Abbildung 1
Im modern ausgestatteten Geruchs- und Geschmackslabor des DIfE können bis zu 10 Probanden gleichzeitig computerunterstützt untersucht werden

Die Nahrungsmittelindustrie ist daher bemüht, den Gehalt dieser Stoffe in unseren Nahrungsmitteln zu verringern. Der Einsatz sogenannter Bitterblocker wäre ein Ausweg aus diesem Dilemma, erforderte jedoch die genaue Kenntnis der Wechselwirkung solcher Stoffe mit den Geschmacksrezeptoren auf der menschlichen Zunge. Unerlässliche erste Schritte dafür wären die Isolierung aller humanen Bitterrezeptoren und der Aufbau einer Messmethode, um sie funktionell zu charakterisieren. Das erste Problem haben wir gelöst, indem wir durch Analyse des humanen Genomprojektes die TAS2R-Genfamilie für menschliche Bitterrezeptoren vollständig isolieren konnten. Die kodierenden Abschnitte aller TAS2R-Gene dienten anschließend zur Herstellung von DNA-Konstrukten, die die Expression der rekombinanten Proteine an der Zelloberfläche ermöglichten und ihren zytochemischen Nachweis erlaubten (Abb. 2).
Unsere weiteren Arbeiten lösten weitgehend das zweite Problem, denn sie zeigten, dass transfizierte Zellen, die das humane TAS2R16-Gen exprimierten, selektiv durch bittere β-Glucopyranoside stimulierbar waren und mit einer Erhöhung der intrazellulären Calciumkonzentration reagierten. Diese Substanzklasse schließt Verbindungen wie Salicin und Amygdalin ein. Beide sind seit langer Zeit sehr gut bekannt.

Salicin wird aus der Rinde von Weiden extrahiert und findet in der Heilkunde durch seine schmerzlindernde und fiebersenkende Wirkung Verwendung. Aus dem Zyangiftstoff Amygdalin der Bittermandel wird das in der Küche gebräuchliche Bittermandelaroma freigesetzt. Alle Daten unserer verschiedenen In-vitro-Versuche entsprachen genau denen, die wir an Versuchspersonen erheben konnten (Abb. 3). Aus dieser Übereinstimmung geht hervor, dass der TAS2R16-Rezeptor ein

* zeitweise während des Berichtszeitraums

menschlicher Bittergeschmacksrezeptor für β-Glucopyranoside ist. Diese Schlussfolgerung wird weiter unterstützt durch den Befund, dass die TAS2R16-mRNA nur in Geschmacksknospen auf der menschlichen Zunge nachweisbar war (Abb. 4).

Unsere Versuche erlaubten auch Vorhersagen darüber, welche strukturellen Eigenschaften Stoffe aufweisen müssen, um TAS2R16 zu aktivieren und um als bitter wahrgenommen zu werden. Unerlässlich ist die β-Konfiguration der glykosidischen Bindung und die äquatoriale Position der Hydroxylgruppe an dem C4-Atom des Pyranoserings. Größere hydrophobe Aglykons ergeben bessere Agonisteneigenschaften als kleinere. Das kleinste Aglykon, ein Wasserstoffatom, kommt in der süß schmeckenden Glucose vor, die den hT2R16 nicht aktiviert.

Pyranoserings mit dem Rezeptor, die daraufhin die an sich geschmacklosen Aglykons in die Bindungstasche ziehen. TAS2R16 koppelt die Erkennung einer spezifischen chemischen Struktur an die bewusste Wahrnehmung bitterer Geschmacks. Der Rezeptor ist in der Lage, eine ganze Klasse von Verbindungen zu erkennen. Würde dieses Verhalten von den anderen TAS2R-Rezeptoren geteilt, dann könnten wir leichter erklären, wie wir die zahlreichen Bitterstoffe mit einem begrenzten Satz von Rezeptoren wahrnehmen können. Die funktionelle Expression von Bittergeschmacksrezeptoren ist eine wichtige Voraussetzung für die Isolierung oder Entwicklung von Bitterblockern, die zur besseren Akzeptanz mancher Lebensmittel und Medikamente führen.

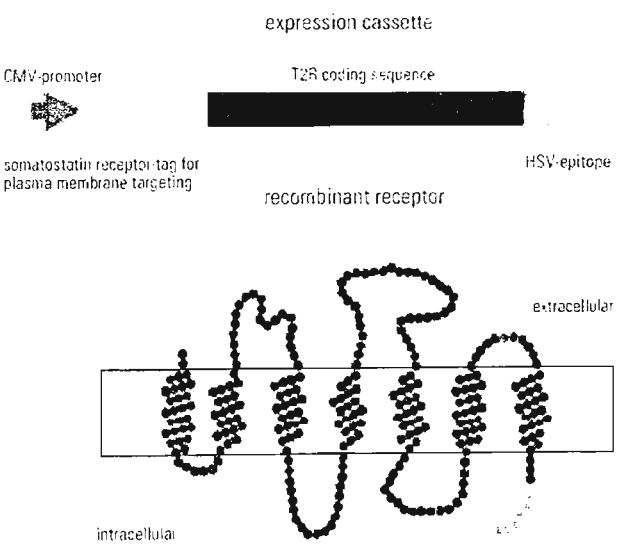


Abbildung 2
Schematische Darstellung der Expressionskassette sowie der rekombinanten Rezeptorproteine zum Nachweis menschlicher Bitterrezeptoren

Hydrophile Substitutionen im Aglykon verschlechtern die Aktivierbarkeit des Rezeptors, während die Stellung der Hydroxylgruppe an C2. Substitutionen an C6 oder das Ersetzen des Sauerstoffs der glykosidischen Bindung durch Schwefel keinen nennenswerten Einfluss aufweisen. Unsere Beobachtungen werden durch ein kürzlich vorgeschlagenes Modell gestützt. Das erklärt die Bitterkeit von β-Glucopyranosiden durch die Wechselwirkung von zwei der Hydroxylgruppen des

Molekularbiologie des Geruchsinns

Dietmar Krautwurst, Kristin Schmiedeberg, Elena Chirokova, Monika Silkeit

Olfaktorische Rezeptoren (OR) ermöglichen es Wirbeltieren, tausende Geruchsstoffe zu unterscheiden. Wir identifizierten Schlüsselaromastoffe(SAS)-erkennende OR in funktionellen Expressionsstudien mit einer OR-cDNA-Bibliothek, exprimiert in HEK293/15-Zellen. Der SAS 2,3-Diethyl-5-methylpyrazin aus

Consistent with its role as a bitter taste receptor, we detected mRNA for TAS2R16 in human taste buds of the vallate papillae, which are specialized morphologic structures for taste reception of the tongue (Fig. 4). Our data also disclosed some structural requirements of the compounds that activate TAS2R16. The β configuration of the glycosidic bond and the equatorial position of the hydroxyl group at C4 of the pyranose are equally crucial for TAS2R16 activation in vitro and human bitter taste. Moreover, large hydrophobic aglycons mediate better agonist properties than the small ones. The hydrogen atom in this position, which occurs in the sweet tasting molecule glucose, prevents agonist activity at hTAS2R16. Hydrophilic ring substitutions correlate inversely to agonist potency. The glycosidic oxygen atom does not seem to be of particular importance because it can be replaced by sulfur. The steric orientation of the hydroxyl group in C2 is of little importance, since mannosides act as receptor agonists as well. Substitutions at C6 had only little effect on agonist properties. The moderate potency of amygdalin is likely due to the hydrophilic nitrile group of its aglycon and not to the α-1,6 glycosidic addition of another glucose molecule. Recent data on the bitter unit of glucopyranosides support our conclusions. This work showed that the aglycons are tasteless and that a hydrogen acceptor and donor site set up by two of the hydroxyl groups of the glucose moiety forms the bitter unit. TAS2R16 links the recognition of a specific chemical structure to the conscious perception of bitter taste. TAS2R16 is tuned to numerous β-glycopyranosides. If this broad tuning is replicated by the other TAS2Rs, it helps explain how humans endowed with a small number of bitter receptors can perceive the multitude of bitter compounds. Functional expression of TAS2Rs will be of value for the design and isolation of bitter masking substances that could block the bitterness of chemoprotective phytonutrients or medicine.

Molecular biology of olfaction

Olfactory receptors (OR) enable vertebrates to discriminate thousands of odorants. We identified cognate ligand/OR pairs in functional expression screenings using a mouse OR cDNA library and selected key food odorants (KFO) in HEK293/15 cells. The KFO 2,3-diethyl-5-methylpyrazine from raw and heated food was used to screen 96 mouse OR chimeras expressed in HEK293/15 cells for intracellular Ca^{2+} signals, employing a FLIPR system. By functional and in silico homology analysis, we identified 3 responding mouse and 2 human OR with different efficacies to induce intracellular

Figure 2
Schematic representation of the expression cassette and recombinant receptor

Ca^{2+} signals upon application of 2 different y smelling alkyl- and methoxypyrazines.
We employed the KEO (-)citronellal and have identified 4 cognate (-)citronellal/OR pairs by FLIPR screening of our 170 mouse OR chimeras.
In silico homology analysis revealed another 10 and 7 homologous genes in mouse and human, respectively, that could be allocated to 3 families. These genes have been cloned, their expression in situ was confirmed by RNA hybridization and RT-PCR, and their expression in human cell lines was confirmed by immunocytochemistry.
For 7 of 14 mouse OR and 5 of 7 human OR an activation by (-)citronellal could be confirmed, and for 2 mouse OR and 3 human OR stable cell lines were established. EC_{50} based odor profiles and mutation studies will clarify structure function relations of OR. Since the majority of OR triggers odorant induced Ca^{2+} signals via a cAMP/cyclic nucleotide gated channel (CNGC) signalling pathway in olfactory sensory neurons, we established a human HeLa cell line stably expressing the olfactory CNGA2 channel.
By conducting Ca^{2+}, CNGCA2 serves as a reporter for a variety of stimuli that increases the intracellular concentration of cyclic nucleotides, such as isoproterenol, atrionatriuretic peptide, and (-)citronellal. This cell line will facilitate the functional identification and characterization of OR, and thus will increase an understanding of odorant coding.

Central regulation of feeding behavior

A major contribution to the regulation of feeding behavior is made by the adipose tissue hormone leptin that signals to the hypothalamus. In recent work we have functionally identified, in the rat model, the hypothalamic neurons that respond to leptin through immunohistochemical detection of the nuclear translocation of the signaling molecule STAT3. The identified neurons are largely located in the mediobasal hypothalamus and form part of an orexigenic-anorectic network. In the reporting period we have analyzed the relation of the leptinoceptive neurons to the somatostatin signaling system by double label immunohistochemistry. We observed almost complete lack of somatostatin expression in leptin responsive hypothalamic neurons, suggesting that, in the rat, leptin does not directly regulate somatostatinergic neurons. However, somatostatin containing fibers and termini were frequently opposed to STAT3 positive neurons. In marked contrast, widespread expression of somatostatin receptor subtypes occurred in the leptin responsive neurons of various hypothalamic nuclei, including those of the

Figure 3
Dose-response relation of the β-glucopyranoside helicin and intracellular calcium concentrations in TAS2R16 expressing cells and the intensity of bitter taste in test persons

* during part of the reporting period

rcher und erhitzten Lebensmitteln wurde auf 96 Maus-OR-Chimären für das Auftreten intrazellulärer Ca^{2+}-Signale in HEK293T15-Zellen mit dem Fluorescence-Imaging-Plate-Reader (FLIPR) getestet. Durch funktionelle und In-silico-Homologieanalysen identifizierten wir 3 Maus- und 2 menschliche OR. Diese induzierten in transienten Expressionsstudien nach Gabe von Alkyl- oder Methoxypyrazinen verschiedener Geruchsqualitäten intrazelluläre Ca^{2+}-Signale mit unterschiedlichem Wirkungsgrad. Wir benutzten den SAS (-)Citronellal und identifizierten 4 funktionale (-)Citronellal/OR-Paare durch FLIPR-Screening gegen 170 Maus-OR-Chimären. Eine In-silico-Homologieanalyse ergab weitere 10 bzw. 7 homologe Gene in Maus und Mensch. Diese Gene wurden molekular kloniert, die Expression im Gewebe durch in-situ-Hybridisierung und RT-PCR, und in menschlichen Zelllinien durch Immunocytochemie bestätigt.

zyklische Nukleotide gesteuerten Kanal, CNGA2, exprimierten. Dieser dient als Reporter für eine Vielzahl von Stimuli, die die intrazelluläre Konzentration zyklischer Nukleotide erhöhen, z. B. Isoproterenol, atrionatriuretisches Peptid und (-)Citronellal. Diese Zelllinie wird die funktionelle Identifizierung von OR erleichtern, und dadurch das Verständnis der Geruchsstoffkodierung ermöglichen.

Anne-Sophie Carlo, Martina Pyrski*, Zara Stepanyan**

Das Adipozytenhormon Leptin leistet wichtige Beiträge zur Kontrolle der Nahrungsaufnahme durch seine Wirkungen auf den Hypothalamus. Im Modell Ratte haben wir vor einiger Zeit eine Nachweismethode für die Nervenzellen entwickelt, die auf Leptin

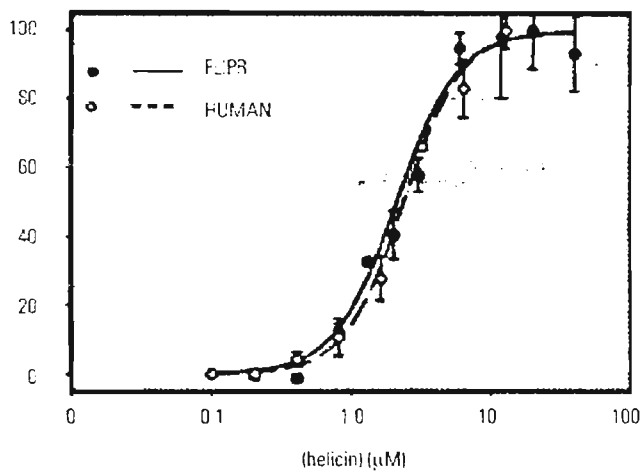


Abbildung 3
Konzentrations-Wirkungsbeziehung des β-Glucopyranosids Helicin und der intrazellulären Calciumkonzentrationen in TAS2R16-exprimierenden Zellen bzw. der Intensität des Bittergeschmacks von Testpersonen

Für 7 von 14 Maus-OR und 5 von 7 menschlichen OR wurden (-)Citronellalabhängige Ca^{2+}-Signale gemessen. Für 2 Maus- und 3 menschliche OR wurden stabile Zelllinien etabliert. EC_{50}-basierende Geruchsstoffprofile und Mutationsstudien werden zur Aufklärung von Struktur-Funktionsbeziehungen der OR führen. Da OR Ca^{2+}-Signale in olfaktorischen Neuronen mehrheitlich durch den cAMP-Signalweg auslösen, etablierten wir eine menschliche HeLa-Zelllinie, die den durch

reagieren und die daher als Bestandteil orexigener Netzwerke identifiziert werden konnten. Im Berichtszeitraum haben wir eine mögliche Beziehung dieser leptinsensitiven Nervenzellen zu Somatostatin untersucht. Eine Wechselwirkung dieses Neuropeptids mit Leptin könnte eine wichtige Rolle bei den veränderten Wachstumshormonspiegeln spielen, die im Zusammenhang mit Adipositas und Kachexie beobachtet werden.
Auf anatomischer Ebene konnten wir keine

* zeitweise während des Berichtszeitraums

Somatostatinexpression in den leptinsensitiven Neuronen beobachten. Jedoch stellten wir fest, dass im mediobasalen Hypothalamus verschiedene Somatostatinrezeptortypen auf den leptinsensitiven Zellen lokalisiert sind und dass sie von somatostatinhaltigen Fasern und Termini umgeben sind. Das könnte auf einen modulierenden Einfluss von Somatostatin auf den anorektischen Effekt von Leptin hindeuten.

Regulation von Somatostatinrezeptoren
Nicole Brune, Ara Kocharyan

Zwar wirken Peptidhormone vorwiegend durch Aktivierung von zelloberflächenständigen Rezeptoren, doch ist weitgehend unbekannt, wie diese im Verlauf der Biosynthese dorthin gelangen. Wir haben festgestellt, dass der Somatostatin-Typ-3-Rezeptor (sst3) in transfizierten Zellen vorwiegend auf der Plasmamembran lokalisiert ist, wohingegen der Typ-1-Rezeptor (sst1) besonders auf intrazellulären Membrankompartimenten zu finden ist. Die Analyse entsprechender Rezeptorchimären führte zur Identifizierung des aminoterminalen Bereichs des sst3, der für die Zielsteuerung zur Zelloberfläche verantwortlich ist.
Eine andere Sequenz im Aminoterminus des sst1 bedingt dagegen die Zielsteuerung in die intrazellulären Kompartimente.
Die Universalität der Plasmamembranzielsteuerungssequenz des sst3 zeigte sich dadurch, dass sie auch die völlig unverwandten Bittergeschmacksrezeptoren an die Zelloberfläche leitet. Ohne diese Sequenz gelangen die Bittergeschmacksrezeptoren nicht an die Zelloberfläche und entziehen sich ihrer funktionellen Identifizierung.

mediobasal hypothalamus that are known to be involved in the regulation of feeding behavior. These anatomical data suggest somatostatin to be a modulator of leptin s anorectic action.

Regulation of somatostatin receptors

Although peptide hormone receptors commonly exert their actions at the plasma membrane, the cellular mechanisms that route the receptor proteins to the cell surface are not well characterized. In transfected cells, sst3 somatostatin receptors are present almost exclusively at the cell surface, while sst1 receptors localize largely at intracellular vesicular compartments. Various chimeric receptors were constructed between rat sst3 and sst1, and analyzed by immunocytochemical methods following transfection in two different cell lines. The results demonstrate that the amino-terminal domain of sst3 suffices to guide the chimeric receptors to the cell surface. Quite in contrast, chimeras that lack this sequence but contain instead the amino-terminus of sst1 localize in intracellular vesicular compartments. Based on these results we used the sst3 targeting sequences to direct human bitter taste receptors to the cell surface (see above) that otherwise fail to reach this compartment and evade functional identification.

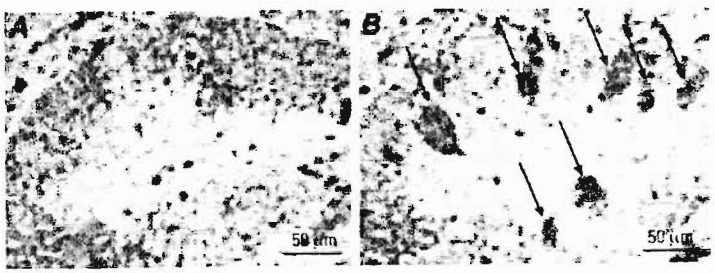


Abbildung 4
Nachweis von TAS2R16 mRNA in humanen Geschmackspapillen durch In-situ-Hybridisierung. (A) Kontrolle, sense Probe. (B) antisense Probe. Balken, 50 µm

Figure 4
Detection of TAS2R16 mRNA by in situ hybridization in human taste buds. (A) control, sense cRNA probe; (B) antisense probe. Scale bar, 50 µm

Drittmittelprojekte
External Funding

Titel: Isolierung von Genen, die an zentralen Hunger und Sättigungsmechanismen beteiligt sind
Finanzierung: Institut Danone für Ernährung
Laufzeit: 07/00-12/02

Titel: Combinatorial high throuput screening of odorants versus an expression library of olfactory receptors by means of functional reconstitution of olfactory signal transduction molecules in mammalian cell lines
Finanzierung: Internationale Stiftung für Ernährungsforschung und Ernährungsaufklärung
Laufzeit: 11/00-10/02

Titel: Aufklärung der Geruchsstoffspezifität olfaktorischer Rezeptoren und Rezeptorsubfamilien durch funktionelle Expression einer Rezeptorbibliothek im Säugerzellsystem
Finanzierung: DFG
Laufzeit: 07/00-06/02

Titel: Somatostatin and its receptors in brain function and dysfunction
Finanzierung: EU
Laufzeit: 01/00-12/03

Titel: Molekulare Klonierung, Charakterisierung und Struktur-Funktions-Beziehungen von olfaktorischen Rezeptoren für den Schlüsselaromastoff 'Weinlacton'
Finanzierung: BMBF
Laufzeit: 05/02-04/04

Titel: Functional expression of members of a mouse olfactory receptor library
Finanzierung: Daimler Benz-Stiftung
Laufzeit: 05/00-05/02

Ausgewählte Publikationen
Selected Publications

Originalarbeiten/Original Papers

Ammon, C., Schäfer, J., Kreuzer, O. J., Meyerhof, W.: Presence of a plasma membrane targeting sequence in the amino-terminal region of the rat somatostatin receptor 3. Arch. Physiol. Biochem. 110, 137-45 (2002).

Baumeister, H., Scharfke, M., Haebel, S., Meyerhof, W.: A two-dimensional map and database of soluble nuclear proteins from HepG2 cells as reference for identification of nutrient-regulated transcription factors. Eur. J. Nutr. 40, 168-177 (2001).

Bufe, B., Hofmann, T., Krautwurst, D., Raguse, J.-D., Meyerhof, W.: The human TAS2R16 receptor mediates bitter taste in response to beta-glucopyranosides. Nature Genetics 32, 397-401 (2002).

Florian, S., Wängler, K., Schmehl, K., Jacobasch, G., Kreuzer, O. J., Meyerhof, W., Brigelius-Flohe, R.: Cellular and subcellular localization of gastrointestinal glutathione peroxidase in normal and malignant human intestinal tissue. Free Radic. Res. 35, 655-663(2001).

Hübschle, T., Harre, E. M., Meyerhof, W., Pehl, U., Roth, J., Gerstberger, R.: The central pyrogenic action of interleukin-6 is related to nuclear translocation of STAT3 in the anteroventral preoptic area of the rat brain. J. Therm. Biol. 26, 299-305 (2001).

Hübschle, T., Thom, E., Watson, A., Roth, J., Klaus, S., Meyerhof, W.: Leptin-induced nuclear translocation of STAT3 immunoreactivity in hypothalamic nuclei involved in body weight regulation. J. Neurosci. 21, 2413-2424 (2001).

Kreuzer, O. J., Krisch, B., Dery, O., Bunnett, N. W., Meyerhof, W.: Agonist-mediated endocytosis of rat somatostatin receptor subtype 3 involves beta-arrestin and clathrin Coated Vesicles. J. Neuroendocrinol. 13, 279-287 (2001).

Schärfke, M., Baumeister, H., Meyerhof, W.: Method for the immunological detection of silver-stained proteins on nitrocellulose membranes. Biotechniques 30, 266-268, 270, 272 (2001).

Stevens, D. R., Seifert, R., Bufe, B., Müller, F., Kremmer, E., Gauss, R., Meyerhof, W., Kaupp, U. B., Lindemann, B.: Hyperpolarization-activated channels HCN1 and HCN4 mediate responses to sour stimuli. Nature 413, 631-635 (2001).

Übersichtsarbeiten/Reviews

Klaus, S., Meyerhof, W.: Hunger entsteht im Gehirn - Die Neurobiologie des Essverhaltens. Teil 1. Ernährung im Fokus 1, 142-147 (2001).

Klaus, S., Meyerhof, W.: Hunger entsteht im Gehirn - Die Neurobiologie des Essverhaltens. Teil 2. Ernährung im Fokus 1, 176-180 (2001).

Buchbeiträge/Articles in Books

Krautwurst, D., Chirokova, E.: The olfactory receptor expression library as a tool for structure-function studies. In: Rothe, M. (ed.): Flavour 2000. Perception, release, evaluation, formation, acceptance, nutrition, health. Proceedings of the 6th Wartburg Aroma Symposium, Wartburg-Stiftung. Eigenverlag Bergholz-Rehbrücke, 13-24 (2001).

Adipositas (krankhaftes Übergewicht) ist wegen der dramatischen Zunahme von Häufigkeit und Schweregrad die wichtigste ernährungsbedingte Erkrankung. Adipositas verursacht das sog. Metabolische Syndrom mit Hypertonie, Cholesterin-Stoffwechselstörung und Insulinresistenz, und kann im weiteren Verlauf zu lebensverkürzendem Typ-2-Diabetes führen. Wirksame therapeutische Methoden (Arzneimittel, nicht-medikamentöse Therapien) lassen sich leichter entwickeln, wenn molekulare Ursachen und Pathophysiologie einer Erkrankung bekannt sind.

Die Arbeit der Abteilung hat deshalb zum Ziel, die Genetik, Pathobiochemie und Pathophysiologie der Adipositas aufzuklären. Wir wollen neue Zielsysteme (Proteine, Signaltransduktionswege) zu ihrer Behandlung identifizieren.

Insulinresistenz und Typ-2-Diabetes: Identifikation von Suszeptibilitätsgenen in einem Mausmodell

Reinhart Kluge, Leona Plum, Katja Schmolz, Mandy Grothe, Annette Schürmann

Entstehung und Verlauf der Adipositas sind ernährungsbedingt und werden zudem durch andere Lebensbedingungen (z.B. die körperliche Aktivität) beeinflusst, basieren aber vor allem auf einer komplexen genetischen Prädisposition. Diese Prädisposition besteht aus einem Netzwerk variabler Gene (polygene Erkrankung), dessen einzelne Komponenten unbekannt und beim Menschen sehr schwer identifizierbar sind. Am DIfE wird daher ein Mausmodell (die New-Zealand obese (NZO) Maus) genutzt, das das polygene Metabolische Syndrom des Menschen sehr exakt abbildet.

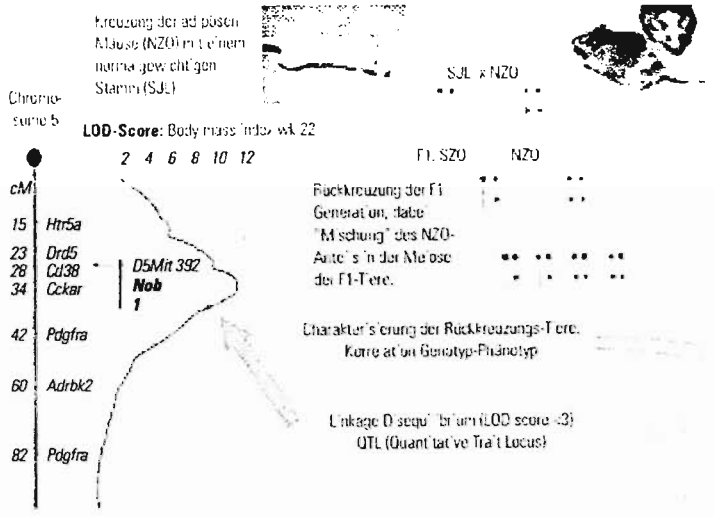


Abbildung 1
Methodik zur Lokalisation von Adipositasgenen. Durch Kreuzung von adipösen NZO-Mäusen mit einem normalgewichtigen Stamm und anschließende Rückkreuzung der F1-Generation wird eine Population mit vollständig gemischten Genomen generiert. Durch Vergleich der Körpergewichte von Tieren mit verschiedenen Genotypen (bestimmt mit über das gesamte Genom verteilten Mikrosatelliten-Markern) lassen sich chromosomale Abschnitte identifizieren, die mit Übergewicht assoziiert sind (sog. Kopplungsungleichgewicht: QTL = Quantitative Trait Loci).

Dies soll geschehen durch:
1. Identifikation von Suszeptibilitätsgenen für Insulinresistenz und Typ-2-Diabetes in einem Mausmodell,
2. durch Untersuchung der Rolle gastrointestinaler Hormone für die Gewichtsregulation sowie
3. durch Aufklärung der molekularen Grundlagen von Nährstofftransport und Insulinwirkung

Identifikation von Suszeptibilitäts-Loci für Adipositas und Diabetes

Krankheitsauslösende Gene lassen sich lokalisieren, indem man das Genom des erkrankenden Mausstamms durch Kreuzung mit dem eines gesunden Stamms mischt. In der Kreuzungspopulation vergleicht man die Eigenschaften der Tiere (‚traits') mit dem Genotyp für sog. polymorphe Marker (s. Abb. 1).

Department of Pharmacology
Head: Prof. Dr. Dr. Hans-Georg Joost

Because of the dramatic increase in incidence and severity, obesity is the most important nutritional disease. Obesity is the main cause of the metabolic syndrome with hypertension, hypercholesterolemia, and insulin resistance, and may lead to life shortening type 2 diabetes. At present, the German public health insurances spend about 6 billion euro (a third of their budget for prescription drugs) for the pharmacotherapy of the entire syndrome; conservative estimates project a doubling of this amount within the next 5 years. This trend will, if it continues, decrease the average life expectancy, and will cause a severe financial crisis of the public health system.

The development of a novel therapy (drugs or others) is largely facilitated by knowledge as to the molecular mechanism and the pathophysiology of a disease. Therefore, it is the aim of the department to study the genetic basis, the pathobiochemistry, and the pathophysiology of obesity in order to identify new targets (proteins, signal transduction pathways) for its treatment. Specifically, it is intended to (1) identify susceptibility genes for insulin resistance and type 2 diabetes in a mouse model, (2) investigate the role of gastrointestinal hormones in body weight regulation, and (3) study the molecular basis of nutrient transport and its regulation by insulin.

Insulin resistance and type-2 diabetes: identification of susceptibility genes in a mouse model

Obesity is dependent on nutritional conditions and on other life style parameters, e.g., physical activity. However, its main basis is the complex, polygenic predisposition. This predisposition consists of a network of variant genes which are unknown and very difficult to identify in humans. Therefore, a mouse model (New Zealand obese mice, NZO) that closely resembles the human metabolic syndrome is used at the DIfE.

Identification of susceptibility loci for obesity and diabetes

Disease genes can be located by crossing the affected mouse strain with a healthy strain, thereby 'mixing' their genomes. Subsequently, the phenotypic characteristics ('traits') of the backcross population are correlated with the genotype as determined with polymorphic markers spread over the whole genome (Fig. 1). With this procedure, chromosomal regions are found (QTL, quantitative trait loci), which are in 'linkage disequilibrium', meaning that a certain genotype (e.g., homozygosity for the NZO allele) is significantly associated with

Figure 1:
Methodology for the localization of obesity genes. Crossing of obese NZO mice with a lean control strain and the subsequent backcross of F1 results in a population with 'mixed' genomes. By comparison of body weights of animals with different genotypes (as determined with microsatellite markers), chromosomal regions (QTL) are identified that are associated with higher body weight (linkage disequilibrium; QTL = quantitative trait locus).

a phenotypic characteristic (e.g., higher body weight). Recently, we identified several QTL for obesity (e.g., Nob1 on chromosome 5, LOD Score > 4 for statistical significance) in the backcross of NZO with the lean Swiss/Jim Lambert mouse strain (SJL, Fig. 1). In addition, we identified a QTL for diabetes (Nidd/SJL on chromosome 4, LOD Score > 4) which is responsible for the destruction of the insulin producing cells in the islets of Langerhans (Fig. 2).

Interaction of obesity and diabetes genes

Data from the backcross of NZO and SJL contributed to our understanding of the interaction of obesity and diabetes genes. In a dose dependent manner, the prevalence of diabetes in the backcross population was dependent on the early obesity of the animals (Fig. 2). Diabetes genes in the Nidd/SJL locus sensitized the mice to the effect of early obesity: Control animals (non gene carriers) became diabetic only if their body weight at 12 weeks was >50 g, whereas carriers of the Nidd/SJL allele developed hyperglycaemia at lower body weights. With a 12 week body weight exceeding 55 g, 90% the Nidd/SJL carriers but only 50% of the controls exhibited a decompensation of the blood glucose regulation. Below a threshold of 40g (12 weeks body weight), Nidd/SJL was not diabetogenic.

The effect of dietary fat on the prevalence of diabetes -

The fat content of the diet markedly accelerated the development of diabetes and aggravated the effect of Nidd/SJL. Under a high-fat diet, carriers of the diabetogenic allele developed diabetes earlier than comparable animals fed a standard diet. This result is paradigmatic for the interaction of the genetic basis with nutritional parameters.

The aim of the ongoing project is to identify the genes responsible for the effects of the susceptibility loci. At present, congenic mouse lines are generated which harbor portions of the QTLs on a different genetic background (transfer of Nob1 to NZB, transfer of Nidd/SJL to C57BL) in order to further narrow down the chromosomal regions which contain the responsible genes. Information as to the function of the genes located in the critical regions is obtained by analysis of their expression (tissue distribution, differences between NZO and SJL). In addition, we are trying to identify variants by sequencing candidate genes located in the QTL.

So lassen sich chromosomale Abschnitte finden (QTL: quantitative trait loci), die sich im 'Kopplungsungleichgewicht' befinden, d.h. ein bestimmter Genotyp (z.B. beide Allele des Abschnitts von NZO) ist signifikant mit einem phänotypischen Merkmal (z.B. höheres Körpergewicht) assoziiert. Auf diese Weise gelang es, im Kreuzungsmodell aus dem adipösen NZO- und dem normalgewichtigen Swiss/Jim-Lambert-Stamm (SJL) mehrere QTL für Adipositas (z.B. Nob1 auf Chromosom 5, LOD-Score > 4 als Maß für die statistische Signifikanz) zu identifizieren (s. Abb.1). Wir fanden zudem einen QTL für Diabetes (Nidd/SJL auf Chromosom 4, LOD-Score > 4), der für die progrediente Zerstörung der insulinproduzierenden Zellen in den Langerhans'schen Inseln verantwortlich ist (s. Abb. 2).

Die Charakterisierung des Rückkreuzungsmodells lieferte außerdem einen wichtigen Beitrag zum Verständnis der Interaktion von Adipositas- und Diabetes-Genen. Die Daten zeigten, dass die Diabetes-Prävalenz der Rückkreuzungstiere von der frühen Adipositas der Tiere abhängig ist (Abb 2); es besteht also eine 'Dosisabhängigkeit' zwischen Adipositas und Diabetes-Prävalenz. Diese Abhängigkeit wird nun von den im Locus Nidd/SJL befindlichen Diabetes-Genen verändert: Kontrolltiere (nicht-Genträger) entwickeln erst ab einem 12-Wochen-Körpergewicht von 50 g einen Typ-2-Diabetes (Prävalenz ca. 50 %), während bei den Genträgern von Nidd/SJL bereits mit niedrigeren Körpergewichten eine Dekompensation der Blutzuckerregulation auftritt. Ist das 12-Wochen-Gewicht größer als 55 g, dekompensiert die Blutzuckerregulation bei 90% der Nidd/SJL-Träger und nur bei 50% der Kontrollen. Unterhalb einer Schwelle von 40 g (Gewicht nach 12 Wochen) wirkte Nidd/SJL nicht diabetogen.

Der Fettanteil der Nahrung hatte einen erheblichen Einfluss auf den Zeitpunkt der Diabetesentstehung und verstärkte die Wirkung von Nidd/SJL. Unter einer fettreichen Diät entwickelte sich der Diabetes in Trägern des diabetogenen Allels viel früher und war schwerer als in vergleichbaren Tieren unter einer Standarddiät. Dieser Befund zeigt exemplarisch die Interaktion der genetischen Grundlage mit der Ernährung. Ziel der gegenwärtigen Arbeit ist es, die für die Effekte der Suszeptibilitätsloci verantwortlichen Gene zu identifizieren. Zur Zeit werden hierzu die Loci durch die Zucht kongener Linien (Übertragung von Nob1 auf NZB sowie von Nidd/SJL auf C57BL) weiter eingegrenzt. Informationen zur Funktion der varianten Gene sollen durch Untersuchung der Expression (Gewebsverteilung, Unterschiede zwischen NZO und SJL) der in den kritischen Regionen liegenden Gene gewonnen werden. Zudem versuchen wir, die Varianten durch Sequenzierung von Kandidatengenen zu identifizieren.

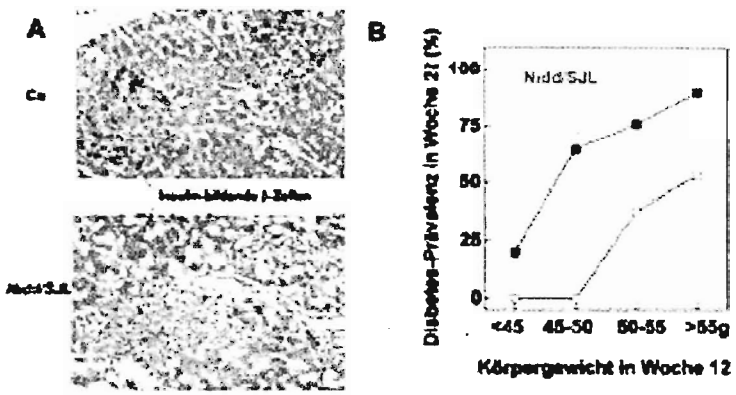


Abbildung 2
Charakterisierung des diabetogenen QTL (Nidd/SJL. A) Einfluss des diabetogenen Allels Nidd/SJL auf die Inselmorphologie von NZO x F1 (SJL x NZO)-Rückkreuzungstieren. Die Insulin-produzierenden β-Zellen des Pankreas (braune Färbung) normoglykämischer (Co) und diabetischer (Nidd/SJL)-Mäuse wurden mit Hilfe eines Antikörpers gegen Insulin nachgewiesen. B) Interaktion des diabetogenen Allels Nidd/SJL mit früher Adipositas. Rückkreuzungstiere (NZO x F1 (SJL x NZO)), die das Nidd/SJL-Allel tragen, zeigen bei einem 12-Wochen Gewicht > 45 g eine höhere Diabetesprävalenz als Nicht-Genträger.

Figure 2:
Characterization of the diabetes QTL (Nidd/SJL. A) Effect of the diabetogenic allele Nidd/SJL on islet morphology in NZO x F1 (SJL x NZO) backcross animals. The insulin-producing pancreatic β- cells (brown staining) of normoglycaemic (Co) and diabetic (Nidd/SJL) mice were visualized by staining with an antibody against insulin. B) The Nidd/SJL allele enhances the effect of early obesity on the prevalence of diabetes.

Die Rolle gastrointestinaler Botenstoffe bei der Regulation des Körpergewichts: Neue Modellansätze zur Behandlung der Adipositas

Matthias Tschöp, Tamara Castañeda, Hella Jürgens, Waltraud Haaß

Hauptprojektziel der Arbeitsgruppe ist es, durch Modifikation der Wirkung bekannter (GLP-1, GIP, Amylin) und neuentdeckter Botenstoffe (Ghrelin und PYY(3-36)) neue therapeutische Optionen zur Behandlung der Adipositas und ihrer Folgeerkrankungen zu erschliessen. Zunächst sollen die Mechanismen der Wirkung dieser im Magen und/oder Darm gebildeten Botenstoffe auf die Energiebilanz am Tiermodell aufgeklärt werden, bevor über eine Blockierung oder Verstärkung dieser Wirkung die neuroendokrine Steuerung von Appetit, Energieverbrauch und Körperzusammensetzung modifiziert werden konnte.

Adipositas. PYY(3-36), ist ein ebenfalls erst kürzlich entdecktes gastrointestinales Hormon, das über eine hypothalamische Wirkung die Nahrungsaufnahme hemmt und das Körperfett verringert.
Zur Zeit untersuchen wir, welche neuronalen Netzwerke vor allem im Hypothalamus durch die erwähnten Hormone moduliert werden. Wir prüfen zudem, ob eine Blockade der Ghrelinwirkung mittels spezifischer Antikörper oder Rezeptorantagonisten - ebenso wie die Erhöhung der zirkulierenden PYY(3-36) - Spiegel einen effektiven Ansatz für die Therapie der Adipositas und somit auch ein Mittel zur Prävention ihrer Folgeerkrankungen darstellen kann. Zusätzlich wird geprüft, ob eine auf Appetitstimulation und Fettaufbau zielende Therapie, z.B. mit Ghrelin oder PYY-Antagonisten, für die Behandlung von Tumorkachexie oder Magersucht eingesetzt werden kann.

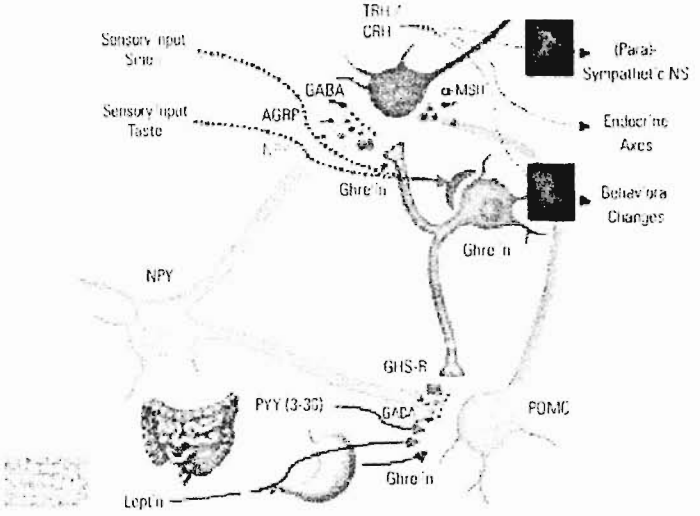


Abbildung 3
Ein besseres Verständnis der Signalwege, die Appetit, Energieverbrauch und -reserven (Körperfettmasse) regulieren, könnte, so hofft man, die Basis für die Entwicklung neuer Therapeutika z.B. gegen Adipositas und Diabetes Typ 2 sein. Kerngebiete im Hypothalamus und Hirnstamm erhalten aus der Peripherie Informationen zur Körperfettmasse, um den Organismus an sich ändernde Umweltbedingungen anzupassen. Die Energiehomöostase soll erhalten und letztlich auch ein einmal erreichter Körperfettmassen-'Stellwert' gehalten werden. Dabei scheint im Magen produziertes, im Blutkreislauf zirkulierendes Ghrelin gemeinsam mit den Sättigungsfaktoren Peptid YY (PYY) und Leptin dem Gehirn mitzuteilen, wann akute oder chronische Energiereserven notwendig werden oder verbraucht werden können. Im Zentralnervensystem produziertes Ghrelin hingegen scheint für die Feinmodulation der Wirkung von Hunger induzierenden Neuropeptiden (wie z.B. Agouti-Related Protein (AGRP), oder Neuropeptid Y (NPY)) und sättigungsfördernden Neuropeptiden (wie z.B. Cocain-Amphetamin regulated Transcript (CART) oder Proopiomelanocortin (POMC)) verantwortlich zu sein.

Ghrelin ist ein kürzlich entdecktes gastrointestinales Peptidhormon, das hypothalamische Steuerungssysteme über den akuten Status der Energieaufnahme informiert. Die Ghrelinexpression und -sekretion werden proportional zum Energiebedarf reguliert. Die chronische exogene Zufuhr von Ghrelin führt über eine erhöhte Nahrungsaufnahme und eine verminderte Fettoxidation zur

Transport von Nährstoffen: Molekulare Grundlagen und hormonelle Regulation

Annette Schürmann, Hadi Al-Hasani, Andrea Scheepers, Jörg Mascher

Nährstoffe müssen nach Aufnahme in den Organismus mehrere jeweils von Zellmembranen abgetrennte Kompartimente durch-

The role of gastrointestinal peptides for the regulation of the body weight: New targets for the treatment of the obesity

The aim of this project is to modify the effects of known (GLP 1, GIP, amylin) and recently discovered hormones (ghrelin, PYY(3 36)) in order to identify new therapeutic options for the treatment of obesity and its co morbidity. The effects of the hormones on the energy balance are analyzed in animal models, and agonists or antagonists of their receptors are employed in order to modify the neuroendocrine control of appetite, energy consumption and body composition. The recently discovered gastrointestinal peptide hormone ghrelin is a crucial regulator of energy homeostasis. Expression and secretion of ghrelin mirror the energy demand of the organism. Chronic treatment with ghrelin increases food intake, decreases fat oxidation and leads to obesity. PYY(3 36) is a second recently discovered gastrointestinal hormone which reduces food intake and body fat mass through hypothalamic effects. At present, we investigate the neuronal networks of the hypothalamus that are modulated by the indicated hormones. In addition, we are trying to inhibit the effects of ghrelin with specific antibodies or receptor antagonists - and also by application of PYY(3-36) - as an option for the therapy of obesity. In addition, we will test whether a therapy with ghrelin or antagonists of PYY can stimulate appetite and increase body fat in patients with tumor cachexia or anorexia nervosa.

Transport of nutrients: Molecular basis and hormonal regulation

After absorption, all nutrients have to permeate several cell membrane barriers between different compartments within the body in order to be utilized in target tissues. This process requires specific membrane proteins that catalyze vectorial transport of substrates across membranes

Figure 3:
It is hoped that a better understanding of the signaling networks that regulate appetite, energy expenditure, and energy reserves (body fat) could be the basis for development of new therapeutics to deal with obesity and type-2 diabetes, for example. From the periphery, core areas of the hypothalamus and brain stem receive information on body fat status, thus enabling the organism to adapt to changing environmental conditions. Energy homeostasis should be maintained and also, in the end, a given body-fat "setting," once it has been reached. Apparently the ghrelin that is secreted gastrically and circulates in the blood-stream, together with the satiety factors peptide YY (PYY) and leptin, informs the brain whenever acute or chronic energy reserves are required or can be used. On the other hand, the ghrelin produced in the central nervous system apparently is responsible for the fine modulation of hunger-inducing neuropeptides (e.g., agouti-related protein, AGRP, or neuropeptide Y, NPY) and satiety neuropeptides (e.g., cocain-amphetamine regulated transcript, CART, or pro-opiomelanocortin, POMC).

Glucose is an important nutrient whose supply, distribution and metabolism is crucial for whole body energy balance. Glucose is generated mainly by cleavage of di- and polysaccharides within the small intestine. It is subsequently transported into the mucosa cell by sodium dependent glucose transporters (SGLT). Export of glucose into the serum and uptake by liver and peripheral tissues are catalyzed by glucose transporters of the GLUT family. The uptake of glucose into adipose tissue and muscle is controlled by insulin.
Insulin induces the translocation of GLUT4 glucose transporters from intracellular compartments to the plasma membrane, resulting in an increased rate of cellular glucose uptake. Because obesity associated insulin resistance impairs the insulin mediated glucose uptake, the Department of Pharmacology investigates the proteins that are involved in the mechanism of GLUT4 translocation.

Recently, we have cloned several novel members of the GLUT family that are currently under investigation of their specific biological functions. GLUT8, a high affinity glucose transporter (Km = 2 mM) is expressed in spermatozoa as well as in insulin-sensitive tissues (differentiation-dependent in fat cells). The transporter contains an N-terminal di-leucine motif, which is responsible for retention of the protein in an intracellular compartment. Mutation of this motif to di-alanine or co-expression of a dominant-negative dynamin mutant leads to constitutive expression of GLUT8 on the cell surface. However, the physiological stimulus that induces translocation of GLUT8 is at present unknown.

GLUT11, a low affinity glucose transporter, is expressed in heart, skeletal muscle, pancreas, kidney, and placenta. Alternative splicing of its gene *(SLC2A11)* generates three isoforms that differ in their N-terminal sequences and may have tissue-specific functions. Our objective is to study the role of GLUT8 and GLUT11 in glucose homeostasis in vivo. Therefore, we will generate mice with targeted disruption of the genes in fat and muscle, and will analyze the phenotype of these so-called knockout-mutants.

Figure 4:
Model of glucose transporter trafficking in insulin-sensitive cells. Binding of insulin to its receptor induces a signaling cascade, which finally leads to the translocation of GLUT4 vesicles to the plasma membrane. In a dynamin-dependent step, GLUT4 vesicles fuse with the membrane, thereby increasing glucose uptake. The di-leucine motif in the N-terminus of GLUT8 retains this transporter in intracellular compartments. Mutation of this motif (GLUT8-LL/AA), or co-expression of GLUT8 with an inhibitory mutant of dynamin (Dyn-K44A), translocates GLUT8 to the plasma membrane. The endogenous stimulus which induces translocation of GLUT8 is still not known. (IR = insulin receptor substrates; PI 3-Kinase = phospho-inositide 3-kinase; PDK1 = phospho-inositide dependent kinase; PKB/Akt = protein kinase B).

queren, um in den verschiedenen Geweben genutzt werden zu können. Hierzu ist ein gerichteter Transport durch die Membranen erforderlich, der von spezifischen Membranproteinen katalysiert wird. Ein wichtiger Nahrstoff, dessen Zufuhr, Verteilung und Metabolismus die Energiebilanz des Organismus maßgeblich bestimmt, ist Glucose. Glucose entsteht hauptsächlich durch Spaltung von Di- und Polysacchariden im Dünndarm, wird durch den Natriumabhängigen Glucosetransporter (SGLT = sodium dependent glucose transporter) in die Mukosazelle, anschliessend durch Mitglieder der GLUT-Familie in das Serum und vom Serum in die Zellen der verschiedenen Gewebe transportiert. Die Aufnahme von Glucose in Fettgewebe und Muskel steht unter der Kontrolle von Insulin. Insulin bewirkt eine Erhöhung der Zahl von Glucosetransportern GLUT4 in der Plasmamembran durch Translokation intrazellulär gespeicherter Transporterproteine; dadurch steigt die Geschwindigkeit, mit der Glucose in das Zellinnere transportiert wird, an. Bei der zusammen mit Adipositas auftretenden Insulinresistenz sind diese Mechanismen gestört. In der Abteilung Pharmakologie wird deshalb untersucht, welche Proteine die Translokation des GLUT4 katalysieren, und über welche Strukturmerkmale sie den Glucosetransporter erkennen. Kürzlich gelang es uns zudem, mehrere neue Mitglieder der GLUT-Familie zu klonieren, die genaue Funktion dieser Glucosetransporter wird zur Zeit weiter aufgeklärt. GLUT8 transportiert Glucose mit hoher Affinität (km = 2 mM) und wird in Spermatozoen, aber auch in insulinempfindlichen Geweben (differenzierungsabhängig in Fettzellen) exprimiert. Das Protein besitzt ein Di-Leucin-Motiv im N-Terminus, das für eine intrazelluläre Retention des Proteins verantwortlich ist. Wird dieses Motiv gegen Di-Alanin ausgetauscht oder GLUT8 mit einer dominant negativen Dynamin-Mutante coexprimiert, lässt sich GLUT8 an der Zelloberfläche nachweisen. Bislang ist allerdings der physiologische Stimulus, der eine Translokation von GLUT8 induziert, nicht bekannt. GLUT11 besitzt nur eine geringe Affinität zu Glucose und wird im Herzen und Skelettmuskel sowie in Pankreas, Niere und Plazenta exprimiert. Durch alternatives Spleißen des GLUT11-Gens *(SLC2A11)* entstehen drei Isoformen, die sich nur in ihrem N-Terminus unterscheiden und möglicherweise eine gewebsspezifische Rolle spielen. Ziel unserer Arbeitsgruppe ist es nun, die Rolle von GLUT8 und GLUT11 für die Glucose-Homöostase in vivo zu untersuchen. Dazu sollen die Gene der Transporter spezifisch im Muskel und im Fettgewebe der Maus ausgeschaltet und der Phänotyp dieser sog. knockout-Mutanten analysiert werden.

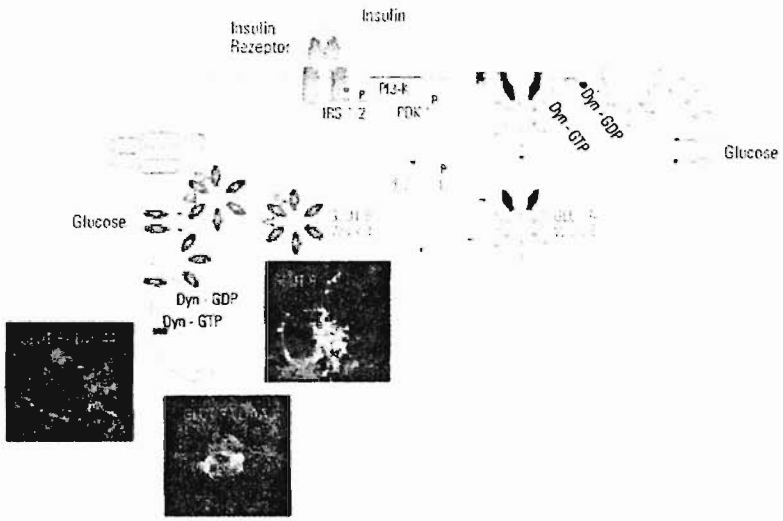


Abbildung 4
Modell der Bewegung von Glucosetransportern in insulinempfindlichen Zellen. Die Bindung von Insulin an den Insulinrezeptor induziert eine Signalkaskade, die schließlich u. a. die Translokation von GLUT4-Vesikeln zur Plasmamembran induziert. In einem Dynamin-abhängigen Schritt verschmelzen die GLUT4-Vesikel mit der Membran, so dass die Glucoseaufnahme ansteigt. Das Di-Leucin-Motiv im N-Terminus des GLUT8 hält diesen Transporter in intrazellulären Kompartimenten. Wird dieses Motiv mutiert (GLUT8-LL/AA), oder GLUT8 mit einer inhibitorischen Dynamin-Mutante (Dyn-K44A) co-exprimiert, lässt sich GLUT8 in der Plasmamembran nachweisen. Der endogene Stimulus, der die Translokation von GLUT8 induziert, ist noch unbekannt. (IRS = insulin receptor substrate; PI3-Kinase = phospho-inositide-3-kinase; PDK1 = phospho-inositide-dependent kinase; PKB/Akt = protein kinase B).

Ausgewählte Publikationen
Selected Publications

Originalarbeiten

Al-Hasani, A., Kunamneni, R. K., Dawson, K., Hinck, C. S., Müller-Wieland, D., Cushman, S. W.: Roles of the N- and C-termini of GLUT4 in endocytosis. J. Cell Sci. 115, 131-140 (2002)

Bahrenberg, G., Behrmann, I., Barthel, A., Hekerman, P., Heinrich, P. C., Joost, H.-G., Becker, W.: Identification of the critical sequence elements in the cytoplasmic domain of leptin receptor isoforms required for janus kinase/signal transducer and activator of transcription activation by receptor heterodimers. Mol. Endocrinol. 16, 859-872 (2002)

Barthel, A., Kruger, K. D., Roth, R. A., Joost, H.-G.: Concentration-dependent stimulatory and inhibitory effect of troglitazone on insulin-induced fatty acid synthase expression and protein kinase B activity in 3T3-L1 adipocytes. N-S Arch. Pharmacol. 365, 290-295 (2002)

Barthel, A., Schmoll, D., Kruger, K. D., Roth, R. A., Joost, H.-G.: Regulation of the forkhead transcription factor FKHR (FOXO1a) by glucose starvation and AICAR, an activator of AMP-activated protein kinase. Endocrinology 143, 3183-3186 (2002)

Mueller, A. G., Moser, M., Kluge, R., Leder, S., Blum, M., Büttner, R., Joost, H.-G., Schürmann, A.: Embryonic lethality caused by apoptosis during gastrulation in mice lacking the gene of ARF-related protein 1 (ARFRP1). Mol. Cell. Biol. 22, 1488-1494 (2002)

Ortlepp, J. R., Breuer, J., Eitner, F., Kluge, K., Kluge, R., Floege, J., Hollweg, G., Hanrath, P., Joost, H.-G.: Inhibition of the renin-angiotensin system ameliorates genetically determined hyperinsulinemia. Eur. J. Pharmacol. 436, 145-150 (2002).

Pass, G. J., Becker, W., Kluge, R., Linnartz, K., Plum, L., Giesen, K., Joost, H.-G.: Effect of hyperinsulinemia and type 2 diabetes-like hyperglycemia on expression of hepatic cytochrome p450 and glutathione S-transferase isoforms in a New Zealand obese-derived mouse backcross population. J. Pharmacol. Exp. Ther. 302, 442-450 (2002).

Plum, L., Giesen, K., Kluge, R., Junger, E., Linnartz, K., Schürmann, A., Becker, W., Joost, H.-G.: Characterisation of the mouse diabetes susceptibilty locus Nidd/SJL islet cell destruction, interaction with the obesity QTL Nob1, and effect of dietary fat. Diabetologia 45, 823-830 (2002).

Schürmann, A., Axer, H., Scheepers, A., Doege, H., Joost, H.-G.: The glucose transport facilitator GLUT8 is predominantly associated with the acrosomal region of mature spermatozoa. Cell Tissue Res. 307, 237-242 (2002)

Schurmann, A., Koling, S., Jacobs, S., Saftig, P., Krauß, S., Wennemuth, G., Kluge, R., Joost, H.-G.: Reduced sperm count and normal fertility in male mice with targeted disruption of the ADP-ribosylation factor like (Arl4) gene. Mol. Cell. Biol. 22, 2751-2768 (2002).

Tschöp, M., Flora, D. B., Mayer, J. P., Heiman, M. L.: Hypophysectomy prevents ghrelin-induced adiposity and increases gastric ghrelin secretion in rats. Obes. Res. 10, 991-999 (2002)

Tschöp, M., Morrison, K.: Weight loss at high altitude. Adv. Exp. Med. Biol. 502, 237-49 (2002)

Tschöp, M., Statnick, M., Suter, T., Heiman, M. L.: Growth hormone-releasing peptide-2 (GHRP-2) increases fat mass in mice lacking neuropeptide Y (NPY): Indication for a crucial mediating role of hypothalamic Agouti-related protein (AGRP). Endocrinology 143, 558-568 (2002)

Übersichtsarbeiten

Joost, H.-G., Bell, G. I., Best, J. D., Birnbaum, M. J., Charron, M. J., Chen, Y. T., Doege, H., James, D. E., Lodish, H. F., Moley, K. H., Moley, J. F., Mueckler, M., Rogers, S., Schürmann, A., Seino, S., Thorens, B.: Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators. Am. J. Physiol. Endocrinol. Metab. 282, E974-E976 (2002)

Buchbeiträge

Joost, H.-G.: Metabolisches Syndrom und Typ-2-Diabetes: Interaktion von Genen und Lebensbedingungen. In: Einfluss von Genomprojekt und Pharmacogenetics auf die medizinische Entwicklung. Eds. A. Rosenthal, U. Wahn, und H. Wolf. Schattauer Verlag (Stuttgart, New York), pp. 69-87 (2002)

Drittmittelprojekte
External Funding

DFG Forschergruppe 441
Mechanismen der normalen und gestörten Insulinwirkung
(Sprecher: Prof. Dr. Dr. Hans Georg Joost)
Teilprojekt 1: Insulinresistenz und Typ 2 Diabetes. Identifikation von Suszeptibilitätsgenen in einem Mausmodell
Laufzeit: 07/01-06/04
Teilprojekt 2: Rolle der Glucosetransporter GLUT8 und GLUT1 im normalen und gestörten Glucosestoffwechsel
Finanzierung: DFG
Laufzeit: 07/01-06/04

Longterm effects of ghrelin and its receptor agonists on energy balance
Finanzierung: Eli Lilly International Foundation
Laufzeit: 07/02-06/03

Rolle der Ras ähnlichen GTPase ARF related protein 1 (ARFRP1) in Differenzierung und Morphogenese
Finanzierung: DFG
Laufzeit: 01/03-12/04

Spiegelmers and obesity
Finanzierung: NOXXON Pharma AG
Laufzeit: 11/02-10/03

Die Abteilung Pharmakologie wurde mit Dienstantritt des wissenschaftlichen Direktors im Januar 2002 zunächst als Arbeitsgruppe eingerichtet. Die hier dargestellten Forschungsergebnisse stammen deshalb z.T. noch aus der Arbeit der Gruppe am Institut für Pharmakologie und Toxikologie der RWTH Aachen. M. Tschöp schloss sich der Abteilung im Mai 2002 an; die von ihm beschriebenen Ergebnisse sind deshalb z.T. am Forschungszentrum der Fa. Eli Lilly in Indianapolis, USA, entstanden.

The Department of Pharmacology was established with the inauguration of Prof. Joost as scientific director in January 2002. Therefore, parts of the results presented here were obtained at the previous affiliation of the group (Institute of Pharmacology and Toxicology, RWTH Aachen). M. Tschöp joined the department in May 2002; the results described by him were in part obtained at the research center of Eli Lilly, Indianapolis, USA.

Leitung: Prof. Dr. Susanne Klaus

Laboratory of Physiology of Energy Metabolism

Head: Prof. Dr. Susanne Klaus

The macronutrients in food, i.e., proteins, carbohydrates, and fats, serve as fuel and as organic building material for humans. The energy metabolism of humans, i.e., the integration of energy provision and energy expenditure, is of great importance for body composition. In adipose tissue, the adipocytes store energy and, moreover, play a regulative role. In the Laboratory of Physiology of Energy Metabolism, research centers around the mechanisms for regulation of body weight and body composition. The etiopathogenesis of obesity within the framework of the metabolic syndrome is to be unrevealed at molecular, cellular, and biochemical levels, including the role played by macronutrients. Basic research into the development of obesity will be carried out by investigations into the development and function of adipose tissue.

Impact of perinatal nutrition on energy metabolism and body composition in later life

Nutrition during critical phases of early development influences the development of different organ systems and can therefore lead to permanent metabolic changes in later life. There is some evidence that this so called 'nutritional programming' during fetal development might be involved in the increasing prevalence of obesity in all industrial countries. Interestingly, both malnutrition of the mother during the last trimester of pregnancy, and increased nutrient intake of the offspring during nursing can increase the risk of obesity. There is evidence that, in particular, a large and rapid increase in body weight during the early postnatal period is a risk factor for developing obesity. In order to elucidate possible physiological mechanisms, we studied the impact of perinatal nutrition on development of energy metabolism and body composition in two different rat models. As outlined below, our data provide evidence that the maternal diet in the prenatal period, specifically a high-protein diet during gestation, rather than the postnatal diet of the offspring while being nursed, leads to development of obesity in later life.

In the rat model, it has been shown that nutritional programming by low maternal protein intake during gestation results in intra-uterine growth restriction followed by metabolic disturbances, which in turn can lead to hypertension and insulin resistance. Although there is some epidemiological evidence that high protein intake during early development might also influence the later development of adiposity, direct experimental evidence is still lacking. Therefore, we examined whether high dietary-protein exposure in utero and/or during postnatal life affects adiposity (Fig.1).

Figure 1:
Measurment of energy expenditure in mice using indirect calorimetry

Proteine, Kohlenhydrate und Fette, die Makronährstoffe aus der Nahrung, sind für den Menschen Brennstoff und organischer Baustoff. Der Energiestoffwechsel des Menschen, d. h. die Integration von Energiezufuhr und Energieverbrauch, ist für die Körperzusammensetzung von großer Bedeutung. Adipozyten im Fettgewebe dienen als Energiespeicher, haben darüber hinaus aber auch regulierende Funktionen.

In der Arbeitsgruppe Physiologie des Energiestoffwechsels liegt der Forschungsschwerpunkt auf Regulationsmechanismen für das Körpergewicht und die Körperzusammensetzung. Auf molekularer, zellularer und biochemischer Ebene soll die Ätiopathogenese von Adipositas im Rahmen des Metabolischen Syndroms aufgeklärt werden und die Rolle der Makronährstoffe dabei. Durch Untersuchungen zur Entwicklung und Funktion von Fettgewebe und Fettzellen wird Grundlagenforschung zur Entstehung von Adipositas betrieben.

beeinflussen. Man nimmt an, dass dieser "Programmierungs-Effekt" auch eine Rolle bei der weltweit zunehmenden Adipositas spielt. So kann z. B. sowohl eine Mangelernährung der Mutter während des letzten Schwangerschafts-Drittels als auch eine erhöhte Nahrungszufuhr während der Stillperiode das Adipositas-Risiko der Nachkommen erhöhen. Vieles deutet darauf hin, dass insbesondere eine sehr schnelle und große Gewichtszunahme in den ersten Monaten nach der Geburt kritisch ist. Um die zugrunde liegenden physiologischen Regulationsmechanismen zu entschlüsseln, haben wir an zwei verschiedenen Rattenmodellen den Einfluss der perinatalen Ernährung auf die Entwicklung des Energiestoffwechsels im späteren Leben untersucht. Unsere Ergebnisse weisen darauf hin, dass die pränatale Ernährung eine größere Rolle spielt als die postnatale Ernährung, und dass speziell eine Hochprotein-Diät während der Gestation das Adipositas-Risiko erhöhen kann.

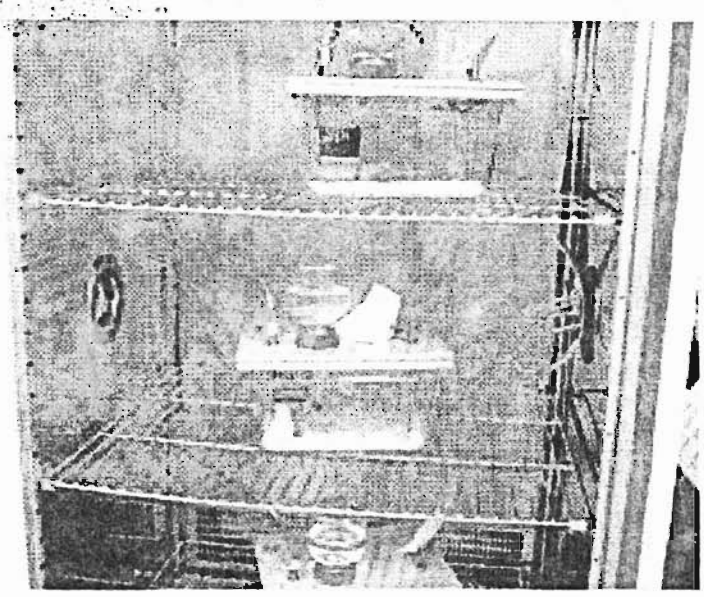


Abbildung 1
Messung des Energieumsatzes von Mäusen in vivo durch indirekte Kalorimetrie.

Einfluss perinataler Ernährung auf Adipositas und Energiestoffwechsel

Maren Daenzer, Cornelia C. Metges, Sylvia Ortmann, Klaus-Jürgen Petzke, Petra Wiedmer

Die Ernährung während kritischer Entwicklungsphasen z. B. der Fötalentwicklung oder auch der frühen postnatalen Entwicklung hat einen Einfluss auf die Entwicklung verschiedener Organsysteme und kann so dauerhaft den Stoffwechsel im späteren Leben

Ein etabliertes Tiermodell des "Nutritional Programming" ist eine maternale Proteinrestriktion während der Gestation, die in einer intrauterinen Wachstumsretardierung resultiert. Nachfolgend entwickeln sich im erwachsenen Tier metabolische Störungen wie Bluthochdruck und Insulinresistenz. Obwohl es epidemiologische Hinweise darauf gibt, dass auch die erhöhte Zufuhr von Protein im frühen Leben spätere Auswirkung auf z. B. Fettakkumulation hat, gibt es kaum experimentelle

Befunde dazu. Wir haben daher am Rattenmodell untersucht, welche metabolischen Auswirkungen eine erhöhte Proteinzufuhr prä- und/oder postnatal hat (Abb.1). Weibliche Ratten wurden verpaart und entweder mit Hochprotein-Diät (HP = 40 % Protein) oder Normalprotein (AP = 20 % Protein) gefüttert. Die männlichen Nachkommen wurden nach der Geburt von Müttern gesäugt, die entweder HP- oder AP-Diät erhielten. Daraus resultierten 4 Gruppen mit unterschiedlicher Diät prä- und postnatal (AP-AP, AP-HP, HP-AP, HP-HP). Die postnatalen Diäten wurden bis zur 9. Woche beibehalten. Überraschenderweise hatten die Jungen von HP-Müttern ein reduziertes Geburtsgewicht. HP-AP-Ratten zeigten von der 3. bis 6. Woche ein erhöhtes Körpergewicht, im Gegensatz zu HP-HP-Tieren. Die pränatale HP-Exposition resultierte zudem in einem erhöhten Körperfettgehalt und erniedrigtem Energieumsatz (Abb. 2).

sein könnte. Männliche neugeborene Ratten wurden dazu in Würfen von 2 (SL) oder 12 (NL) Tieren aufgezogen. Nach dem Absetzen wurde im Alter von 5, 8 und 12 Wochen die Körperzusammensetzung und der Energieumsatz (TEE) gemessen. SL-Ratten hatten ein erhöhtes Gewicht von Woche 2-5 und wiederum von Woche 10-12. Fettfreie Körpermasse und Körperfett, sowie TEE waren höher bei den SL-Tieren im Alter von 5 Wochen. TEE wurde in allen Altersgruppen bei SL und NL durch dieselbe lineare Korrelation beschrieben. Im Alter von 12 Wochen waren bis auf einen leicht erhöhten Fettgehalt bei SL keine Unterschiede zwischen SL- und NL-Tieren mehr zu finden. Daraus schließen wir, dass die postnatale Überernährung keinen langfristigen Einfluss auf den Energiestoffwechsel im späteren Leben hat, während pränatale Ernährungseinflüsse offensichtlich langerfristige Auswirkungen haben können.

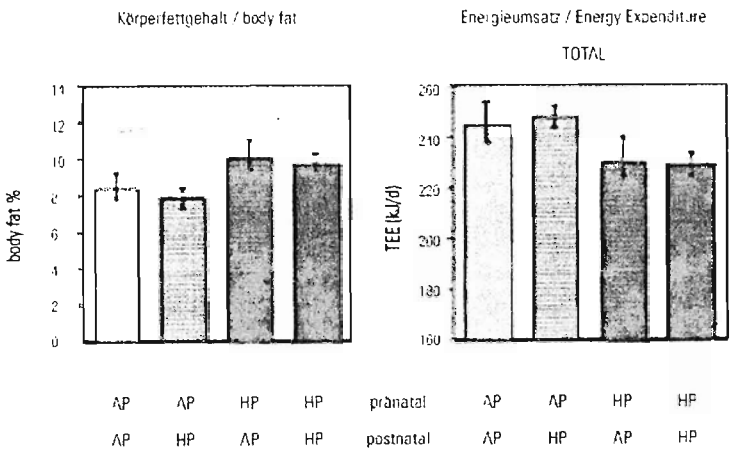


Abbildung 2
Körperfettgehalt und Energieumsatz von 8 Wochen alten Ratten, die prä- und postnatal Hochprotein (HP) oder Normalprotein (AP) erhielten. Eine pränatale Hochproteinzufuhr führt zu einem erhöhten Körperfettanteil und erniedrigtem Energieverbrauch im Alter von 8 Wochen.

Diese Ergebnisse deuten darauf hin, dass eine pränatale Hochprotein-Zufuhr lang andauernde Auswirkungen auf das Körpergewicht und die Energiehomöostase hat. Postnatale Überernährung (PNO), verursacht durch Reduktion der Wurfgröße während der Laktation, führt zu erhöhtem Gewicht und Körperfett. PNO-Ratten dienen als Modell für moderates Übergewicht und Hyperinsulinämie. Wir untersuchten, ob ein verringerter Energieumsatz Ursache des Übergewichts

Fettgewebe als thermogenes und endokrines Organ: Analyse von Genexpression und Fettgewebe-Stoffwechsel in vivo

Frauke Adams, Stéphane Boeuf, Michael Boschmann

In den letzten Jahren hat sich herausgestellt, dass Fettgewebe nicht nur ein passiver Energiespeicher ist, sondern ein Organ, das eine aktive Rolle im Energiestoffwechsel spielt.

Two groups of female rats were mated and pair-fed isocaloric high protein (40% protein, HP) or adequate protein (20% protein; AP) diets throughout pregnancy. The male offspring were suckled by foster mothers pair fed HP or AP diets, resulting in four prenatal/ postnatal groups (AP AP, AP HP, HP AP, HP HP). Subsequently, they were pair fed the same diets their nurses had received during lactation, until 9 weeks of age. Surprisingly, HP offspring had a reduced birth weight. HP AP rats had a higher body weight than AP AP controls in weeks 3 to 6, in contrast to HP HP. Prenatal HP exposure resulted in an increased total and relative fat mass and decreased energy expenditure in week 9 (Fig.2). Postnatal HP alone had no significant effect on body composition or metabolic rate. Therefore, prenatal exposure to a high protein level seems to reprogram body weight and energy homeostasis.

Early postnatal over nutrition (PNO) induced by restricting litter size in rats leads to increased body weight and body fat in later life. PNO rats are used as an animal model of moderate obesity and early hyperinsulinemia. We investigated if the increased adiposity could be due to decreased energy expenditure. Male new born rats were raised in litters of two (SL) or twelve pups (NL), weaned at 4 weeks of age and subsequently fed ad libitum. Body weight (BW) was recorded continuously until 12 weeks of age. Daily energy intake, total daily energy expenditure (TEE), and body composition were measured at 5, 8, and 12 weeks of age. SL rats displayed increased BW compared to NL rats from week 2 to 5 and again from week 10 to 12. Lean body mass, body fat and protein content, and TEE were increased in SL rats at week 5. The same linear correlation described the relationship between BW and TEE in NL and SL rats. At week 8 and 12 no differences in energy metabolism could be found, but total fat content was higher in SL rats at week 12. Energy balance, i. e. assimilated energy minus energy expenditure, did not differ in SL and NL at any time measured. We conclude that although PNO rats display increased adiposity in early life, energy metabolism in later life does not seem to be affected significantly.

Adipose tissue as thermogenic and endocrine organ: analyse of gene expression and adipose tissue metabolism in vivo

White adipose tissue (WAT) is not only a passive energy-storing organ, but it contributes actively to regulation of energy metabolism by secreting numerous hormones and signal molecules. In addition to the well-known WAT, there is a second

Figure 2:
Body fat and energy expenditure in 8-week-old rats exposed to different levels of dietary protein prenatally or postnatally. Data are means (SEM) Prenatal high-protein (HP) exposure resulted in increased proportion of body fat and decreased energy expenditure at 8 weeks of age. AP, normal protein level

...ue of adipose tissue, i.e., brown adipose tissue (BAT), which is the source of thermogenesis in small and in newborn mammals. Its function is therefore the opposite of WAT, i.e., energy expenditure instead of energy storage. Using different methodological approaches, we examined the impact of age on gene expression and tried to identify genes involved in the differentiation of brown preadipocytes.

The adipose tissue renin angiotensin system has been implicated in the regulation of adipocyte growth and differentiation. We studied the influence of age, body weight, body fat and its anatomical localization, and diet on the expression of angiotensinogen (AGT) and angiotensin II type 1 (AT1) receptor genes in epididymal WAT and liver of control and PNO rats (see above). Gene expression was measured at the age of 4, 8, and 12 weeks by real time RT PCR. At the age of 12 weeks, AGT expression was significantly decreased in both tissues. Furthermore, expression of the AT1 receptor gene was significantly decreased in liver but not in WAT at 12 weeks of age. Postnatal overfeeding had no effect. At the age of 24 weeks, AGT expression was significantly greater in epididymal than in subcutaneous adipose tissue, whereas no site specific differences could be found for the AT1 receptor. We conclude that age and depot-specific mechanisms are of more importance for the expression of AGT and AT1-receptor genes during the first 12 weeks of age than a short period of overfeeding.
BAT thermogenesis can prevent the development of obesity in certain rodent models; however, the adult human has only very little BAT. The recruitment of BAT precursor cells could therefore be envisaged as a means of fighting human obesity. So far, not much is known about the genetic control of the differentiation and determination of brown preadipocytes (pAds). We therefore tried to identify differentially expressed genes involved in brown and white pAd development. This study was performed in collaboration with the groups of Dr. Martin Klingenspor (Marburg) and Dr. Jaap Keijer (Wageningen, Holland). Using representational difference analysis and cDNA microarray screening, we identified a total of twelve genes showing differential expression in white and brown pAds (Table 1). The expression profile of these genes was analyzed during preadipocyte differentiation, and in vivo in comparison to references genes. The expression pattern in vivo proved somewhat different from the expression pattern during cell culture. We conclude that brown and white pAds can be distinguished by expression levels of several genes, confirming their differential determination. A possible role of these genes in functional differentiation of brown and white adipocytes needs to be established (Tab.1)

Table 1:
Differently expressed genes in white and brown preadipocytes. The differences are only in the expression of a few genes with structural, metabolic, or unknown functions

z.B. durch die Bildung von einer Reihe von Hormonen und Signalstoffen. Zudem existiert neben dem bekannten weißen Fettgewebe (WAT) auch das braune Fettgewebe (BAT), das bei Kleinsäugern und Neugeborenen Ort der Wärmebildung ist, d.h. im Gegensatz zum WAT nicht Energie speichert, sondern Energie verbraucht. Mit verschiedenen methodischen Ansätzen untersuchten wir die altersabhängige Genexpression im WAT und versuchten Gene zu identifizieren, die bei der Differenzierung von braunen Adipocyten eine Rolle spielen. Das Renin-Angiotensin-System im WAT ist involviert in Adipocyten-Wachstum und Differenzierung. Wir haben den Einfluss von Alter, Gewicht, Körperfett, und Diät auf die Genexpression von Angiotensinogen (AGT) und Angiotensin-II-type-1-Rezeptor (AT1-R) im epididymalen WAT und der Leber von normalen und PNO-Ratten (s. o.) im Alter von 4, 8 und 12 Wochen untersucht.

Thermogenese im braunen Fettgewebe kann die Entwicklung von Übergewicht bei verschiedenen Tiermodellen verhindern. Der erwachsene Mensch hat nur wenig BAT übrig, eine mögliche Neurekrutierung könnte jedoch ein Weg sein, um Übergewicht zu bekämpfen. Bisher ist wenig bekannt über die genetische Kontrolle der Differenzierung und Determinierung von braunen Fettzellen. Wir hatten daher zum Ziel, Gene zu identifizieren, die dabei relevant sind. Dieses Projekt wurde in Zusammenarbeit mit Dr. Martin Klingenspor (Marburg) und Dr. Jaap Keijer (Wageningen, Holland) durchgeführt. Durch die Anwendung der Methode der "representational difference analysis" kombiniert mit einem Screening auf cDNA-Microarrays konnten wir insgesamt 12 Gene identifizieren, die unterschiedlich in weißen und braunen Präadipocyten exprimiert werden (Tab. 1)

Function	expression W/B	gene
complement	8.9	complement factor B
	3.5	complement component C3
	3.9	complement component C2
metabolism	3.9	delta-6-fatty-acid desaturase (FADSD6)
Function	**expression B/W**	**gene**
structure	2.9	fibronectin
	2.3	alpha actinin 4 (Actn4)
	2.1	metagridin (MDC15 gene)
transcription	2.4	Necdin
	2.2	Vigilin
	2.1	small nuclear ribonucleoprotein polypeptide A (Snrpa)
unknown	2.2	hepatocellular carcinoma associated protein (HCAP)
	2.0	new (B26)

Genes with a higher expression in white (expression W/B) and brown preadipocytes (expression B/W), respectively.

Tabelle 1
Unterschiedliche Genexpression in weissen und braunen Präadipozyten: Sie unterscheiden sich nur in der Expression von wenigen Genen, die strukturelle, metabolische oder noch weitgehend unbekannte Funktion haben.

Mit 12 Wochen war die AGT-Expression in beiden Geweben verringert. Die AT1-R-Expression mit 12 Wochen war nur in der Leber verringert. Postnatale Überernährung hatte keinerlei Einfluss. Im Alter von 24 Wochen war die AGT-Expression größer im epididymalen als im subkutanen WAT. Wir schließen daraus, dass Alter und Lokalisation des Fettgewebes eine größere Rolle für die Genexpression von AGT und AT1-R spielen als eine kurze Periode der Überernährung.

Zusätzlich analysierten wir die Expressions-Profile dieser Gene während der Adipocyten-Differenzierung im Vergleich zur Expression im weißen und braunen Fettgewebe in vivo. Die Expressionsmuster in Zellkulturen waren leicht verschieden von denen in vivo. Es zeigte sich, dass weiße und braune Preadipocyten durch die Expression bestimmter Gene unterschieden werden können, wobei die exakte Rolle dieser Gene im Fettgewebe noch etabliert werden muss (Tab.1)

Norepinephrin-Transporter-Funktion und autonome Stoffwechselkontrolle

Genetische Variabilität und zahlreiche Medikamente beeinflussen die Funktion des Noradrenalin-Transporters (NET), wobei die Konsequenzen einer NET-Inhibierung für den Metabolismus noch weitgehend unverstanden sind. Wir haben einen Versuch an 15 Gesunden durchgeführt, die entweder 8 mg des selektiven NET-Inhibitors Reboxetin oder Placebo erhielten. Energieumsatz (EE) und Substratoxidation (SO) wurden vor und während intravenöser Isoproterenol(Iso)-Infusion mittels indirekter Kalorimetrie bestimmt. Der WAT-Metabolismus wurde durch eine Microdialyse vor und während lokaler Isoproterenol-Perfusion untersucht. Basaler EE und SO unter Reboxetin und Placebo unterschieden sich nicht.

Bei 1 µg/min Iso war der EE in Männern und Frauen sowohl unter Reboxetin als auch mit Placebo erhöht. Die Kohlenhydratoxidation war unter Reboxetin erhöht. Basaler und iso-stimulierter Blutfluss im Fettgewebe war unter Reboxetin etwa 2fach erhöht. Zudem war der iso-stimulierte Glucosestoffwechsel und die Lipidmobilisation unter Reboxetin konsistent erhöht.

Wir schließen daraus, dass eine akute NET-Inhibierung die Glucoseaufnahme und -oxidation im Fettgewebe erhöht. Während die Fettmobilisation erhöht wird, ist die ß-adrenerg stimulierte Lipidoxidation erniedrigt. Dieser Effekt kann nicht durch erhöhte systemische oder lokale Noradrenalin-Konzentrationen erklärt werden, sondern wahrscheinlich durch eine Sensitivierung des Fettgewebes gegenüber β-adrenerger Stimulation.

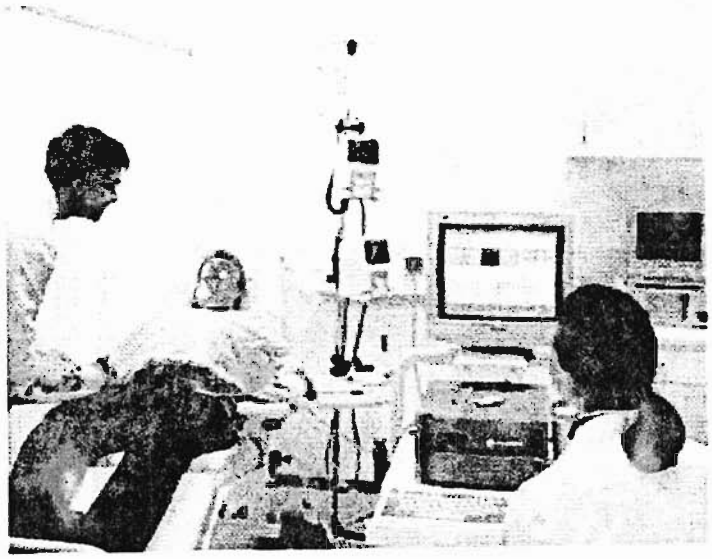


Abbildung 3
Energieumsatzmessung am Menschen

Norepinephrine transporter function and autonomic control of metabolism

Genetic variability, numerous medications, and some illicit drugs influence norepinephrine transporter (NET) function; however, the metabolic consequences of NET inhibition are poorly understood. We performed a randomized, double blind, cross over trial in 15 healthy subjects who ingested 8 mg of the selective NET inhibitor reboxetine or placebo (Fig 3). Energy expenditure (EE) and substrate oxidation rates (SO) were determined by indirect calorimetry before and during intravenous infusion of isoproterenol (iso). Adipose tissue metabolism was studied by microdialysis before and during local isoperfusion. At rest, EE and SO did not differ between reboxetine and placebo treatment. At 1 µg/min iso, EE was significantly increased in men (+15%) and women (+20%) with both reboxetine and placebo treatment. However, carbohydrate oxidation rate was significantly higher with reboxetine only. Baseline and iso-stimulated adipose tissue blood flow was increased approximately 2-fold with reboxetine. Furthermore, glucose supply and metabolism as well as lipid mobilization were consistently stimulated in adipose tissue under reboxetine compared to placebo. We conclude that acute NET inhibition increases glucose uptake and metabolism in adipose tissue. While lipid mobilization is increased, overall lipid oxidation is decreased during beta adrenergic stimulation. This effect cannot be explained by increased systemic or adipose tissue norepinephrine concentrations. Instead, NET inhibition may sensitize adipose tissue to beta-adrenergic stimulation.

Figure 3:
Measurement of energy expenditure in humans.

Drittmittelprojekte
External Funding

Titel: Einfluss einer Hochproteindiät unterschiedlicher Proteinqualität während der Schwangerschaft auf die Entwicklung der Föten Untersuchungen am Rattenmodell
Finanzierung: Institut Danone für Ernährung
Laufzeit: 2/02 1/04

Titel: Analytische Bearbeitung von Mikrodialyse-Proben
Finanzierung: Medipart Biotechnik
Laufzeit: 2002

Titel: Pharmakogenetik und Physiologie des beta-2 Rezeptors beim Menschen
Finanzierung: DFG
Laufzeit: 01/01 12/02

Titel: Mikrobielle Lysinsynthese und -absorption im monogastrischen Tiermodell (Schwein)
Finanzierung: DFG
Laufzeit: 8/98 10/01

Titel: Stabilisotopenmessungen Aminosäurekatabolismus und Fibrinogensynthese bei Insulinresistenz der Leber - Effekte nichtveresterter Fettsäuren"
Finanzierung: DFG
Laufzeit: 01/01 12/01

Titel: Molekulare Grundlagen zur funktionellen Differenzierung brauner und weißer Adipocyten
Finanzierung: DFG
Laufzeit: 04/00 06/02

Titel: Short term Bedrest Study, Integrative Physiology
Finanzierung: BMBF
Laufzeit: 01/00 06/03

Titel: Bestimmung von Proteinverdaulichkeit und Proteinanteil energiereicher Diät Nahrung
Finanzierung: Almased
Laufzeit: 09/00 05/01

Titel: Wirkung potentieller Anorektika auf Parameter des Energiehaushaltes bei der Ratte
Finanzierung: Aventis
Laufzeit: 06/99 08/01

Ausgewählte Publikationen
Selected Publications

Originalarbeiten/Original Papers

Adams, F., Wiedmer, P., Gorzelniak K., Engeli, S., Klaus S., Boschmann M.: Age-related changes of renin-angiotensin system genes in white adipose tissue of rats. Horm. Metab. Res. 34, 715-720 (2002)

Bockes, G., Hennig, U., Petzke, K. J., Elsner, A., Junghans, P., Nürnberg, G., Metges, C. C.: Contribution of intestinal microbial lysine to lysine homeostasis is reduced in minipigs fed a wheat gluten-based diet. Am. J. Clin. Nutr. 76, 1317-1325 (2002)

Boeuf, S., Keijer, J., Franssen-van Hal, N. L. W., Klaus, S.: Individual variation of adipose gene expression and identification of covariated genes by cDNA microarrays. Physiol. Genomics 11, 31-36 (2002)

Boeuf, S., Klingenspor, M., van Hal, N. L. W., Schneider, T., Keijer, J., Klaus, S.: Differential gene expression in white and brown preadipocytes. Physiol. Genomics 7, 15-25 (2001).

Boschmann, M., Adams, F., Klaus, S.: In situ metabolic and hemodynamic response to dexfenfluramine in white adipose tissue of rats. Ann. Nutr. Metab. 45, 24-29 (2001)

Boschmann, M., Jordan, J., Schmidt, S., Adams, F., Luft, F. C., Klaus, S.: Gender-specific response to interstitial angiotensin II in human white adipose tissue. Horm. Metab. Res. 34, 726-730 (2002).

Boschmann, M., Krupp, G., Luft, F. C., Klaus, S., Jordan, J.: In vivo response to a pha-1 adrenoreceptor stimulation in human white adipose tissue. Obes. Res. 10, 555-558 (2002)

Boschmann, M., Schroeder, C., Christensen, N. J., Tank, J., Krupp, G., Biaggioni, I., Klaus, S., Sharma, A. M., Luft, F. C., Jordan, J.: Norepinephrine transporter function and autonomic control of metabolism. J. Clin. Endocrinol. Metab. 87, 5130-5137 (2002).

Daenzer, M., Ortmann, S., Klaus, S., Metges, C. C.: Prenatal high protein exposure decreases energy expenditure and increases adiposity in young rats. J. Nutr. 132, 142-144 (2002)

Daenzer, M., Petzke, K. J., Baquette, B. J., Metges, C. C.: Whole-body nitrogen and splanchnic amino acid metabolism differ in rats fed mixed diets containing casein or its corresponding amino acid mixture. J. Nutr. 131, 1965-1972 (2001)

Gaudichon, C., Bos, C., Morens, C., Petzke, K. J., Mariotti, F., Everwand, J., Benamouzig, R., Daré, S., Tomé, D., Metges, C. C.: Ileal losses of nitrogen and amino acids in humans and their importance to the assessment of amino acid requirements. Gastroenterology 123, 50-59 (2002).

Klaus, S., Seivert, A., Boeuf, S.: Effect of the beta-3-adrenergic agonist CI316,243 on functional differentiation of white and brown adipocytes in primary cell culture. Biochim. Biophys. Acta 1539, 85-92 (2001)

Kunz, I., Schorr, U., Rommling, K., Klaus, S., Sharma, A. M.: Habitual fat intake and basal fat oxidation in obese and non-obese Caucasians. Int. J. Obes. Relat. Metab. Disord. 26, 150-155 (2002).

Metges, C. C., Daenzer, M., Petzke, K. J., Elsner, A.: Low-abundance plasma and urinary ^{15}N urea enrichments analyzed by gas chromatography-combustion-isotope ratio mass spectrometry. J. Mass. Spectrom. 37, 489-494 (2002)

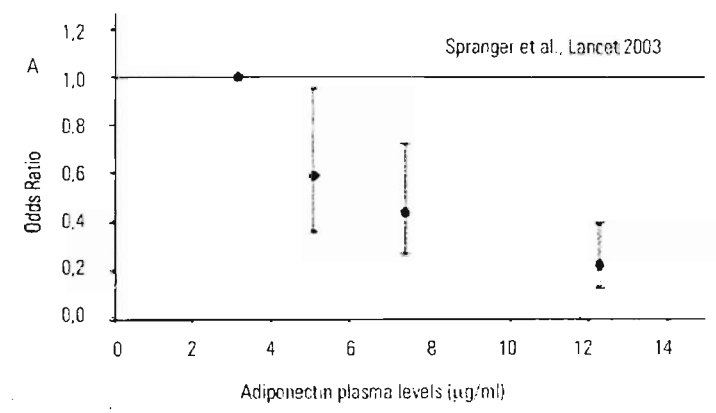
Wiedmer, P., Ortmann, S., Klaus, S.: Energy metabolism of young rats after early postnatal overnutrition. Br. J. Nutr. 88, 301-306 (2002).

Abteilung Klinische Ernährung

Leitung: Prof. Dr. Andreas F. H. Pfeiffer

Die Abteilung Klinische Ernährung (KLE) befasst sich mit der Rolle nahrungsabhängiger Komponenten in der Pathogenese der häufigsten Zivilisationskrankheiten. Genetisch programmiert scheint z.B. Übergewicht die Konstellation des Metabolischen Syndroms (MESY) zu erzeugen. Dessen Komponenten beschreiben einen häufigen Cluster von Risikokomponenten, die die Grundlage für Atherosklerose, Typ-2-Diabetes und zumindest einige Krebsformen bilden. Nach der WHO-Definition zählen dazu: gestörter Zuckerstoffwechsel, zentrale Adipositas, Dyslipidämie mit niedrigem HDL-Cholesterin und erhöhten Triglyceriden, Hypertonie, Mikroalbuminurie und Fibrinolysestörung. Die Aufklärung der Mechanismen des Metabolischen Syndroms bietet den Schlüssel für gezielte Präventionsstrategien und steht deshalb im Fokus unserer Studien.

auch antiatherogene Eigenschaften zu besitzen. In Kooperation mit der Abteilung Epidemiologie wurde die Bedeutung des Adiponectins prospektiv in der EPIC-Studie als Vorhersageparameter für das Neuauftreten eines Diabetes mellitus untersucht. Es zeigte sich, dass Adiponectin ein starker Schutzfaktor gegen den Typ-2-Diabetes ist. Wir suchen nach Strategien, Adiponectin zu erhöhen und verwenden es als Biomarker. Tatsächlich zeigt sich, dass Adiponectin durch Insulin gesenkt wird.
Dies konnten wir durch Insulininfusionen bei konstantem Blutzucker direkt nachweisen. Dagegen hatten Fettinfusionen, die einen starken Anstieg freier Fettsäuren bedingen, keinen Einfluss. Bei Menschen mit hohen Insulinspiegeln infolge einer Insulinresistenz sinken die Adiponectinspiegel, wie dies auch bei Typ-2-Diabetes beobachtet wird.



nach Adjustierung für Alter und Geschlecht

Abbildung 1
Adiponectin schützt vor Typ-2-Diabetes: je höher die Adiponectinspiegel sind, desto geringer ist das Risiko für die Entwicklung eines Typ-2-Diabetes - Daten aus der prospektiven EPIC-Potsdam-Studie

Bio- und Genmarker für das Metabolische Syndrom

Matthias Möhlig, Joachim Spranger, Martin Osterhoff

Fettgewebe reguliert über Produkte wie freie Fettsäuren und Botenstoffe den Stoffwechsel. Ein neu beschriebener Botenstoff, der ausschließlich aus weißem Fett stammt, ist das Adiponectin. Es scheint die Insulinwirkung in der Leber und im Muskel zu regulieren und

Eine Gewichtsabnahme führt dagegen zu einem Anstieg des Adiponectins, was wir auch bei massiv übergewichtigen Patienten, die durch Einsatz eines Magenbandes erheblich an Gewicht abnahmen, sehen konnten (in Kooperation mit Prof. Schernthaner, Wien). Gegenwärtig untersuchen wir die genauere Wirkung von nahrungsinduziertem Insulinanstieg auf die Adiponectinspiegel bei 30 Patienten mit frühem Typ-2-Diabetes. Nahrung, die einen geringeren Insulinanstieg

Department of Clinical Nutrition

Head: Prof. Dr. Andreas F. H. Pfeiffer

The Department of Clinical Nutrition investigates the role of nutrient dependent factors in the pathogenesis of major diseases of industrialized countries. Obesity appears to be a central constellation inducing the components of the metabolic syndrome, which includes important genetic effects. The metabolic syndrome describes the frequent clustering of the risk components which provide the common soil for atherosclerosis, type 2 diabetes and at least several types of cancer. The metabolic syndrome, as defined by the WHO (Zimmet & Alberti, 1998), includes the following components: disturbed glucose metabolism, central obesity, dyslipidemia with low HDL cholesterol and raised triglycerides, hypertension, microalbuminuria, and disturbed fibrinolysis. A better understanding of the mechanisms eliciting the metabolic syndrome will offer a key for targeted strategies of prevention. The focus of our studies is therefore an understanding of these mechanisms.

Genetic and biological markers of the metabolic syndrome

Adipose tissue appears to regulate metabolism by releasing free fatty acids and certain signaling molecules. Adiponectin represents such a signal, which is exclusively produced by white fat.
It appears to regulate insulin action in liver and muscle and, additionally, it has antiatherogenic properties. In cooperation with the Department of Epidemiology, we performed a prospective nested case-control study within the EPIC Study investigating the predictive value of adiponectin for the risk of type 2 diabetes. Indeed, adiponectin turned out to be a strong protective factor against type 2 diabetes. We are currently searching for strategies to increase adiponectin, which is used as a biomarker. These investigations showed that adiponectin is lowered by insulin but not by fatty acids. This was demonstrated by hyperinsulinaemic euglycemic clamps in the presence or absence of additional infusion of fat. This implies that elevated levels of insulin as observed in the metabolic syndrome will lower the levels of adiponectin, which is indeed observed in type 2 diabetes. Loss of weight led to a marked increase in adiponectin in morbidly obese patients that were treated by gastric banding to lower their body mass index by approximately 15 points starting with a BMI of 50 (a study in cooperation with Prof. Schernthaner, Vienna). Currently we are investigating the effects of postprandial rises in insulin on adiponectin in patients with early type 2 diabetes. Foods with a lower glycemic index might be favorable by avoiding extensive suppression of adiponectin due to a reduced release of insulin.

Figure 1
Adiponectin protects from type-2 diabetes: the higher the level of adiponectin, the lower the risk of developing type-2 diabetes -- Data from the prospective EPIC-Potsdam Study

The role of genetic variants as represented by single nucleotide polymorphisms (SNPs) in predicting the risk of obesity, type 2 diabetes, and other diseases represents another focus of our interests. Preliminary results show that a specific haplotype within the adiponectin promoter affects circulating levels of adiponectin and may thereby have a strong effect on the risk of diabetes as modified by obesity.

Fat cells also secrete substances which increase the risk of type 2 diabetes. A hypothesis arousing intensive interest is that a subclinical inflammation increases the risk of type 2 diabetes. Markers of inflammation such as C-reactive protein (CRP) are known to be elevated prior to development of type 2 diabetes. This may be triggered by an increased expression of pro-inflammatory cytokines and by reduced expression of anti-inflammatory cytokines. In the EPIC study, we observed that the combined elevation of interleukins IL-1 β and IL-6 were most valid in predicting the risk of type 2 diabetes. This risk is also linked to genetic variants, which we are currently investigating (Fig. 1). As described by others, we also observed that TNF α (tumor-necrosis factor α) is elevated in obese subjects and may provide a link between obesity and insulin resistance. However, in contrast to IL-1 β and IL-6, TNF-α did not exert an independent effect on the risk of type 2 diabetes.

Ghrelin is a novel neuroendocrine appetite-regulating factor that is primarily secreted by gastric cells. Together with M. Tschöp, Department of Pharmacology, we investigated the role of macronutrients in modulating ghrelin levels. In our first investigations, the postprandial decrease in ghrelin was primarily influenced by the increase in insulin rather than by free fatty acids. The connection between glucose absorption and insulin increase is the subject of current investigations.

Molecules within the vascular wall that are regulated by nutrients appear to represent a link between nutrition and atherosclerosis. It is known that proteolytic systems (plasminogen activator system) and adhesion molecules play a role in this regard. The interaction of urokinase and vitronectin with the classical glycolipid-anchored receptor of urokinase (uPAR) regulates migration of vascular smooth muscle cells and probably influences the development of atherosclerosis. The team headed by J. Spranger observed that uPAR is differentially regulated by insulin, glucose, and various free fatty acids. Further details of its metabolic regulation are currently being investigated in vivo in humans. Glucose toxicity refers to the phenomenon that

durch einen niedrigen "glykämischen Index" aufweist, könnte auf diesem Wege vorteilhaft sein. Weiterhin wird von der Arbeitsgruppe der Zusammenhang zwischen Adipositas, Typ-2-Diabetes und genetischen Veränderungen untersucht. So zeigen vorläufige Ergebnisse der Projektgruppe, dass ein bestimmter Haplotyp von Adiponectin-Promotor-SNPs die zirkulierenden Adiponectin-Spiegel beeinflusst und dadurch einen massiven Einfluss auf das BMI-abhängige Diabetesrisiko hat.
Fettgewebe sezerniert auch Substanzen, die in erhöhten Konzentrationen vermutlich zu einem erhöhten Diabetesrisiko führen. Derzeit besonders intensiv diskutiert wird die Theorie, dass eine chronische Entzündungsreaktion an der Entstehung des Typ-2-Diabetes beteiligt ist. Entzündungsmarker wie z. B. CRP sind auch vor Auftreten eines Typ-2-Diabetes erhöht.

erhöhten Diabetesrisiko assoziiert. Dies wird auch durch individuelle Genvarianten modifiziert, die wir aktuell untersuchen (Abb. 2). Weiterhin konnte von uns gezeigt werden, dass TNF-alpha insbesondere bei adiposen Patienten erhöht ist und so eine Verbindung zwischen Adipositas und Insulinresistenz darstellen könnte. Im Gegensatz zu IL-1β und IL-6 zeigt sich allerdings in unseren Untersuchungen kein unabhängiger Effekt auf das Diabetesrisiko. Ghrelin ist ein neu entdeckter, appetitregulierender neuroendokriner Faktor, der vorwiegend im Magen gebildet wird. In Zusammenarbeit mit der Abteilung Pharmakologie (M. Tschöp) untersuchten wir Makronährstoffe auf mögliche Effekte auf Ghrelin. Der Abfall von Ghrelin nach der Nahrungsaufnahme erscheint weniger durch Änderung der freien Fettsäuren beeinflusst als vielmehr durch den Insulinanstieg als Folge der Glucoseresorption.

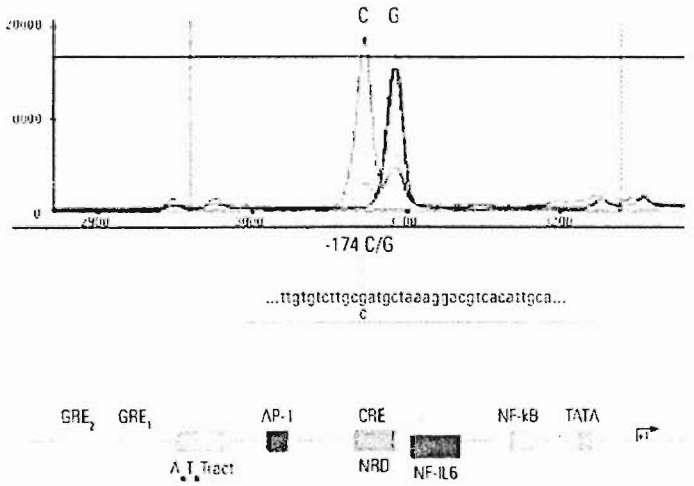


Figure 2
In the EPIC cohort examined, a rise in interleukin-6 is associated with a higher risk of diabetes. The interleukin-6 promoter polymorphism shown and its possible effects on the risk of diabetes are under study.

Abbildung 2
Ein Erhöhung von Interleukin 6 ist in der untersuchten EPIC-Kohorte mit einem erhöhten Diabetesrisiko assoziiert. Mögliche Effekte des dargestellten Interleukin-6-Promotor Polymorphismus auf das Diabetesrisiko werden derzeit untersucht.

Diskutiert werden die gesteigerte Expression von pro-inflammatorischen Zytokinen und eine verminderte Freisetzung antiinflammatorischer Zytokine wie Interleukin-6 (IL-6), Tumor-Nekrose-Faktor-(TNF)-alpha und IL-1β. In einer Zusammenarbeit mit der Abteilung Epidemiologie untersuchten wir Laborparameter des von dieser Abteilung gesammelten und charakterisierten EPIC-Kollektivs und fanden insbesondere eine kombinierte Steigerung von IL-1β und IL-6 mit einem

Bestimmte Moleküle in der Gefäßwand stellen Bindeglieder zwischen Ernährung und Atherosklerose entstehung dar, sie werden durch Nahrungskomponenten reguliert. Proteolytische Systeme (Plasminogen-Aktivierungssystem) und bestimmte Adhäsionsproteine sind hierbei beteiligt. Die Interaktion von Urokinase und Vitronectin mit dem klassischen Glykolipid-verankerten Urokinase-Rezeptor (uPAR) führt zur Migration von vaskulären glatten Muskelzellen und stellt

vermutlich ein wichtiges Bindeglied bei der Entstehung der Atherosklerose dar. So konnte in Makrophagen durch die Arbeitsgruppe von Joachim Spranger gezeigt werden, dass uPAR durch Insulin, Glucose sowie gesättigte oder ungesättigte Fettsäuren differentiell reguliert wird; weitere metabolische Parameter werden in vivo am Menschen untersucht. Glucosetoxizität beschreibt das Phänomen, dass bei persistierender Hyperglykämie eine progrediente Abnahme der Insulinsekretion zu beobachten ist. In der Arbeitsgruppe Osterhoff/Möhlig konnte gezeigt werden, dass eine Reduktion der Calzium/Calmodulin-abhängigen Protein-Kinase-II(CaMK II)-Expression in Insulinomzellen der Ratte zu einer erheblichen Verringerung des Insulingehalts und der Insulinsekretion führt. Der geringere Insulingehalt der Zellen begründet sich in einer verminderten Aktivierung des Insulin-Promotors und folglich einer verminderten Insulin-Genexpression.

Zeit erhöhte Ca^{2+}-Konzentrationen, wie sie z.B. durch chronisch erhöhten Blutzucker ausgelöst werden, zu einer Abnahme der CaMK-II-Aktivität, der PDX-1 Aktivität und schließlich zu einer Verringerung der Insulin-Genexpression führen.

Mechanismen der Krankheitsentstehung durch Alterationen der Redox-Balance

Michael Ristow, Frank Isken

Oxidation und Reduktion sind grundlegende Prozesse im Zellstoffwechsel. Sie dienen der intrazellulären Signaltransduktion sowie der Detoxifizierung von metabolischen Nebenprodukten, sogenannten freien Radikalen oder *reactive oxygen species* (ROS).
Die Bildung von ROS ist bei der Entstehung von Krebsformen, Diabetes mellitus, Arteriosklerose, Bluthochdruck und Neurodegeneration beteiligt.

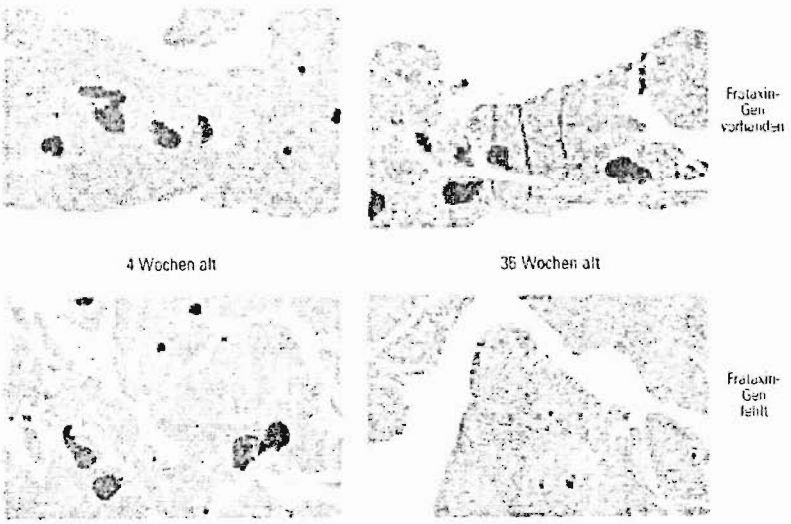


Abbildung 3
Pankreatische Betazellen der Maus: wird das Gen für das Friedreich-Ataxie-Protein Frataxin ausgeschaltet, so entwickeln die Tiere einen Verlust an Insulinsekretionskapazität und dadurch einen stetig progredienten Diabetes mellitus.

Wahrscheinlich führt die Aktivierung von CaMK II in β-Zellen direkt oder indirekt zu einer Aktivierung des β-zell-spezifischen Transkriptionsfaktors PDX-1, welcher an den Insulin-Promotor bindet, und so die Insulin-Genexpression und Insulin-Verfügbarkeit in der Zelle erhöht. Die Aktivierung von CaMK II könnte also einen "Nachschubmechanismus" anstoßen, der eine Insulin-Sekretion über längere Zeit ermöglicht. Für diesen Mechanismus spricht die Erkenntnis, dass über lange

Die Mitarbeiter der Projektgruppe Ristow beschäftigen sich mit der Entwicklung von Mausmodellen für diese komplexen Zusammenhänge. Gene der Maus, denen eine Funktion in der Regulation des Redox-Systems zugeschrieben wurde (Vitamin E-Transporter Protein *TTP*, Friedreich-Ataxie-Protein *Frataxin*), wurden, zum Teil auch gewebsspezifisch, inaktiviert. Pathogenese und präventive Therapie einer Redox-Imbalance kann an diesem physiologischen Modell

persistent hyperglycemia induces a progressive decline of insulin secretion. The group of Osterhoff/Möhlig demonstrated that a reduction of the expression of calcium/calmodulin dependent protein kinase II (CaMK-II) in rat insulinoma cells causes a dramatic reduction of the insulin content. The reduction of cellular insulin levels was due to a reduced activation of the insulin promoter and a reduced insulin gene expression. This was associated with an altered regulation of the transcription factor PDX-1, which binds to the insulin promoter and thereby increases insulin gene expression within the cell. The CaMK II-system appears to activate the backup production of insulin needed for prolonged insulin secretion. Since downregulation of CaMK-II is caused by persistent increases in cellular calcium, as induced by chronically elevated blood sugar, this mechanism provides another hypothesis to explain glucose toxicity.

Pathogenesis of diseases related to alterations of redox metabolism

Oxidation and reduction are essential mechanisms in cellular metabolism. They are involved in intracellular signal transduction as well as in the elimination of free radicals or reactive oxygen species (ROS) generated as side products in metabolism. It is currently believed that ROS are involved in the pathogenesis of diabetes mellitus, atherosclerosis, hypertension, neurodegenerative diseases, as well as certain types of cancer.
The group of M. Ristow develops mouse models to investigate these complex associations.
To this end, genes involved in the regulation of redox systems were inactivated (vitamin E transport protein, TTP), some of them in conditional knockouts only in specific tissues such as the Friedreich ataxia protein, frataxin. The pathogenesis of redox related diseases can be investigated in these physiological models. The deletion of the frataxin gene exclusively in pancreatic beta cells of the mouse is associated with markedly increased production of ROS in beta cells and leads to a steadily progressive loss of beta cells, resulting in diabetes mellitus (Fig 3). In contrast to other animal models, this closely mimics the development of type 2 diabetes (Fig.4). It is quite possible that this model is pathogenetically closely related to human type-2 diabetes.
The role of oxidative stress in man is investigated in parallel studies which are performed in the "Siegfried Thannhauser" Metabolic Ward in our associated Department of Endocrinology, Diabetes, and Nutrition at the Benjamin Franklin Medical Center in Berlin.

Figure 3
Pancreatic beta cells of the mouse: when the gene for the Friedreich ataxia protein, frataxin, is deleted, the animals lose the capacity to secrete insulin and thus develop a steadily progressive diabetes mellitus.

These studies focus on the formation of free radicals associated with rapid changes in blood sugar and free fatty acids as occurs postprandially. Hyperglycemia and hyperlipidemia induce a rapid formation of reactive oxygen species that are generated in metabolism. The modulation of ROS formation is of great interest in the pathogenesis of nutrition related diseases because they can be influenced by the choice of nutrients consumed. In particular, fruit and vegetables are known to contain antioxidants. The consumption of foods with a low glycemic index represents a promising strategy to prevent these diseases. Signaling induced by oxidative stress appears to involve changes in intracellular calcium and, particularly, the activation of CaMK II and prolonged oxidative stress due to hyperglycemia reduce insulin secretion. The research group Osterhoff/Mählig is currently developing a mouse knockout model with a tissue specific knockout of CaMK II to investigate these mechanisms in more detail.

Metabolomics - strategies to identify health-promoting metabolites

Fat, carbohydrates, and protein induce hormonal responses and are metabolized to products which exert further actions in the organism. For example, the postprandial rise of blood sugar and lipids induces specific hormonal and metabolic responses. These are known to affect the risk of cardiovascular disease, particularly in the context of impaired glucose tolerance and dislipidemia. Thus, the metabolic phenotype interacts strongly with the effects of nutrients on the health of the organism.

Apart from these macronutrient aspects, nutritional components of fruit, vegetables, and spices are known to exert direct effects on specific molecules within the biochemistry of the organism and thereby alter their function, comparable to the action of pharmaceuticals. Examples are antioxidants, flavonoids, phytoestrogens, phytosterols, and carbohydrates such as acarbose. Very little is known about the bioavailability of such molecules after ingestion of food of plant origin. In cooperation with the Max Planck Institute for Plant Genetics in Golm, we are currently establishing a technique to analyze a large number of metabolites in blood. These techniques have been established in Golm to analyze plant metabolites and have now been adapted to the human situation. This will help to identify biologically available plant metabolites to allow their definition and further investigation with regard to metabolic targets and metabolic processes affected.

untersucht werden. Beispielsweise erzeugt die Unterbrechung des Frataxin-Gens ausschließlich in pankreatischen Betazellen der Maus einen stetig progredienten Diabetes mellitus durch Verlust der Inselzellen (Abb. 3), der sich im Gegensatz zu den bisherigen Tiermodellen als neuer physiologischer Mechanismus herausstellt (Abb. 4). Pathogenetische Parallelen zum Typ-2-Diabetes des Menschen sind wahrscheinlich.
Parallel zu diesen Forschungen im Tiermodell werden in der klinischen Ambulanz der KLE im DIfE, sowie auf der Stoffwechselstation "Siegfried Thannhauser" am Universitätsklinikum Benjamin Franklin in Berlin Untersuchungen zur Entstehung von oxidativem Stress im Menschen durchgeführt. Uns interessiert die Bildung freier Radikale durch kurzfristige Veränderungen des Blutzuckerspiegels sowie der Konzentration freier Fettsäuren im Stoffwechsel.

Metabolomics - Strategien zur Identifikation gesundheitsbeeinflussender Stoffwechselprodukte
Joachim Spranger

Fette, Kohlenhydrate und Eiweiße lösen eine hormonelle Antwort aus und werden in Metaboliten umgewandelt, die auch eigene Wirkungen haben. Beispiele sind der Anstieg von Blutzucker und Blutfetten nach Mahlzeiten, die eine Hormon- und Stoffwechselantwort auslösen.
Diese beeinflussen das Risiko von Gefäßerkrankungen besonders dann, wenn ein gestörter Zuckerstoffwechsel und/oder eine Fettstoffwechselstörung besteht. Nahrungskomponenten besonders von Obst, Gemüse und Kräutern haben direkte Wirkungen auf bestimmte Moleküle im Organismus und verändern deren Funktion, ähnlich der Wirkung von Medikamenten.

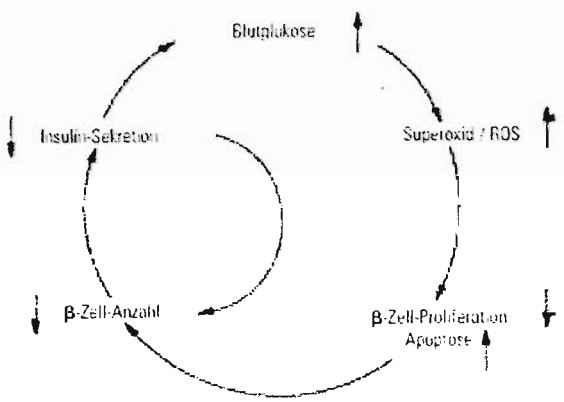


Abbildung 4
Mögliche Regelkreis für die Entstehung des Diabetes mellitus.

Bei Hyperglykämie und Hyperlipidämie fallen durch Aktivierung des metabolischen Umsatzes vermehrt ROS an. Angesichts der möglicherweise zentralen Stellung von Radikalen in der Pathogenese ernährungsabhängiger Erkrankungen ist deren Beeinflussung durch Nahrungskomponenten von großem Interesse und unmittelbar mit den beiden anderen Abteilungsschwerpunkten verbunden. Auch CaMK II ist in die Vermittlung von Signalen, die durch oxidativen Stress ausgelöst werden, involviert und oxidativer Stress vermindert die Insulin-Sekretion. Anhand eines CaMK-II-K.O.-Mausmodells soll in der Arbeitsgruppe Osterhoff/Mählig untersucht werden, welche Effekte eine Reduktion der CaMK-II-Expression in einem lebenden Tier, besonders bei unterschiedlichen Ernährungsformen, hat.

Beispiele hierfür sind Antioxidanzien, Flavonoide, Phytoöstrogene, Phytosterole. Aufbauend auf neuen massenspektrometrischen Screeningtechniken aus dem Max-Planck-Institut für Pflanzenphysiologie in Golm (Direktor Prof. Willmitzer) arbeiten wir kooperativ an der Analyse von Metaboliten im Blut. Die neuen Verfahren erlauben das Erfassen einer Vielzahl von niedermolekularen Verbindungen, und zwar auch von solchen, deren genaue Zusammensetzung noch nicht bekannt ist. Die Struktur kann dann aber in einem zweiten Schritt angegangen werden. Die aktuellen Arbeiten etablieren zunächst diese Technik. In naher Zukunft soll sie dann zur Identifikation von tatsächlich resorbierten Pflanzeninhaltsstoffen und deren Metaboliten verwendet werden.

Figure 4
Possible regulatory scheme for development of diabetes mellitus

Ausgewählte Publikationen
Selected Publications

Originalarbeiten/Original Papers

Assert, R., Kotter, R., Schiemann, U., Goretzki, P., Pfeiffer, A. F.: Effects of the putatively oncogenic protein kinase Calpha D294G mutation on enzymatic activity and cell growth and its occurrence in human thyroid neoplasias. Horm. Metab. Res. 34, 311-317 (2002).

Assert, R., Scherk, G., Bumbure, A., Piraqs, V., Schatz, H., Pfeiffer, A. F.: Regulation of protein kinase C by short term hyperglycaemia in human platelets in vivo and in vitro. Diabetologia 44, 188-195 (2001).

Hering, S., Isken, E., Knabbe, C., Janott, J., Jost, C., Pommer, A., Muhr, G., Schatz, H., Pfeiffer, A. F.: TGFbeta1 and TGFbeta2 mRNA and protein expression in human bone samples. Exp. Clin. Endocrinol. Diabetes 109, 217-226 (2001).

Kopp, R., Ruge, M., Rothbauer, E., Cramer, C., Kraeming, H. J., Wiebeck, B., Schildberg, F. W., Pfeiffer, A.: Impact of epidermal growth factor (EGF) radioreceptor analysis on long-term survival of gastric cancer patients. Anticancer Res. 22, 1161-1167 (2002).

Möhlig, M., Spranger, J., Otto, B., Ristow, M., Tschop, M., Pfeiffer, A. F.: Euglycemic hyperinsulinemia, but not lipid infusion, decreases circulating ghrelin levels in humans. J. Endocrinol. Invest. 25, RC36-38 (2002).

Roudovitch, N., Nauck, M. A., Schatz, H., Pfeiffer, A. F.: Modified hyperinsulinaemic, eu- and hypoglycaemic clamp technique using lispro-insulin for insulinoma diagnostic. Exp. Clin. Endocrinol. Diabetes 109, 397-401 (2001).

Schiemann, U., Konturek, J. W., Osterhoff, M., Assert, R., Rembiasz, K., Pfeiffer, D., Schatz, H., Domschke, W., Pfeiffer, A.: Decreased expression of epidermal growth factor receptor and mRNA of its ligands in Helicobacter pylori-infected gastric mucosa. Scand. J. Gastroenterol. 36, 23-31 (2001).

Shoichet, S. A., Baumer, A. T., Stamenkovic, D., Sauer, H., Pfeiffer, A. F., Kahn, C. R., Müller-Wieland, D., Richter, C., Ristow, M.: Frataxin promotes antioxidant defense in a thiol-dependent manner resulting in diminished malignant transformation in vitro. Hum. Mol. Genet. 11, 815-821 (2002).

Spranger, J., Möhlig, M., Osterhoff, M., Buhnen, J., Blum, W. F., Pfeiffer, A. F.: Retinal photocoagulation does not influence intraocular levels of IGF-I, IGF-II and IGF-BP3 in proliferative diabetic retinopathy-evidence for combined treatment of PDR with somatostatin analogues and retinal photocoagulation? Horm. Metab. Res. 33, 312-316 (2001).

Spranger, J., Osterhoff, M., Reimann, M., Möhlig, M., Ristow, M., Francis, M. K., Cristofalo, V., Hammes, H. P., Smith, G., Boulton, M., Pfeiffer, A. F.: Loss of the antiangiogenic pigment epithelium-derived factor in patients with angiogenic eye disease. Diabetes 50, 2641-2645 (2001).

Spranger, J., Kroke, A., Möhlig, M., Bergmann, M. M., Ristow, M., Boeing, H., Pfeiffer, A.F.: Adiponectin and protection against type 2 diabetes mellitus. Lancet 361:226-8 (2003).

Ziemssen, F., Schnepf, R., Pfeiffer, A. F.: [Die SSCP-(Single Strand Conformation Polymorphism-)Analyse zum Nachweis von Punktmutationen. Eine Technik und ihre Grenzen am Beispiel der Diagnostik dominant vererbter diabetesformen (MODY)]. Med. Klin. 96, 515-520 (2001).

Übersichtsarbeiten/Reviews

Pfeiffer, A. F., Emig, Ch.: Prävention mit Vitaminen und Spurenelementen. Dtsch. Med. Wochenschr. 127, 2251-2252 (2002).

Buchbeiträge/Articles in Books

Spranger, J., Pfeiffer, A.: Diabetic microvascular complications. In: Mogensen, C. (ed.): Hypertension & Diabetes. Volume 1. London, Lippincott Williams & Wilkins, pp. 57-68 (2002).

Sonstige/Others

Bahr, V., Diederich, S., Pfeiffer, A. F. H.: The metabolic syndrome X and peripheral cortisol synthesis. Exp. Clin. Endocrinol. Diabetes 110, 313-318 (2002).

Ziemssen, F., Bellanne-Chantelot, C., Osterhoff, M., Schatz, H., Pfeiffer, A. F.: To: Lindner, T., Cockburn, B. N., Bell, G. I. (1999): Molecular genetics of MODY in Germany. Diabetologia 42, 121-123, Diabetologia 45, 286-287, discussion 287-288 (2002).

Drittmittelprojekte
External Funding

Titel: Investigation of PKC dependent regulation of Pigment Epithelium Derived Factor (PEDF)
Finanzierung: Eli Lilly Foundation
Laufzeit: 06/01-11/02

Titel: Ist durch eine fettsäurengesteuerte Abnahme von UCP 2 in humanen Beta Zellen eine Reduktion der Hyperinsulinämie bei Adipositas erzielbar?/Can a fatty acid regulated decrease in UCP 2 in human β cells serve to reduce hyperinsulinemia in obesity?
Finanzierung: Institut Danone für Ernährung
Laufzeit: 10/00-12/02

Titel: Identifizierung von Polymorphismen im PPARγ coactivator 1 Gen: Gibt es eine Assoziation mit Adipositas oder Diabetes mellitus Typ 2?
Finanzierung: Deutsche Diabetes Gesellschaft
Laufzeit: 02/01-06/02

Titel: Mögliche Glucoseintoleranz durch (β zell-spezifische Expression der cre Rekombinase und ihre Konsequenzen für die Bewertung pankreasspezifischer Gen Inaktivierungen
Finanzierung: Deutsche Diabetes Gesellschaft
Laufzeit: 07/02-06/03

Titel: Beeinflussung der oszillierenden Insulinsekretion des Menschen durch Störungen des mitochondrialen Energiestoffwechsels bei Friedreich-Ataxie
Finanzierung: Deutsche Diabetes Gesellschaft
Laufzeit: 02/01-06/02

Titel: Dominant-negative Inaktivierung eines Regulators des nicht-oxidativen Glukosestoffwechsels im transgenen Tiermodell im Schwerpunkt: Mono- und polygene Krankheiten des Menschen: Definition und molekulare Pathogenese
Finanzierung: Fritz Thyssen Stiftung
Laufzeit: 01/02-12/03

Titel: Untersuchungen der insulinsekretionsstimulierenden Wirkung von neuen β-zytotropen Substanzen
Finanzierung: Universitätsklinikum Benjamin Franklin, Freie Universität Berlin

Titel: Gewebsspezifische Inaktivierung von Frataxin, einem mitochondrialen Regulator des oxidativen Energiestoffwechsels/Tissue-specific inactivation of frataxin,a mitochondrial regulator of energy metabolism
Finanzierung: DFG
Laufzeit: 11/00-06/03

Titel: Etablierung eines Mausmodells mit Überexpression der Kalzium/Calmodulin-abhängigen Proteinkinase II δ 2 in den beta Zellen des Pankreasinselorgans
Finanzierung: Deutsche Diabetes Gesellschaft
Laufzeit: 04/01-12/03

Leitung: Prof. Dr. Hans-Joachim Franz Zunft

Department of Intervention Studies

Head: Prof. Dr. Hans-Joachim Franz Zunft

The research of the department is focused on the role of food products, food ingredients, and food choice in preventing nutrition dependent chronic diseases, i.e., overweight, obesity, hypertension and hyperlipoproteinemia, as well as cardiovascular and gastrointestinal disorders. Based on its methodology, the department can be characterized as an experimental epidemiological group using intervention studies, particularly with volunteers who maintain their habitual way of life.
We especially investigate

- markers of disease risk
  - anthropometric variables (body composition),
  - clinical variables in blood, urine, feces,
  - physiological parameters (energy expenditure, endothelial function),
- physical condition
  - physical activity,
  - sensory sensitivity,
  - state of health and well being,
- nutritional behavior
  - sensory preferences/acceptance,
  - consumer attitudes.

In our research, we cooperate with partners both within and outside of the DIfE, (1) to integrate a broad spectrum of competence and (2) to acquire the necessary statistical validity by increasing the number of volunteers. Therefore, the department is involved in several international projects, most of them funded by the framework programs of the European Union.

Age-dependent changes in sensory physiology and food choice

The EU-funded project, HealthSense, has set a target for delivering results to increase the acceptance of a healthy diet by elderly people. Therefore, the sensory determinants of food choice will be investigated in different work packages. These include studies on taste and smell sensitivity, preferences for selected food products, and the dependency of these variables on age and cultural background. With the results obtained, it is planned to increase the acceptance of specified foods by the elderly. Our department is involved in three different work packages of this project:
Representative samples of elderly people in all member states of the EU were questioned about their attitudes towards health and nutrition. The majority of senior citizens rank health highest among the motives for food selection. On the other hand, 80 % are convinced that they already follow a healthy diet. This is clearly in contrast to the real nutritional situation in all these countries. Our results demonstrate an incorrect selfreflection of personal nutritional behavior.

Figure 1
Age-dependent decrease in sense of smell
Test: Detection and identification of 16 different odors by 313 German subjects
Conclusion: The ability to detect and identify odors diminishes with increasing age.

Die experimentell-epidemiologische Forschungsarbeit der Abteilung richtet sich darauf, die Bedeutung von Lebensmitteln, ihren Inhaltsstoffen sowie ihrer Zusammenstellung zu bestimmten Kostformen für die Prävention ernährungsabhängiger chronischer Erkrankungen zu erkennen und zu bewerten. Als Zielgrößen werden vorrangig Erkrankungen des Metabolischen Syndroms (Übergewicht, Adipositas, Hypertonie, Fettstoffwechselstörungen), kardiovaskuläre sowie gastrointestinale Faktoren betrachtet.

Bevorzugte Studienform sind Interventionsstudien mit Personen, die ihre habituelle Lebensweise grundsätzlich beibehalten. Die speziellen Untersuchungen richten sich auf

- Marker des Erkrankungsrisikos:
  anthropometrische Variablen (Körperzusammensetzung),
  Messgrößen in Körperflüssigkeiten,
  physiologische Größen (Energieumsatz, Endothelfunktion),
- den Körperzustand
  - körperliche Aktivität,
  - sensorische Sensibilität,
  - Gesundheitsstatus und Wohlbefinden,
- das Ernährungsverhalten:
  - sensorische Akzeptanz,
  - Verbrauchereinstellungen.

Bei der Verfolgung ihrer Forschungsziele arbeitet die Abteilung mit zahlreichen Partnern zusammen, die meisten Projekte sind in Rahmenprogramme der EU eingebunden.

Sensorische Physiologie und Nahrungsauswahl im höheren Lebensalter

Stephan Hoyer, Ulrike Simchen, Hans-Joachim Franz Zunft

Das von der EU geförderte Projekt *HealthSense* will mit seinen Erkenntnissen die Akzeptanz einer gesundheitsfördernden Ernährung bei älteren Menschen erhöhen. Dazu wird in verschiedenen Arbeitspaketen untersucht, wie sich die sensorischen Determinanten der Kostwahl (Geruch, Geschmack, Präferenz zu Lebensmitteln) mit dem Alter verändern und ob und wie diese Beziehungen innereuropäisch differieren. Im ersten Vorhaben sind ältere Menschen in allen EU-Ländern repräsentativ nach ihrer Einstellung gegenüber dem gesundheitlichen Wert ihrer Ernährung befragt worden. Die Mehrzahl der Befragten gab an, sich bei der Lebensmittelauswahl hauptsächlich vom Gesundheitsaspekt leiten zu lassen und diesen auch zu berücksichtigen. Dies widerspricht jedoch den tatsächlichen Verzehrsdaten

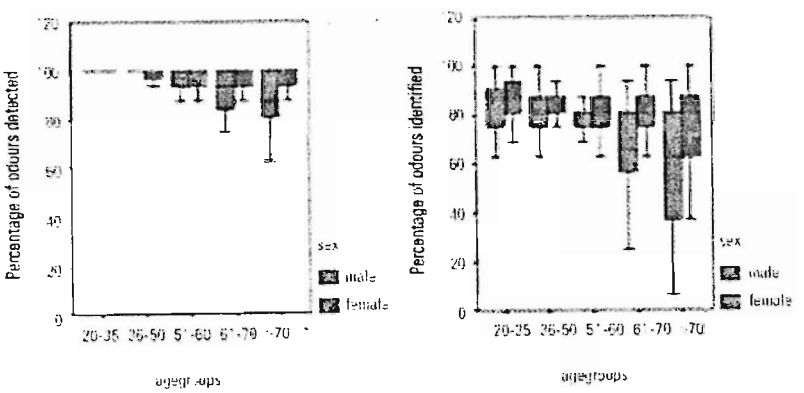


Abbildung 1
Altersabhängige Leistungsabnahme des Geruchsinns
Test: Wahrnehmung und Erkennung von 16 verschiedenen Gerüchen durch 313 deutsche Probanden
Schlußfolgerung: Die Fähigkeit zur Wahrnehmung und Identifikation von Gerüchen sinkt mit zunehmendem Lebensalter.

und zeugt von einer verzerrten Wahrnehmung des eigenen Verhaltens.
Im zweiten Teilprojekt sind Erwachsene im Alter von 20 bis über 70 Jahren auf sensorische Sensitivität (Abb 1) und Präferenz gegenüber Lebensmitteln innerhalb einer Mahlzeit (Suppe, Reis, Kekse) geprüft worden. Die landestypische Esskultur erwies sich auch bei älteren Menschen als vorrangige Bestimmungsgröße für Bevorzugung und Ablehnung von Zubereitungen, Speisen und Kostformen.

Im dritten Teilvorhaben wird untersucht, welchen prädiktiven Wert Beliebtheitsprüfungen im Labor für das Verzehrsverhalten im täglichen Leben besitzen. Dazu wurde ein mobiler Getränkeautomat (Abb. 2) entwickelt und in Einrichtungen der Gemeinschaftsverpflegung für jüngere bzw. ältere Menschen eingesetzt. Dabei zeigte sich, dass Senioren Getränke mit höherer Süße bevorzugen. Vor allem aber präferieren sie - im Gegensatz zu jüngeren Erwachsenen - im Labor andere Getränke als im Langzeitexperiment unter Alltagsbedingungen. Der sensorische Labortest ist demnach ungeeignet, um für ältere Menschen die dauerhafte Akzeptanz gegenüber einem Lebensmittel vorauszubestimmen.

Gesundheitsfördernder Effekt von Synbiotika bei älteren Menschen

Christiana Hanisch, Hans-Joachim Franz Zunft, Michael Blaut[1], Susanne Müller

Dazu sind in den Studienzentren in Stockholm, Potsdam-Rehbrücke, Paris und Camerino (Italien) von Personen im Alter von 25 bis 45 bzw. über 65 Jahren der Lebensmittelverzehr erhoben sowie Stuhlproben untersucht worden.

Dieser Ausgangsuntersuchung folgt in 2 Zentren (Paris, Potsdam-Rehbrücke) eine doppelblind angelegte, randomisierte Cross-over-Studie an älteren Personen (> 65 Jahre). Die Probanden verzehren täglich über je 8 Wochen entweder ein Synbiotikum (Oligofruktose mit Laktobazillen) oder ein Placebo. Derzeit werden die in beiden Studienabschnitten gewonnenen Stuhlproben auf ihre mikrobielle und biochemische Zusammensetzung geprüft. Die Ergebnisse lassen fundierte Aussagen über mögliche günstige Effekte von Prä- und Probiotika für die Darmgesundheit und darüber hinaus für das Wohlbefinden älterer Menschen erwarten.

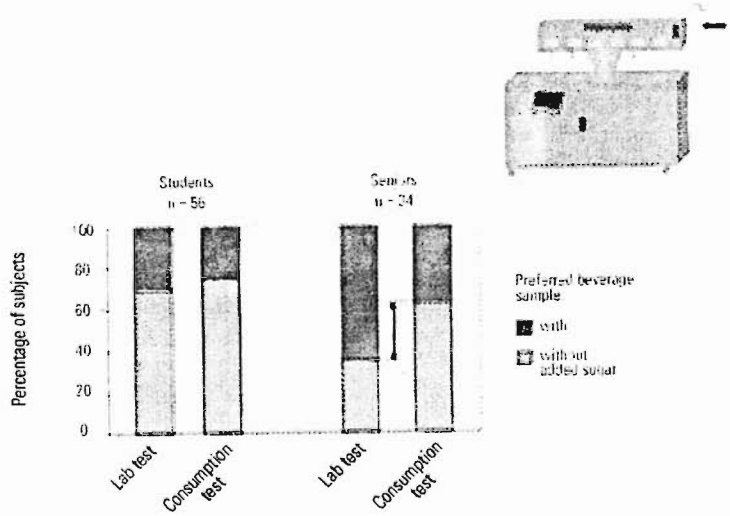


Abbildung 2
Präferenz zum Süßgeschmack von Fruchtsaftgetränken unter Labor- und Alltagsbedingungen

Substrat:	Fruchtsaftgetränke mit oder ohne zugesetzten Zucker
Labortest:	Rangfolgetest
Konsumtest:	Vierwöchige Registrierung der individuellen Saftauswahl und der jeweils konsumierten Menge über eine Chipkarte an einem Getränkeautomaten
Auswertung:	Klassifikation der Versuchspersonen gemäß ihrer Präferenz zu den Varianten mit oder ohne Zuckerzusatz
Schlussfolgerung:	65 % der Senioren bevorzugen im Labor die süßeren Getränke, aber nur 38 % trinken diese am Automaten.

Das von der EU geförderte multizentrische Projekt *Crownalife* fragt nach dem potentiellen Nutzen von Prä- und Probiotika für die Darmgesundheit älterer Menschen in Zusammenarbeit mit der Abteilung Gastrointestinale Mikrobiologie.
Geprüft wird zunächst, ob und wie sich Ernährungsgewohnheiten, Lebensalter sowie kulturelle Zugehörigkeit auf die Zusammensetzung der Darmflora auswirken.

Unlösliche Ballaststoffe aus Johannisbrot senken den Cholesterinspiegel

Corinna Koebnick, Hans-Joachim Franz Zunft

Von löslichen Ballaststoffen ist eine cholesterinsenkende Wirkung bekannt. Wir konnten diesen Effekt erstmalig auch für die unlöslichen Bestandteile des Johannisbrots zeigen. Eine 6wöchige Applikation von 15 g/d einer an Lignin, Polyphenolen und Pinitol

Five centers recruited volunteers, 20 to over 70 years of age, and estimated their sensory sensitivities (Fig. 1) and preferences for products within a meal (soup, rice, biscuit). The results revealed which sensory properties are preferred by elderly people. The hedonic ratings differed between age groups and genders. To a higher degree, however, they differed between countries. Thus, the cultural background has been revealed to be of major importance for sensory preferences in the elderly.

In the third subproject, the predictive value of sensory tests in the laboratory for food consumption in real-life situations was investigated. A mobile vending machine has been developed to measure the quality and quantity of consumed beverages whose hedonic estimates had been determined previously in the laboratory. The results showed that senior citizens preferred higher sugar concentrations than students. Moreover, the lab tests with the elderly but not with students failed to reflect the long-term preference for beverages in the real-life setting (Fig. 2). These results force a reconsideration of how products specifically for the elderly will be designed and evaluated.

Health-promoting effect of synbiotics in the elderly

The EU funded project *Crownalife* is targeted to give information about the potential health benefits of pro- and prebiotics for elderly people. In the first work package, the relation between dietary habits, age, and cultural background on the one hand and fecal composition on the other hand was investigated with subjects (25 - 45 and over 65 years of age) in the study centers in Stockholm, Paris, Potsdam, and Camerino (Italy). This baseline study was followed by a randomized, double-blind, crossover intervention study in the Paris and Potsdam centers. During two 8-week periods, elderly people consumed daily either a synbiotic (oligofructose with lactobacilli) or a placebo.

Figure 2
Preference for sweetness of fruit beverages, in the laboratory and in real-life situations

Substrate:	*Fruit beverages with natural sweetness or with added sugar*
Lab test:	*Hedonic ranking test*
Consumption test:	*4-week registration of individual beverage choice (quality and quantity) via chip card and computerized vending machine*
Evaluation:	*Classification of subjects according to their preference for samples either with or without added sugar*
Conclusion:	*65 % of the senior citizens prefer the sweeter samples in the laboratory, but only 38 % during the 4-week consumption test.*

Fecal samples were collected and their microbial (by FISH, i.e., fluorescence in situ hybridization) and biochemical composition analyzed. The results are expected to deliver new insights into the potential benefits of synbiotics for health and well being in the elderly.

Cholesterol-lowering effect of insoluble carob fiber

Soluble but not insoluble dietary fiber has been reported to lower serum cholesterol levels. Recently, cholesterol reduction could also be shown for a carob pulp preparation containing high amounts of water insoluble fiber and polyphenols in animal experiments. To show whether an analogous effect can be found in humans, a randomized, double blind, placebo controlled, parallel intervention trial with hypercholesterolemic subjects was run together with industrial partners. Carob fiber consumption (15 g/d) over 6 weeks reduced the serum level of LDL cholesterol in both genders by 10.5 (± 2.2 %) and serum triglycerides in females by 11.3 (± 4.5 %) (Fig.3). No effects on HDL levels could be observed. Generally, in females the lipid-lowering effects were more pronounced than in males. The results suggest a potential benefit of carob fiber for prevention and treatment of hypercholesterolemia.

Role of nutrition information for consumer behavior and dietary intake

The relation between nutritional information, consumer behavior and health related dietary quality has been investigated. Preferred sources of nutriton information were experiences of friends, marketing campaigns in supermarkets, and newspaper articles. The highest confidence was placed in information from consumer tests (Stiftung Warentest), in product labeling prescribed by law, and in scientific publications. Subjects using information from these sources were characterized by a more health oriented choice of foods. Although the importance of fruit and vegetable intake is well known, only 15 % of the consumers ate these foods five times a day or more.

Fat consumption in Germany

Data on food consumption in Germany was evaluated to describe the changes in fat consumption in the past decade. According to agricultural statistics and family budget surveys, the consumption of vegetable oil clearly increased after 1995. The pattern of fatty acids, however, marginally shifted to a slightly higher level of polyunsaturated fatty acids. The ratio between poly-, monounsaturated, and saturated fatty acids is averages 16:38:46. This is far from the recommendation of the German Nutrition Society (DGE), which will not be reached within the next years on the basis of recent consumption trends.

Figure 3
Reduction of LDL cholesterol level during the 6-week consumption of 15 g/d of insoluble dietary fiber from carob pulp (carob fiber)

reichen Fraktion (Carob-Fiber) führten in einer randomisierten, doppelblinden, placebokontrollierten Studie (gemeinsam mit Partnern aus der Industrie durchgeführt) bei Hypercholesterinämikern zu einer Senkung des LDL-Spiegels im Serum von 10,5 ± 2,2 % (Abb.3). Bei den teilnehmenden Frauen reduzierte sich der Triglyceridspiegel um 11,3 ± 4,5 %. Der HDL-Spiegel veränderte sich nicht. Die Effekte waren bei den Frauen ausgeprägter als bei den Männern. Damit besitzen Carobprodukte ein hohes Potential in Prävention und Behandlung von ernährungsabhängigen Erkrankungen, insbesondere bei Hypercholesterinämie.

Ernährungsinformation, Ernährungsverhalten und Lebensmittelverzehr
Gottfried Ulbricht

Um die Frage zu beantworten, welcher Verbraucher ernährungsbezogene Informationen beziehen und wie sich diese auf die gewählte Ernährung auswirken, führten wir im Raum Berlin-Brandenburg eine Befragung durch. Als bevorzugte Informationsquellen erwiesen sich Hinweise von Freunden und Bekannten, Angebotspräsentationen in Supermärkten sowie Berichte in Tageszeitungen und Zeitschriften. Ein größeres Vertrauen genießen jedoch Berichte der Stiftung Warentest, die gesetzlich vorgeschriebenen Verpackungsaufschriften sowie wissenschaftliche Publikationen. Personen, die ihre Ernährungsinformationen aus diesen vertrauenswürdigen Quellen beziehen, zeichnen sich durch eine stärker gesundheitsorientierte Kostwahl aus. Die Bedeutung des Obst- und Gemüseverzehrs für eine gesunde Ernährung ist weithin bekannt, bei Frauen mehr als bei Männern. Dennoch wurde der gewünschte Verzehr von 5 mal täglich Gemüse und Obst nur von 15 % erreicht.

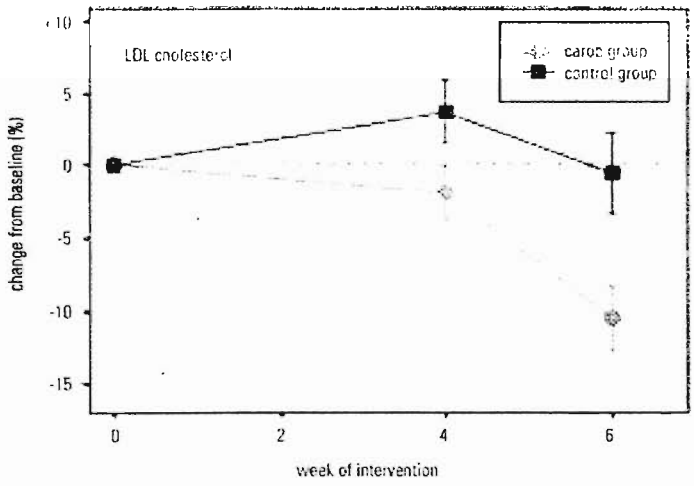


Abbildung 3
Absenkung des LDL-Cholesterinspiegels bei 6-wöchigem Verzehr von 15 g/d eines unlöslichen Ballaststoffs aus Johannisbrot (Carob Fiber)

Entwicklungstendenz des Fettverzehrs in Deutschland
Gottfried Ulbricht

Zwischen 1990 und 2000 fand gemäß Agrar- und Haushaltsstatistik in Deutschland eine deutliche Verbrauchsverlagerung weg von Margarine und tierischen Fetten hin zu pflanzlichen Ölen statt. Dennoch hat sich das Fettsäuremuster nur geringfügig zugunsten mehrfach ungesättigter Fettsäuren verschoben. Das Verhältnis der mehrfach ungesättigten zu einfach ungesättigten zu gesättigten Fettsäuren liegt im Mittel bei 16 : 38 : 46. Die Fettverzehrsempfehlungen der DGE werden trotz Ernährungsaufklärung immer noch deutlich verfehlt und sind auch bei Beibehaltung der Verbrauchstrends der 90er Jahre mittelfristig nicht erreichbar.

Neue Methode zur Bestimmung der Körperzusammensetzung
Ulrike Trippo, Karen Wagner

Das von der EU geförderte Projekt *BodyLife* zielt auf die Entwicklung und Validierung eines neuen nicht invasiven Gerätes zur Bestimmung der Körperzusammensetzung ab. Das neue Gerät, von französischen und

britischen Projekt-Partnern entwickelt, kombiniert 2 Messprinzipien: Leitfähigkeits- und Ultraschall-Untersuchung. Die Validierung nutzt die in der Abteilung etablierten Labormethoden, wie Hydrodensitometrie (Abb. 4), Air Displacement Plethysmographie (BodPod) und DEXA.
Als Probanden dienen übergewichtige und normalgewichtige Erwachsene unterschiedlichen Alters.
Um auch intraindividuelle Änderungen der Körperzusammensetzung zu verfolgen, wird ein Teil der Probanden in einer Interventionsstudie mit erhöhter sportlicher Aktivität und Umstellung der Ernährungsgewohnheiten zur Gewichtsreduktion angeleitet. Die Ergebnisse führen zu Regressionsgleichungen für spätere bevölkerungsweite Anwendungen der neuen Methode.

New method to estimate body composition
The main objective of the EU funded project *BodyLife* is to develop and validate a new device for measuring body composition. Compared to established methods, the new device is intended to be simpler, more accurate, non invasive, and transportable.
The French and British partners are responsible for the technical development of the device, which combines electromagnetic and innovative ultrasonic techniques. The department will carry out an intervention study with obese and normal weight adults to validate the new device in comparison with established laboratory methods, e.g., hydrodensitometry (Fig. 3), air displacement plethysmography and DEXA. In order to study intraindividual changes in body composition, some of the obese subjects will be shown how to reduce weight and body fat mass by increasing physical activity and changing nutritional habits. The results will deliver regression equations for applications within the general public.

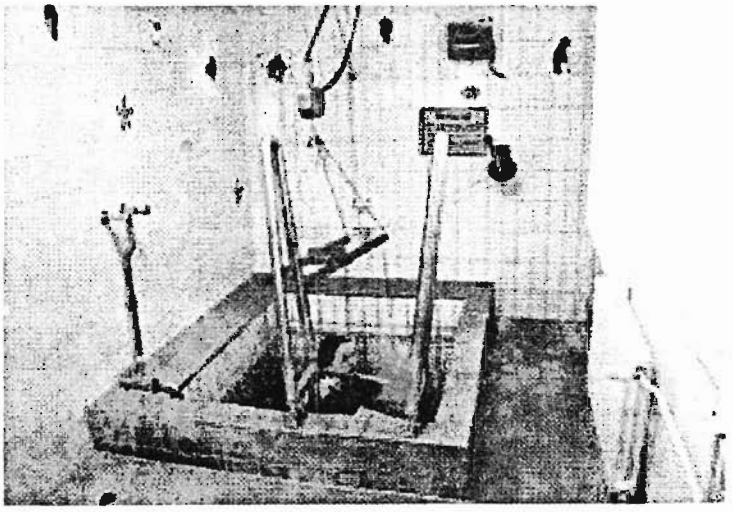


Abbildung 4
Unterwasserwägung (Hydrodensitometrie) – Messmethode zur Erfassung des Körpervolumens zur Bestimmung der Körperzusammensetzung

Figure 4
Underwater weighing (hydrodensitometry) – a method for measuring body volume in order to estimate body composition

Protektive Rolle phenolischer Pflanzenmetabolite in der Genese von Herz-Kreislauf-Erkrankungen
Anja Machowetz, Manja Reimann, Corinna Koebnick

Mit der vorbeugenden Wirkung von sekundären Pflanzenmetaboliten gegenüber Herz-Kreislauf-Erkrankungen befassen sich zwei EU-geförderte Projekte: *Isoheart* widmet sich den vor allem in Sojaprodukten enthaltenen Isoflavonen, ihren möglichen protektiven Wirkungen und der Verbraucherakzeptanz dieser Produkte in Europa. In einer doppelblind angelegten, randomisierten Crossover-Studie werden an postmenopausalen Frauen die Effekte von Isoflavonen auf die Endothelfunktion und andere kardiovaskuläre Zielgrößen untersucht (z. B. mittels Ultraschall, Volumen-oszillometrie, Impedanzkardiographie). Dazu werden auch genomische und proteomische Marker genutzt.
Eurolive richtet sich auf die antioxidativen Eigenschaften von phenolischen Komponenten in nativem Olivenöl. Wesentliche Arbeitsaufgabe ist eine randomisierte, doppelblind angelegte Crossover-Studie an gesunden Nichtrauchern im Alter von 20 bis 60 Jahren.
Geprüft wird die protektive Rolle der phenolischen Substanzen auf die Zusammensetzung der Blutlipide, die Lipidperoxidation und degenerative DNA-Veränderungen (oxydiertes LDL; Oxydationsauffälligkeit der LDL-Subklassen).

Beide Projekte haben 2002 mit Untersuchungen zur Bioverfügbarkeit der eingesetzten Substanzen begonnen. Im Jahre 2003 werden die geplanten Interventionsstudien laufen.

Phenolics and their benefit in cardiovascular diseases
Two other EU funded projects center around the protective role of secondary plant metabolites (nutraceuticals) in cardiovascular diseases (CVD). Isoheart centers around isoflavone intake and risk reduction regarding coronary heart disease. The physiological effects of phytoestrogens and their consumer acceptability will be elucidated. In a double-blind randomized cross-over intervention study for a total of 16 weeks among postmenopausal women, isoflavone effects on endothelial function and other cardiovascular parameters will be investigated by using genomic or proteomic markers.

The aim of Eurolive is to demonstrate the antioxidative properties of phenolic compounds in virgin olive oil. Therefore, a randomized double blind crossover study will be run for 15 weeks using healthy non-smoking men, aged 20-60 years. The results will answer the question whether olive oil phenolics have beneficial effects on disorders associated with oxidative damage, i. e., blood lipids, lipid peroxidation (oxydized LDL, succeptibility to oxydation of LDL-subclasses and degenerative DNA alterations. Thus, the project may help to explain the health related benefits of the Mediterranean diet.

Both projects started in 2002. After estimating bioavailability, a suitable selection of foods has been established for use within the intervention trials. Recruiting has been completed and the trials will start in early 2003.

Drittmittelprojekte
External Funding

Titel: Erstellung eines Compartmodells des Süßrezeptors zur Vorhersage der Süßintensität beliebiger Mischungen ausgewählter süßer Verbindungen in wässriger Lösung/Establishment of a compartment model of the receptor for sweetness to predict the sweetness intensity of mixtures of selected sweet compounds dissolved in water
Finanzierung: AiF
Laufzeit: 06/99-05/01

Titel: Bewertung des ernährungsphysiologischen Effekts sowie der Akzeptanz neuartiger Getreideprodukte ohne Zöliakietoxizität
Finanzierung: BMBF
Laufzeit: 01/00-02/03

Titel: Herstellung eines neuartigen, gesundheitsförderlichen Kohlenhydratkonzentrats aus Abfallstoffen der Weizenstärkegewinnung durch integrierten Einsatz biotechnologischer Verfahren
Finanzierung: Deutsche Bundesstiftung Umwelt
Laufzeit: 05/00-04/03

Titel: Präventionsprogramm gegen ernährungsbedingte Risiken und Krankheiten in einem sozial belasteten Stadtteil
Finanzierung: MWFK Brandenburg
Laufzeit: 01/00-12/01

Titel: CROWNALIFE. Functional Food, Gut Microflora and Healthy Ageing (QLRT-2000-00067), gemeinsam mit GAMI
Finanzierung: EU
Laufzeit: 01/01-12/03

Titel: BODY LIFE Intelligent System Monitoring the Body Composition for better Healthy Life Style and Illness Prevention (IST-2000-25410)
Finanzierung: EU
Laufzeit: 01/01-12/03

Titel: ISOHEART: Isoflavones for reducing risk of coronary heart diseases among postmenopausal women
Finanzierung: EU
Laufzeit: 01/02-12/04

Titel: EUROLIVE: The effect of olive oil consumption on oxidative damage in European population
Finanzierung: EU
Laufzeit: 01/02-12/04

Titel: *HealthSense* Healthy ageing: how changes in sensory physiology, sensory psychology and socio-cognitive factors influence food choice
Finanzierung: EU
Laufzeit: 02/00-01/03

Titel: Absenkung der Fettresorption beim Menschen durch den wasserlöslichen Ballaststoff Konjakmannan (Zweckbetrieb)
Finanzierung: Meistermarken
Laufzeit: 10/00-02/01

Ausgewählte Publikationen
Selected Publications

Originalarbeiten/Original Papers

Koebnick, C., Heins, U. A., Wickramasinghe, S. N., Ratnayake, I. D., Hathorn, T., Pahlberg, A. B., Hoffmann, I., Dagnelie, P. C., Lindemans, J., Leitzmann, C.: Longitudinal levels of vitamin B12 and vitamin B12 binding proteins during normal pregnancy. Clin. Chem. 48, 926-933 (2002).

Kromeyer-Hauschild, K., Wabitsch, M., Kunze, D., Geller, F., Geiß, H. C., Hesse, V., von Hippel, A., Jaeger, U., Johnsen, D., Korte, W., Menner, K., Müller, G., Müller, J. M., Niemann-Pilatus, A., Remer, T., Schaefer, F., Wittchen, H.-U., Zabransky, S., Zellner, K., Ziegler, A., Hebebrand, J.
Perzentile für den Body-mass-Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. Monatsschr. Kinderheilkd. 149, 807-818 (2001).

Raben, A., Astrup, A., Vasilaras, T. H., Prentice, A. M., Zunft, H.-J. F., Formiguera, X., Verboeket-van de Venne, W. P., Poppitt, S. D., Seppelt, B., Johnston, S., Keogh, G. F., Saris, W. H.: [The CARMEN trial: increased intake of carbohydrates--simple or complex--and unchanged blood lipids in overweight subjects]. Ugeskr. Laeger. 164, 627-631 (Danish) (2002).

Ulbricht, G.: Einstellungen zum Obst- und Gemüseverzehr und ihr Einfluß auf die Ernährungsbilanz. Ern. Umschau 49, 305-308 (2002).

Ulbricht, G.: Nutritional information and consumer behaviour - their relationship to nutritional balance. A study conducted in Berlin. Food Service Technol. 2, 5-11 (2002).

Zunft, H.-J. F., Lüder, W., Harde, A., Haber, B., Graubaum, H. J., Gruenwald, J.: Carob pulp preparation for treatment of hypercholesterolemia. Adv. Ther. 18, 230-236 (2001).

Übersichtsarbeiten/Reviews

Koebnick, C., Heins, U., Hoffmann, I., Leitzmann, C.: Die Bedeutung von Vitamin B12 in der Schwangerschaft und daraus resultierende Empfehlungen für die Schwangerschaftsvorsorge. Geburtshilfe Frauenheilk. 62, 227-33 (2002).

Zunft, H.-J. F., Seppelt, B.: Einfache oder komplexe Kohlenhydrate: Welche sollen bevorzugt werden? Gordian. Int. J. Food Technol. 100, 183-185 (2001).

Buchbeiträge/Articles in Books

Ulbricht, G., Zunft, H.-J. F.: Stichworte zu Ernährungspolitik, -ökonomie und -epidemiologie. In: Lexikon der Ernährung, Spektrum Akademischer Verlag, Heidelberg Berlin, (2001).

Zunft, H.-J. F.: Functional Food in der Prävention und Therapie der Adipositas. In: Erbersdobler, H. F., Meyer, A. H. (eds.) Praxishandbuch Functional Food, Behr's Verlag, Hamburg, Abschnitt 10.2, S. 1-28 (2001).

Zunft, H.-J. F. (Übersetzung und Bearbeitung): Nährstoffbedarf und Ernährungsbewertung; Mangelernährung und Beurteilung des Ernährungszustandes, Vitamine und Spurenelemente - Mangel und Überschuss, Adipositas; Essstörungen. In: Haimsons Innere Medizin, ABW Wissenschaftsverlag, 15. Auflage, Band 1, Teil 5, Kap. 73-75, 77, 78, S. 499-522, 533-545 (2002).

Sonstige/Others

Moeseneder, J. M., Seppelt, B., Zunft, H.-J. F.: Ad libitum consumption of a low-fat diet rich in dairy products, fruits and vegetables fails to reduce blood pressure in German adults. FASEB J. 15, A275 (2001).

Seppelt, B., Zunft, H.-J. F.: Body weigth development 6 years after a dietary intervention with low-fat products. Int. J. Obes. 25, Suppl. 2, S51 (2001).

Ulbricht, G.: Relationships between nutritional behaviour and nutrient intake - results of a study, Berlin 1997. In: Edwards, J. S. A., Hewedi, M. M. (eds.) Culinary Arts and Sciences III. Global and Nutritional Perspectives. Bournemouth University (2002).

Ulbricht, G.: Werden regionale Produkte vom Verbraucher bevorzugt? Ergebnisse einer Konsumentenbefragung in Berlin. Berichte der Bundesforschungsanstalt für Ernährung, Karlsruhe, 157-166 (2002).

Abteilung Epidemiologie

Leitung: PD Dr. Heiner Boeing

Das wissenschaftliche Forschungsfeld der Abteilung Epidemiologie ist die Beziehung zwischen Lebensstilfaktoren, biologischen Parametern und Erkrankungsrisiko.
Der besondere Schwerpunkt liegt dabei auf Krebserkrankungen, Herz-Kreislauferkrankungen, Metabolischem Syndrom und Diabetes mellitus.
Ein weiteres Forschungsgebiet ist die Rolle der Veränderung von Ernährungsgewohnheiten und anderer Lebensstilfaktoren für die Entstehung von chronischen Erkrankungen. Methodische Fragestellungen prägen einen Teil des wissenschaftlichen Profils; es gilt, die Qualität der Aussagen zu Ätiologie und Prävention der untersuchten Erkrankungen zu verbessern.

Abbildung 1
Die zu Beginn der EPIC-Studie entnommenen Blutproben wurden in Flüssigstickstoff eingelagert und stehen für biochemische und molekulargenetische Untersuchungen zur Verfügung.

EPIC-Potsdam-Studie

Manuela Bergmann, Wolfgang Fleischhauer, Eva Fischer

Die European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam-Studie, eine prospektive Kohortenstudie mit 27 548 Teilnehmern (Frauen im Alter von 35 bis 64 Jahren und Männer im Alter von 40 bis 64 Jahren), ist Teil einer europäischen Kohortenstudie mit insgesamt ca. 521 000 Studienteilnehmern. Ziel ist, den Einfluss der Ernährung auf die Entstehung von Krebs und andere chronische Erkrankungen zu erforschen.

Nachbeobachtung

Die 1994 begonnene EPIC-Potsdam-Studie ist als Langzeitstudie mit einer Nachbeobachtungszeit von 20 Jahren konzipiert worden. Im Studienzeitraum werden sowohl Daten über das Auftreten von neuen Erkrankungen als auch über den weiteren Verlauf der einmal gemessenen Faktoren (Rauchen, Ernährung, körperliche Aktivität, Medikamenten- und Hormoneinnahme usw.) gesammelt, das Spektrum der erfragten Erkrankungen umfasst 24 chronische Erkrankungen. Der Kontakt zu den Studienteilnehmern erfolgt mit Fragebögen, die maschinell eingelesen und bei Bedarf telefonisch ergänzt werden. In den ersten beiden Runden der Nachbeobachtung (2-Jahresabstand) haben jeweils 96 % der Teilnehmer geantwortet. Gegenwärtig wird der dritte Fragebogen versendet, der einen überarbeiteten Ernährungsfragebogen enthält. Zur Erhebung medizinisch korrekter Erkrankungsdiagnosen arbeitet die Studie mit Gesundheitsämtern, Ärzten, Kliniken und anderen Organisationen eng zusammen.

Department of Epidemiology

Head: PD Dr. Heiner Boeing

The field of research of the Department of Epidemiology is the relation between lifestyle factors, biological parameters, and the risk of chronic diseases. The focus will be mainly on various cancer sites, cardiovascular heart disease, diabetes, and the metabolic syndrome. Another area of interest is the change of dietary habits and other lifestyle factors over time in relation to chronic diseases. Methodological issues are part of the scientific profile of the department with the overall aim to improve the quality of results concerning the etiology and prevention of diseases.

The EPIC-Potsdam Study

The European Prospective Investigation into Cancer and Nutrition (EPIC) Potsdam Study, a prospective cohort study with 27 548 participants (women, age 35-64 years, and men, age 40-64 years), is part of a European cohort study with around 521 000 subjects. The aim of the study is to investigate the effect of nutrition on the development of cancer and other chronic diseases.

Follow-up

The EPIC Potsdam Study started in 1994 and is a long term study with a follow up period of about 20 years. During this time period, data on the incidence of diseases as well as changes in factors assessed during baseline examination (smoking status, diet, physical activity, medication and hormone use) are generated. Occurence data are collected for 24 chronic diseases from study participants. Contact with the study participants is maintained with mailed follow up questionnaires, which are scanned when returned and complemented with information obtained via telephone contact. The response rate of the first two follow-up questionnaires (sent every other year) was 96%. Currently, the third follow-up questionnaire, which includes a revised food frequency question-naire, is being mailed. The EPIC Potsdam Study collaborates with a number of local health-related institutions to determine if diseases reported have been diagnosed correctly.

Biological samples

During the baseline examination, 1994-1998, blood samples (30 ml) were fractioned and divided into portions, and frozen in -196 °C liquid nitrogen. A portion of the remaining material was also stored at -80 °C (Fig. 1). The assessment of the genetic factors affecting the development of diseases requires the exact isolation of DNA material. Up until now, DNA has been extracted from approximately 5000 blood samples from incident cases of chronic diseases. The variability of the genetic information and its association with both intermediate biomarkers and disease risk will be examined.

Figure 1
Blood samples collected during the baseline examination samples were frozen in liquid nitrogen and may be used for biochemical and molecular-genetical research

International EPIC Study
Scientists from the 23 administrative EPIC centers in 10 countries analyze the data on an European level. Preliminary results are available for the most frequent cancer sites such as colorectal, breast, lung, prostate, stomach, kidney, and cervical cancer. In addition, the exposure factors such as anthropometric data, physical activity, hormone use, and reproductive behavior are assessed.

Results
Methodological research
The comparability of dietary assessments obtained by use of country specific food frequency questionnaires remains an important issue in dietary assessment studies. Therefore, standardized 24-hour dietary recalls were collected in a representative sample of around 8% of the EPIC study participants. These short term recalls serve as a reference standard for calibration of the food frequency questionnaire data. We developed a new non-linear calibration method to ensure that center-specific data are mixed correctly.

Another methodological problem is the assessment of dietary patterns reflecting specific dietary habits. Within a given population, dietary patterns integrate food items which are highly correlated with each other. The use of dietary patterns, instead of individual food items, may improve the statistical models when investigating diet-disease relations. In a project funded by the DFG, different analytical procedures in identifying unique dietary patterns were empirically compared.

Cancer
International research findings indicate that a healthy lifestyle (physical activity, weight control, diet rich in fruit and vegetables, no smoking and alcohol consumption) reduces the risk of cancer. For instance, weight gain is an important factor within the framework of cancer prevention. Recent descriptive analyses of the EPIC data (on a European level) were presented during an international conference on "Nutrition and Cancer" in Lyon, France, in 2001 (IARC Scientific Publications No. 156, 2002) and published in 15 additional articles (Public Health Nutrition, 2002;5(6B): 1147-1162) in December, 2002.

Figure 2
Relative risk of colorectal cancer associated with fibre intake. The relative risk was estimated using three different statistical models:
1-quintile values of fibre
2-fibre intake as a continuous variable
3-fibre intake as a continuous variable from the dietary questionnaires corrected for measurement errors

Source: Lancet 2003, 361:1496-501

Biologische Proben
Bei der Erstuntersuchung zwischen 1994 und 1998 wurden 30 ml Blut fraktioniert und in mehreren Portionen bei -196° C in Flüssigstickstoff eingefroren; ein Restblutanteil wurde zusätzlich bei -80°C eingelagert (Abb.1). Die Erfassung der Bedeutung genetischer Faktoren bei der Krankheitsentstehung setzt eine gezielte Aufarbeitung der Proben zur DNA-Gewinnung voraus. Eine Extraktion der DNA wurde inzwischen bei etwa 5000 Blutproben von Neuerkrankungsfällen vorgenommen, die Variabilität der genetischen Information und ihr Bezug zu intermediären Biomarkern und dem Krankheitsrisiko wird erforscht.

EPIC-Studie International
Die Daten werden auf europäischer Ebene von den Wissenschaftlern der 23 administrativen EPIC-Studienzentren der 10 beteiligten Länder ausgewertet.

Forschungsergebnisse
Methodische Fragestellungen
Kurt Hoffmann, Matthias Schulze, Ute Nöthlings, Dietmar Walter

Ein zentrales methodisches Problem bei Verzehrstudien ist die Vergleichbarkeit von Verzehrsmengen, die aus unterschiedlichen länderspezifischen Ernährungsfragebogen ermittelt werden. Um die Vergleichbarkeit der Daten herzustellen, haben ca. 8 % aller EPIC-Studienteilnehmer standardisierte 24-Stunden-Erinnerungsprotokolle ausgefüllt. Diese Kurzzeitmessungen dienen als Referenzstandard bei einer Kalibrierung der Fragebogendaten. Wir entwickelten ein neues nichtlineares Kalibrierungsverfahren, das nun ein korrektes Durchmischen der Daten verschiedener Zentren sicher stellt. Ein zweites methodisches Problem ist die Bestimmung von Ernährungsmustern, die typische Ernährungsweisen widerspiegeln.

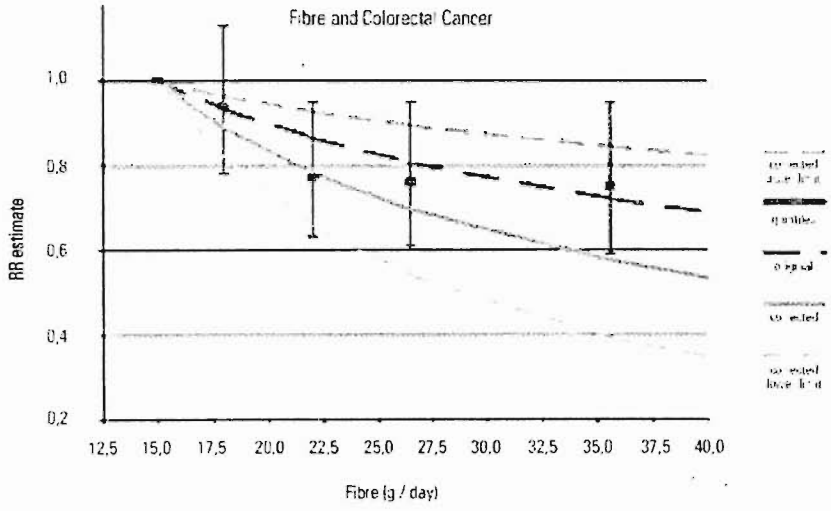


Abbildung 2
Relatives Risiko (RR) des kolorektalen Karzinoms in Zusammenhang mit der Ballaststoffzufuhr. Das relative Risiko wurde anhand von drei verschiedenen statistischen Modellen berechnet:
1. Ballaststoffzufuhr berechnet als kategorische Größe sog. Quintile
2. Ballaststoffzufuhr berechnet als stetige Größe
3. Ballaststoffzufuhr berechnet als stetige Größe und korrigiert für Messfehler.
Nach: Lancet 2003, 361:1496-501

Für häufig auftretende Krebslokalisationen wie Darm, Brust, Lunge, Prostata, Magen, Niere, Gebärmutterhals liegen erste Ergebnisse vor. Außerdem werden Expositionsfaktoren wie Anthropometrie, körperliche Aktivität, Hormoneinnahme und reproduktives Verhalten erfasst.

Sie fassen Lebensmittel zusammen, deren durchschnittliche Verzehrsmengen in der Population hoch korreliert sind. Die Verwendung von Ernährungsmustern anstelle einzelner Lebensmittel bei der Modellierung des Zusammenhanges von Ernährung und chronischen Erkrankungen kann zu einer Verbesserung der Modelle führen. Im Rahmen eines DFG-Projekts wurden die verschiedenen Methoden zur Herleitung von Ernährungsmustern miteinander empirisch verglichen.

Krebserkrankungen
Petra Lahmann, Heiner Boeing, Anja Kroke, Marjorlein Haftenberger

Mittlerweile zeigen weltweit gewonnene Forschungsergebnisse, dass ein gesunder Lebensstil (körperliche Bewegung, Gewichtskontrolle, obst- und gemüsereiche Ernährung, Nikotinverzicht, kein Alkohol) das Krebsrisiko senkt. So ist z.B. die Gewichtszunahme in der Bevölkerung auch im Rahmen der Krebsprävention ein wichtiger Faktor. Erste beschreibende Auswertungen der EPIC-Daten auf europäischer Ebene wurden auf einer internationalen Tagung über das Thema "Ernährung und Krebs" in Lyon, Frankreich, im Jahr 2001 präsentiert (IARC Scientific Publications No. 156, 2002) und in weiteren 15 Artikeln zusammengefasst, die in der Zeitschrift Public Health Nutrition im Dezember 2002 veröffentlicht wurden.

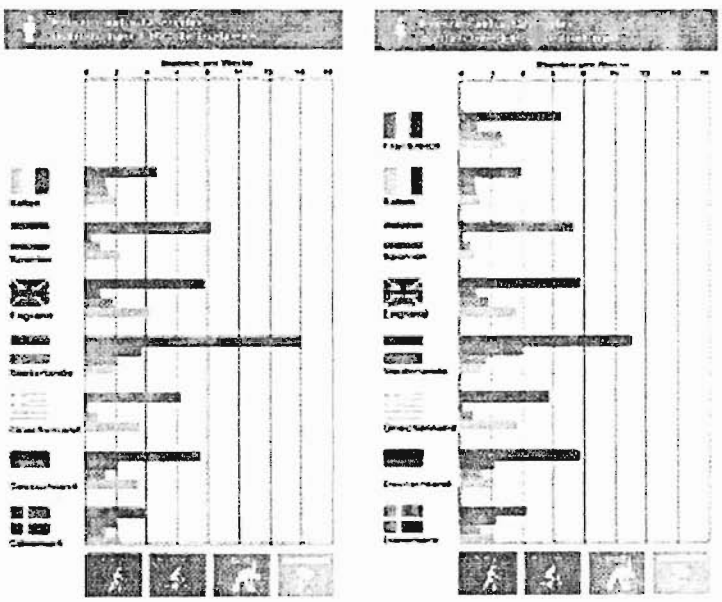


Abbildung 3
Freizeitaktivitäten in den Ländern der EPIC-Studie

In der gesamtdeutschen Kohorte sind bis Ende 2002 insgesamt 1482 Krebserkrankungen aufgetreten, davon 773 in der Potsdamer Kohorte. Für die ersten prospektiven Analysen der EPIC-Daten standen die Bedeutung der Ballaststoffzufuhr und des Obst- und Gemüseverzehrs im Mittelpunkt verschiedener Auswertungen. Die Ergebnisse der für die Messfehler bereinigten EPIC-Auswertung zeigen, dass sowohl bei Männern als auch Frauen eine erhöhte Ballaststoffzufuhr von durchschnittlich 13,0 g/Tag auf mehr als 36,0 g/Tag mit einem bis zu 40 % erniedrigten Darmkrebsrisiko assoziiert ist (Abb.2). Die Herkunft des Ballaststoffes spielt hierbei keine besondere Rolle. Abhängig von der Krebslokalisation sind die Ergebnisse der EPIC-Studie hinsichtlich des Obst- und Gemüseverzehrs unterschiedlich.
Beim Lungenkarzinom lag eine Risikosenkung von 39 % durch erhöhten Obstverzehr (69,0 g versus > 490,0 g/Tag) vor. Für Gemüseverzehr hingegen war keine Wirkung erkennbar. Bei Karzinomen des oberen Verdauungstraktes wurde eine risikosenkende Wirkung der Obst- und Gemüsezufuhr (287 g versus > 456 g/Tag) von 45 % beobachtet, während eine Obst- und Gemüseassoziation beim Prostata- und Brustkrebs nicht festgestellt werden konnte. Ergebnisse zum Zusammenhang zwischen Adipositas und Brustkrebs zeigen, dass bei postmenopausalen Frauen, die sich keiner Hormonersatztherapie unterziehen, Übergewicht ein signifikanter und unabhängiger Risikofaktor für Brustkrebs ist. In einigen wenigen Studien konnte gezeigt werden, dass körperliche Aktivität einen protektiven Effekt auf die Brustkrebsentstehung hat. Dies soll gezielt in der EPIC-Kohorte weiter untersucht werden. Unterschiede im Umfang der Freizeitaktivität werden innerhalb der beteiligten Länder der EPIC-Studie sehr deutlich sichtbar (Abb.3).

Within the two German EPIC cohorts, 1482 incident cancer cases were identified until the end of 2002, including 773 cases in the Potsdam cohort.
The first EPIC analyses focused primarily on investigating the impact of fiber or of fruit and vegetable intake. The findings, corrected for measurement error, indicate that an increase in fiber intake from 13.0 g/day on the average to more than 36.0 g/day is associated with an up to 40% reduced risk of colorectal cancer in both European men and women (Fig.1), irrespective of fiber source. The EPIC findings on fruit and vegetable intake show different relations, depending on the cancer site. A protective effect of 39% risk reduction by increased fruit consumption, (69.0 g versus >490.0 g/day) but not of vegetables was observed for lung cancer. Fruit and vegetables reduced the risk for carcinoma of the upper gastro intestinal tract by 45% (287 g versus >456g/day), this effect was not observed for prostate or breast cancer.

The investigation of body weight and breast cancer risk showed that among postmenopausal women who are not using hormonal replacement therapy, obesity is a significant and independent risk factor for breast cancer. In a few studies it has been reported that physical activity reduces the risk of breast cancer. This aspect will be studied in more depth within the EPIC cohort. The range of leisure physical activity varies substantially among the countries involved in the EPIC Study (Fig. 2).

Left over samples from the VERA Study (biological samples from 2000 subjects) were used for a project on breast cancer. In collaboration with Dr. T. Stürmer and co-workers from the research group headed by Prof. H. Brenner, Zentrum für Altersforschung (Center for Gerontology) in Heidelberg, we were able to identify a genetically determined alloenzyme of the alcohol dehydrogenase that modifies the relation between alcohol consumption and breast cancer risk. In a case control study of colorectal cancer, which was done in collaboration with the Department of Toxicology (DIfE) and a research group from Barcelona, an interaction between the effect of alcohol consumption and alloenzymes of sulfotransferases was documented.

Cardiovascular disease
Cardiovascular diseases - specifically coronary heart disease (CHD) and stroke - are the major causes of death in Germany. Nutrition and lifestyle are affecting the main risk factors for CHD. In the EPIC-Potsdam Study we investigated the relation between dietary patterns and risk of hypertension in 172 incident cases of hypertension. This study was funded by the DFG. A dietary pattern with a high intake of fruit, vegetables, and milk products appears to be associated with a lower risk of hypertension.
Another project based on data of the EPIC-Potsdam

Figure 3
Leisure physical activity in the countries involved in the EPIC-study

cohort is conducted within the BMBF network project "Molecular nutrition research" in collaboration with the University of Magdeburg. Genetic determinants of the homocysteine and folic acid metabolism are used to examine the relation between nutritional and lifestyle factors and the risk of myocardial infarction or stroke.

A population based case control study (Coronare Risikofaktoren für Arteriosklerose bei Frauen / in women from the city of Hamburg was conducted in collaboration with Prof. E. Windler, University Hospital Hamburg Eppendorf. Two hundred cases of cardiovascular heart disease and 255 control women were interviewed in 1999 and 2000. The findings can be translated into a number of preventive measures concerning coronary heart disease in women: a diet low in meat, meat products, and other calorically dense foods but high in fruits and vegetables, regular physical activity, and no smoking are beneficial.

Diabetes and the metabolic syndrome

Important results regarding the pathogenesis of type 2 diabetes were generated in collaboration with the Department of Clinical Nutrition (DIfE). Type 2 diabetes is characterized by a marked insulin resistance due to impaired insulin secretion and decreased plasma insulin concentrations. The research hypothesis under investigation was that type 2 diabetes is a manifestation of an ongoing inflammatory acute phase response that is characterized by alterations in so-called acute phase proteins.

Findings of a case-control study embedded in the EPIC-Potsdam Study indicate that an increase of inflammatory markers in the blood, such as interleukin IL 6 and IL-1β, is associated with an elevated risk of type 2 diabetes. In contrast, adiponectin, an adipocyte derived peptide, reduces the risk. Furthermore, the significance of genetically induced enzyme variants will be studied.

Another complementary study on diabetes is being done in collaboration with Prof. Schrezenmeir and the research group in the network "Molecular nutrition research" in Kiel. The project includes participants in the EPIC Potsdam Study who have been type 2 diabetes patients for several years. The research objective is to examine proteins responsible for transport and binding of fatty acids and their genetic polymorphisms. We study lifestyle factors in relation to the metabolic syndrome, taking into account biochemical and molecular characteristics.

Overweight is an established risk factor for the metabolic syndrome. However, there is lack of prospective data on weight development and the associated manifestations of the metabolic syndrome. Preliminary results of the EPIC Potsdam Study indicate that weight gain in early adulthood (age 25-40 years) has a greater effect with regard to the risk of diabetes than does weight gain after the age of 40 years (38% and 21% increased risk, respectively).

Durch Nutzung der Restseren der VERA-Studie (Biologische Materialien und biochemische Messgrößen von 2000 Probanden liegen vor) als Referenz wurde von T. Stürmer und Mitarbeitern aus der Arbeitsgruppe von Prof. H. Brenner, Zentrum für Altersforschung Heidelberg, und uns der Nachweis erbracht, dass ein genetisch bedingtes Alkoholdehydrogenase das Erkrankungsrisiko für Brustkrebs in Verbindung mit der Alkoholaufnahme modifiziert. Mit der Abteilung Toxikologie des DIfE und einer Gruppe aus Barcelona konnten im Rahmen einer Fall-Kontroll-Studie mit Dickdarmkarzinompatienten eine Interaktion zwischen der Alkoholwirkung und Allozymen der Sulfotransferasen entdeckt werden.

Herz-Kreislauferkrankungen
Kerstin Klipstein-Grobusch, Dagmar Drogan, Manuela Bergmann

Arteriosklerotische Herz-Kreislauf-Erkrankungen – insbesondere die koronare Herzkrankheit (KHK) und der Schlaganfall – zählen in Deutschland zu den führenden Todesursachen. Ernährung und Lebensstil spielen hinsichtlich der Beeinflussung klassischer Risikofaktoren für die KHK eine zentrale Rolle. In einer von der DFG geförderten Studie wurde die Beziehung von Ernährungsmustern zum Hypertonierisiko prospektiv an 172 inzidenten Hypertonikern in der EPIC-Potsdam-Studie untersucht. Insbesondere ein Ernährungsmuster, das durch hohe Aufnahmen von Obst, Gemüse und Milchprodukten gekennzeichnet ist, scheint mit einem verminderten Hypertonierisiko assoziiert zu sein.
Im Rahmen eines BMBF-Netzwerkprojektes zur "Molekularen Ernährungsforschung" mit der Universität Magdeburg werden genetische Determinanten des Homocysteïn- und Folsäuremetabolismus in der EPIC-Potsdam-Studie für Untersuchungen zur Beziehung von Ernährungs- und Lebensstilfaktoren und dem Myokard- bzw. Schlaganfallrisiko herangezogen. Gemeinsam mit Prof. E. Windler vom Universitätsklinikum Hamburg-Eppendorf wurde eine populationsbezogene Fall-Kontroll-Studie (Coronare Risikofaktoren für Arteriosklerose bei Frauen) in Hamburg durchgeführt. 200 an Herz-Kreislauferkrankungen erkrankte Frauen und 255 Kontrollpersonen wurden 1999 und 2000 befragt. Die Ergebnisse zeigen eine Reihe realisierbarer Maßnahmen zur Prävention von Herz-Kreislauf-Erkrankungen bei Frauen auf: eine Ernährung mit weniger Fleisch, Wurst und anderen kaloriendichten Lebensmitteln zugunsten von mehr Obst und Gemüse, begleitet von regelmäßiger körperlicher Aktivität und Verzicht auf Nikotin ist von Vorteil.

Diabetes mellitus und Metabolisches Syndrom
Heiner Boeing, Anja Kroke, Anja Schienkiewitz

Wichtige Ergebnisse zur Pathophysiologie des Diabetes mellitus Typ-2 konnten in Zusammenarbeit mit der Abteilung Klinische Ernährung des DIfE gefunden werden. Gekennzeichnet durch einen relativen Insulinmangel aufgrund einer Störung der Insulinsekretion, geht der Typ-2-Diabetes gewöhnlich mit einer mehr oder weniger ausgeprägten Insulinresistenz einher. Der Vermutung, dass der Typ-2-Diabetes Folge einer andauernden entzündlichen Akut-Phase-Reaktion ist, deren Veränderungen in den Akut-Phase-Proteinen sichtbar wird, wurde zuerst nachgegangen.

In einer in die EPIC-Potsdam-Studie eingebetteten Fall-Kontroll-Studie konnte gezeigt werden, dass erhöhte Entzündungsparameter im Blut, wie Interleukin IL-6, IL-1β mit einem größeren Erkrankungsrisiko für Typ-2-Diabetes assoziiert sind.
Ebenso konnte gezeigt werden, dass z.B. andere Stoffwechselprodukte der Fettzelle wie das Adiponectin risikosenkend wirken. Von wissenschaftlichem Interesse ist auch die Bedeutung genetisch bedingter Enzymvarianten. Ergänzt wird die epidemiologische Erforschung des Krankheitsbildes Typ-2-Diabetes durch die Zusammenarbeit mit Prof. Schrezenmeir und der Forschergruppe im Netzwerk "Molekulare Ernährungsforschung Kiel".

Die gemeinsamen Untersuchungen erstrecken sich auf die Studienteilnehmer der EPIC-Potsdam-Studie, die schon länger an Typ-2-Diabetes erkrankt sind. Erforscht werden Transport- und Bindungsproteine der Fettsäuren und deren genetische Varianten.
Für das Metabolische Syndrom untersuchen wir Lebensstilfaktoren unter Berücksichtigung der biochemischen und molekulargenetischen Befunde. Während die Bedeutung des Übergewichts als Risikofaktor unumstritten ist, gibt es z. B. kaum prospektive Daten zu Gewichtsentwicklung und Diabetesrisiko. Die bisherigen Auswertungen der EPIC-Potsdam-Kohorte dazu ergaben, dass ein Gewichtsanstieg im frühen Erwachsenenalter zwischen 25 und 40 Jahren einen größeren Einfluss auf das Erkrankungsrisiko besitzt, einen Typ-2-Diabetes zu entwickeln, als der Gewichtsanstieg nach dem 40. Lebensjahr (38 % bzw. 21 % erhöhtes Risiko).

Ausgewählte Publikationen
Selected Publications

Originalarbeiten/Original Papers

Berg, G., Bode, G., Blettner, M., Boeing, H., Brenner, H.: Helicobacter pylori infection and serum ferritin: A population-based study among 1806 adults in Germany. Am. J. Gastroenterol. 96, 1014-1018 (2001)

Bianchini, F., Jeckel, A., Vineis, P., Martinez-Garcia, C., Elmstahl, S., van Kappel, A. L., Boeing, H., Chishima, H., Riboli, E., Kaaks, R.: Inverse correlation between alcohol consumption and lymphocyte levels of 8-hydroxydeoxyguanosine in humans. Carcinogenesis 22, 885-890 (2001).

Bingham, S. A., Day, N. E., Luben, R., Ferrari, P., Slimani, N., Norat, T., Clavel-Chapelon, F., Kesse, E., Nieters, A., Boeing, H., Tjonneland, A., Overvad, K., Martinez, C., Dorronsoro, M., Gonzalez, C. A., Key, T. J., Trichopoulou, A., Naska, A., Vineis, P., Tumino, R., Krogh, V., Bueno-de-Mesquita, H. B., Peeters, P.H.M., Berglund, G., Hallmans, G., Lund, E., Skeie, G., Kaaks, R., Riboli, E.: Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. Lancet Vol 361, 1496-1501 (2003).

Dierkes, J., Jeckel, A., Ambrosch, A., Westphal, S., Luley, C., Boeing, H.: Factors explaining the difference of total homocysteine between men and women in the European Investigation Into Cancer and Nutrition Potsdam Study. Metabolism 50, 640-645 (2001).

Hoffmann, K., Kroke, A., Klipstein-Grobusch, K., Boeing, H.: Standardization of dietary intake measurements by nonlinear calibration using short-term reference data. Am. J. Epidemiol. 156, 862-870 (2002).

Hoffmann, K., Boeing, H., Dufour, A., Volatier, J. L., Telman, J., Virtanen, M., Becker, W., De Henauw S.: Estimating the distribution of usual dietary intake by short-term measurements. Eur. J. Clin. Nutr. 56, Suppl 2, S53-S62 (2002).

Imhof, A., Froehlich, M., Brenner, H., Boeing, H., Pepys, M. B., Koenig, W.: Effect of alcohol consumption on systemic markers of inflammation. Lancet 357 (9258), 763-767 (2001).

Klipstein-Grobusch, K., den Breeijen, J. H., Grobbee, D. E., Boeing, H., Hofman, A., Witteman, J. C.: Dietary antioxidants and peripheral arterial disease: The Rotterdam Study. Am. J. Epidemiol. 154, 145-149 (2001)

Kroke, A., Klipstein-Grobusch, K., Hoffmann, K., Terbeck, I., Boeing, H., Helander, A.: Comparison of self-reported alcohol intake with the urinary excretion of 5-hydroxytryptophol, 5-hydroxyindole-3-acetic acid, a biomarker of recent alcohol intake. Br. J. Nutr. 85, 621-627 (2001)

Kroke, A., Liese, A. D., Schulz, M., Bergmann, M. M., Klipstein-Grobusch, K., Hoffmann, K., Boeing, H.: Recent weight changes and weight cycling as predictors of subsequent two years' weight change in a middle-aged cohort. Int. J. Obes. 26, 403-409 (2002).

Oveser, L., Boeing, H.: The use of biomarkers in multicentric studies with particular consideration of iodine, sodium, iron, folate, and vitamin D. Eur. J. Clin. Nutr. 56, Suppl 2, S12-S17 (2002).

Riboli, E., Hunt, K.J., Slimani, N., Ferrari, P., Norat, T., Fahey, M., Charrondiere, U. R., Hemon, B., Casagrande, C., Vignat, J., Overvad, K., Tjonneland, A., Clavel-Chapelon, F., Thiebaut, A., Wahrendorf, J., Boeing, H., Trichopoulos, D., Trichopoulou, A., Vineis, P., Palli, D., Bueno-De-Mesquita, H.B., Peeters, P.H., Lund, E., Engeset, D., Gonzalez, C.A., Barricarte, A., Berglund, G., Hallmans, G., Day, N.E., Key, T.J., Kaaks, R., Saracci, R., EPIC study populations and data collection. Public Health Nutr. 1113-1124 (2002).

Schulz, M., Kroke, A., Liese, A. D., Hoffmann, K., Bergmann, M. M., Boeing, H.: Food groups as predictors for short-term weight changes in men and women of the EPIC-Potsdam cohort. J. Nutr. 132, 1335-1340 (2002)

Schulze, M. B., Hoffmann, K., Kroke, A., Boeing, H.: Dietary patterns and their association with food and nutrient intake in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study. Br. J. Nutr. 85, 363-373 (Erratum: Br. J. Nutr. 87 (2002), 279) (2001)

Stürmer, T., Wang-Gohrke, S., Arndt, V., Boeing, H., Kong, X., Kreienberg, R., Brenner, H.: Interaction between alcohol dehydrogenase II gene, alcohol consumption, and risk for breast cancer. Br. J. Cancer 87, 519-523 (2002).

van den Brandt, P., Voorrips, L., Hertz-Picciotto, I., Shuker, D., Boeing, H., Speijers, G., Guittard, C., Kleiner, J., Knowles, M., Wolk, A., Goldbohm, A.: The contribution of epidemiology. Food Chem. Toxicol. 40, 387-424 (2002)

Drittmittelprojekte
External Funding

Titel: Langzeitstudie zum Einfluss der Ernährung auf die Entstehung von Krebserkrankungen: European Prospective Investigation into Cancer and Nutrition EPIC
Finanzierung: Deutsche Krebshilfe
Laufzeit: 01/00-09/06

Titel: The role of diet in the aetiology of Crohn's disease and ulcerative colitis - a pilot study in a European prospective cohort study
Finanzierung: Kooperation mit Norwich Health Care NHS Trust, Großbritannien
Laufzeit: 07/99-12/01

Titel: Ernährungsmuster als neuer Ansatz zum Verständnis der Rolle der Ernährung in der Ätiologie der essentiellen arteriellen Hypertonie/Nutrional pattern as a new approach to understanding the role of nutrition in the etiology of essential arterial hypertension
Finanzierung: DFG
Laufzeit: 03/00-05/02

Titel: EPIC Studie: European Prospective Investigation into Cancer and Nutrition
Finanzierung: EU
Laufzeit: 04/99-08/03

Titel: EPIC ELDERLY: concerted action: The role of diet on the longevity of elderly Europeans - A study in the context of the European Prospective Investigation into Cancer and Nutrition
Finanzierung: EU
Laufzeit: 01/02-12/04

Titel: Prospektive epidemiologische Untersuchungen zum Einfluss von Ernährungsfaktoren auf das osteoporotische Frakturrisiko
Finanzierung: Institut Danone für Ernährung
Laufzeit: 2001

Titel: Genetische Determinanten des Homocystein und Folsäuremetabolismus, Netzwerk Molekulare Ernährungsforschung: Koordinator Magdeburg
Finanzierung: BMBF
Laufzeit: 02/01-01/05

Titel: Nahrungsfette und Stoffwechsel - Genvariabilität, -regulation, -funktion und funktionelle Lebensmittelinhaltsstoffe, Netzwerk Molekulare Ernährungsforschung: Koordinator Kiel
Finanzierung: BMBF
Laufzeit: 04/02-03/05

Titel: Untersuchung der Lebensqualität von Patienten mit rheumatischer Arthritis und Spondylarthritis
Finanzierung: Industrie über Universität Erlangen
Laufzeit: 08/01-01/03

Titel: Tumorentstehung - hemmende und fördernde Ernährungsfaktoren (Beitrag zum Ernährungsbericht 2004)
Finanzierung: Bundesanstalt für Landwirtschaft und Ernährung
Laufzeit: 12/02-05/03

Leitung: Prof. Dr. Hans-Rudolf Glatt

Department of Nutritional Toxicology

Head: Prof. Dr. Hans Rudolf Glatt

Food not only consists of nutrients, but also contains natural and anthropogenic ***non-nutritive*** components ***(xenobiotics)***. Xenobiotics are major factors which determine smell, taste and look of foods. Many may be absorbed and interfere with functions of the organism. Independent of whether these effects are favorable or harmful in the individual case, an accumulation of xenobiotics has to be avoided. The elimination of xenobiotics usually involves their structural transformation. Although this ***biotransformation*** means a detoxification in principle, it can lead to highly toxic metabolites in some cases. Biotransformation is therefore a central aspect for understanding toxicological effects, in particular as it is extremely variable.

The studies in this department are aimed at the determination of natural and anthropogenic toxic substances in food, the elucidation of their mechanism of action, and the assessment of the type and extent of the resulting health risks. In addition, individual genetic factors and nutritional effects which enhance or reduce the risks should be recognized. Toxicological effects mediated by chemically reactive metabolites are of special interest because, even at low exposure, they may lead to irreversible and cumulating damage. The pathophysiological consequences of this damage – such as cancer, degenerative changes, allergy and malformation or inherited damage in descendants – are only manifested after a latency period, which can be several years or decades or sometimes even generations. Therefore, it is almost impossible to elucidate the causal relationships only by using epidemiological methods. Mechanism based biomarkers for disposition, exposure, internal load and early pathophysiological changes are devised and integrated, in cooperation with the Department of Epidemiology, in studies on relationships between diet and health.

Recombinant test systems for toxicological investigations

Biotransformation is determined primarily by the xenobiotic metabolizing enzymes, whose levels and characteristics may extremely vary between species and tissues, and are also influenced by the individual genetic constitution, diet and other environmental factors. Due to the decisive role of biotransformation, these variations have to be taken into account in experimental and epidemiological investigations. Moreover, an appropriate biotransformation is a fundamental prerequisite for the usage of bacteria and cells in culture for toxicological investigations. Genetic engineering allows the expression of defined enzymes from human directly in cells used for toxicological studies.

Figure 1
Mutagenicity of nitrofen to Salmonella typhimurium TA1538 (circles) and a TA1538-derived strain expressing human sulfotransferase 1A1 (triangles). The decrease in the number of revertant colonies at high dose levels is due to toxicity.

Lebensmittel enthalten nicht nur *Nährstoffe*, sondern auch *nicht-nutritive Komponenten (Fremdstoffe)*. Diese können natürlichen oder anthropogenen Ursprungs sein und unter anderem Geruch, Geschmack und Aussehen von Nahrungsmitteln bestimmen. Viele Fremdstoffe werden absorbiert und wirken auf den Organismus ein. Unabhängig davon, ob die Wirkung im Einzelfall günstig oder ungünstig ist, muss eine Akkumulation vermieden werden. Die Elimination von Fremdstoffen ist oft mit einer stofflichen Umwandlung verbunden. Diese *Biotransformation* bedeutet in der Regel eine Entgiftung, kann aber im Einzelfall zur Bildung von stark toxischen Produkten führen. Sie ist ein zentraler Aspekt für das Verständnis vieler toxikologischer Wirkungen, zumal sie hoch variabel ist.

Die Arbeit der Abteilung hat das Ziel, natürliche und anthropogene Schadstoffe in der Nahrung zu erfassen, deren Wirkungsmechanismen aufzuklären und Art und Höhe der sich ergebenden Gesundheitsrisiken abzuschätzen. Zudem sollen individuelle genetische Faktoren und Nahrungseinflüsse erkannt werden, die Risiken verstärken oder vermindern. Von besonderem Interesse sind Wirkungen, die durch chemisch reaktive Metaboliten vermittelt sind, da diese bereits bei niedriger Exposition zu irreversiblen und kumulierenden Schäden führen können. Die pathophysiologischen Folgen – wie Krebs, degenerative Veränderungen, Allergien und Missbildungen oder Erbschäden in Nachkommen – manifestieren sich dabei typischerweise erst nach einer Latenzzeit, die Jahre, Jahrzehnte oder sogar mehrere Generationen betragen kann. Dies erschwert das Erfassen von ätiologischen Zusammenhängen mit epidemiologischen Methoden. Auf Mechanismen basierende Biomarker für Disposition, Exposition, interne Belastung und frühe pathophysiologische Wirkungen werden in Zusammenarbeit mit der Abteilung Epidemiologie in Untersuchungen über Zusammenhänge von Ernährung und Gesundheit integriert.

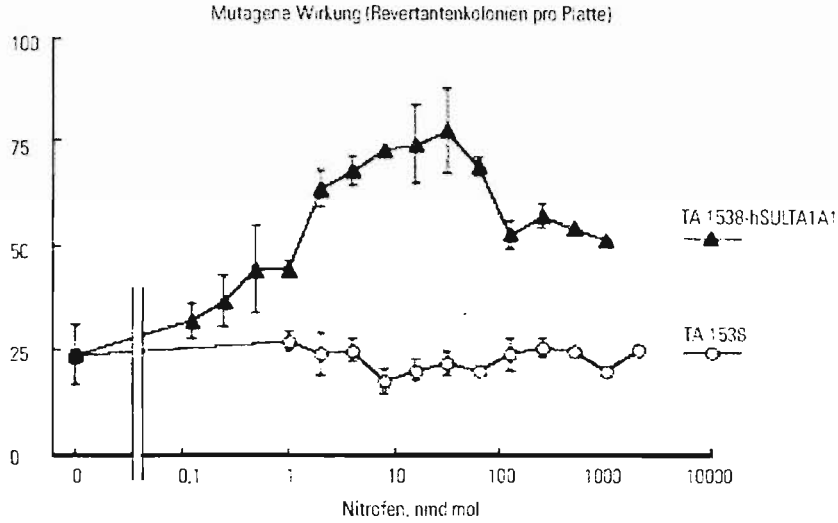


Abbildung 1
Mutagenität von Nitrofen im konventionellen Stamm *Salmonella typhimurium* TA1538 (Kreise) und einem davon abgeleiteten Stamm, der die humane Sulfotransferase 1A1 exprimiert (Dreiecke). Die Abnahme der Zahl der Mutanten im Stamm TA1538-hSULT1A1 bei hohen Dosen beruht auf Toxizität.

Rekombinante Testsysteme für toxikologische Untersuchungen

Eva Muckel, Yungang Liu, Walter Meinl, Susanne Schlichter, Yasmin Sommer, Ulrike Pabel

Die Biotransformation wird in erster Linie durch *fremdstoffmetabolisierende Enzyme* bestimmt. Je nach Spezies, Gewebe, individuellen genetischen Faktoren, Ernährungsweise und anderen Umwelteinflüssen kann

die Ausstattung mit diesen Enzymen und damit die Empfindlichkeit gegenüber Schadstoffen extrem unterschiedlich sein. Die Berücksichtigung der Biotransformation ist außerdem für die In-vitro-Toxikologie unentbehrlich. Hierzu exprimieren wir mit gentechnischen Methoden definierte Enzyme des Menschen in Zellen, die sich für toxikologische Studien eignen. Zur Zeit untersuchen wir vor allem die Sulfotransferasen (SULT), daneben auch Acetyltransferasen und Cytochrome P450. Die SULT zeichnen sich sowohl durch ein hohes Detoxifizierungs- wie auch ein hohes Toxifizierungspotenzial aus (in Abhängigkeit vom Wirkstoff). Wir haben bereits 18 SULT des Menschen und 17 SULT von Versuchstieren in In-vitro-Systemen exprimiert, eine humane Form haben wir überdies als Transgen in die Maus eingeschleust. Diese SULT waren in der Lage, allein oder in

Polymorphismen von SULT

Walter Meinl, Ronny Kollock, Heiko Schneider

Für mehrere SULT des Menschen sind genetische Polymorphismen nachgewiesen. SULT1A1 und 1A2 sind in der Lage, nahrungsrelevante Kanzerogene zu aktivieren und inaktivieren, wobei wir markante Unterschiede zwischen Alloenzymen entdeckten.

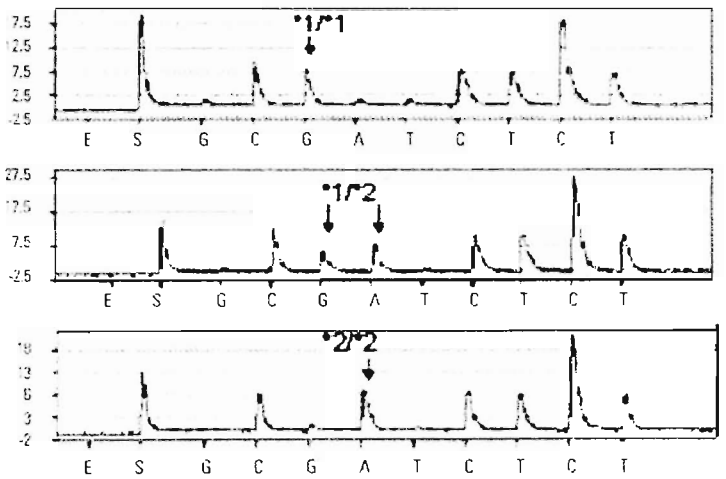


Abbildung 2
Genotypisierung der humanen Sulfotransferase 1A1 durch Pyrosequenzieren des Genabschnittes C^{638}GCTCCCT (kodiert Arg^{213}, hohe Enzymaktivität und -stabilität) bei Allel *1 und C^{638}ACTCCCT (kodiert His^{213}, reduzierte Aktivität/Stabilität) bei Allel *2. Gezeigt sind die Ergebnisse für die drei möglichen Genotypen (homozygot für eine der beiden Formen oder heterozygot). E, S: negative und positive Kontrolle; A, C, G, T: Zugabe des betreffenden Desoxyribonucleotids (Lichtsignal bei Übereinstimmung mit der Sequenz).

Kombination mit Cytochromen P450, über 100 Stoffe (wovon viele in Nahrungsmitteln vorkommen) zu Mutagenen zu aktivieren. Häufig erfolgte die Aktivierung eines bestimmten Stoffes durch eine einzige oder wenige SULT, z. B. nur durch ein humanes Enzym, aber nicht durch Rattenenzyme. So erwies sich das in die Schlagzeilen geratene Nitrofen als inaktiv in konventionellen *Salmonella typhimurium* Stämmen (den Zielzellen des am häufigsten eingesetzten Mutagenitätstests), doch war es bereits in niedrigen Dosierungen mutagen, wenn die SULT1A1 des Menschen im Teststamm exprimiert wurde. Eine wesentlich geringere Mutagenität wurde bei Expression von SULT der Maus oder der Ratte gefunden. Es ist deshalb zu befürchten, dass Nitrofen im Menschen eine stärkere Wirkung entfaltet als in den Tiermodellen, in denen seine kanzerogene und teratogene Wirkung entdeckt wurde.

Zur Genotypisierung setzen wir die Restriktionsanalyse und das Pyrosequenzieren ein. Wir untersuchen, ob sich die Häufigkeit verschiedener SULT-Genotypen zwischen Tumorpatienten und Kontrollprobanden unterscheidet, wobei Ernährungsgewohnheiten mitberücksichtigt werden. In Zusammenarbeit mit der Gruppe von Ellen Kampman (Wageningen) fanden wir, dass Dickdarm-Adenome bei Rauchern mit dem schnellen SULT1A1-Genotyp assoziiert sind.

At present, we focus our research activities in this area on the sulfotransferases (SULT), in addition we are investigating acetyltransferases and cytochromes P450. The class of SULT is characterized by a high detoxification as well as a high toxification potential (depending on the chemical studied). We have expressed 18 human SULT and 17 SULT from laboratory animals in in vitro test systems. Furthermore we have introduced one SULT as a transgene into a mouse.
SULT were capable of activating more than 100 chemicals, including many food borne compounds, to mutagens. The activation of various chemicals occurred by only one or a few SULT, for instance by a human SULT but not by any rat enzyme. An example is nitrofen, which recently was in the headlines in Germany due to a massive contamination in animal feeds and organically grown chicken. Nitrofen was inactive in the conventional *Salmonella typhimurium* strains (the target cells of the most widely used mutagenicity assay) but was mutagenic even at very low dose levels in corresponding strains expressing human SULT1A1. Expression of any of the available rat and mouse SULT led to much weaker activation. Thus, human may be more susceptible towards nitrofen than the rodent species in which its carcinogenic and teratogenic activity had been detected.

Polymorphisms of SULT

Genetic polymorphisms have been detected for several human SULT. We demonstrated that SULT1A1 and 1A2 are capable of activating various diet borne carcinogens, and we detected large differences in the activation potential between alloenzymes. For the determination of polymorphisms we use restriction analyses and pyrosequencing. We are studying whether the frequency of different SULT genotypes varies between tumor patients and control subjects; dietary habits are also taken into account in these studies.
In cooperation with the group of Ellen Kampman (Wageningen) we detected an association between the occurrence of colon adenomas in smokers and the fast SULT1A1 genotype, suggesting a causal role of smoke borne aromatic amines and/or alkylated polyarenes. Together with the Department of Epidemiology, we observed an association between adipositas and the slow SULT1A1 genotype; this influence may be mediated by central neurotransmitters or iodothyronines, which both are substrates of SULT1A1.

Figure 2
*Determination of a genetic polymorphism of human sulfotransferase 1A1 via pyrosequencing of the gene segment C^{638}GCTCCCT with allele *1 (high enzyme activity/stability) and C^{638}ACTCCCT with allele *2 (reduced activity/stability). E, S: negative and positive controls; A, C, G, T: addition of the corresponding deoxyribonucleotide (emitting light signals if matching the sequence).*

Synergisms in chemical mutagenesis/carcinogenesis

Food contains a plethora of components, evoking the question on interaction in noxious effects. Using our in vitro test systems we demonstrated that many benzylic and allylic alcohols are activated to mutagens by SULT. After the administration of a representative of this class, 1-hydroxymethyl pyrene (HMP), to rats we observed strong DNA damage (adducts) in various tissues. However, the analysis of urinary metabolites and metabolism studies in tissue samples indicated that the major part of HMP is metabolized in a harmless way to carboxylic acids. Inhibition of this pathway in the rat by concurrent administration of ethanol or 4-methylpyrazole (an antidote in methanol and glycol poisoning) led to dramatic up to 200-fold increases in the levels of HMP-induced DNA adducts. Although ethanol is not carcinogenic in animal models, alcohol consumption is a clear risk factor for various tumors in humans (especially in smokers). Therefore, indirect mechanisms may be pivotal, such as the synergism of ethanol with HMP (a metabolite of a smoke-borne carcinogen). It is probable that this synergism is not limited to HMP, as the human is exposed to numerous compounds that can be inactivated by dehydrogenases and toxified by SULT.

Insight from biomarkers on the nature and fate of reactive metabolites formed in the organism

Nowadays, acutely toxic chemicals reach relevant levels in foods only in exceptional cases in Western countries. However, the organism is still exposed to numerous natural and anthropogenic chemicals which have a potential to cause cancer in principle. Since the complete avoidance of these compounds is impossible, it is important to assess the risks resulting from individual exposures and to learn which are substantial. We seek to use certain classes of chemicals present in urine (and other biological samples) to characterize the chemical structure, the level and the further processing of the reactive metabolites formed in the organism: reactive metabolites are either detoxified or react in an uncontrolled manner with cellular components. For the detoxification, the conjugation with glutathione represents a major mechanism. The conjugates are usually processed to, and then excreted as mercapturic acids. Important targets for toxicological effects of reactive intermediates are the base residues of the DNA. Modified bases or their nucleosides are released when the DNA is repaired or the cell dies.

Figure 3
Influence of co-treatment with ethanol or 4-methylpyrazole, an inhibitor of alcohol dehydrogenase, on DNA damage (adducts) by 1-hydroxymethylpyrene (HMP) in kidney of rats in vivo (5 animals per group).
From left to right: control; ethanol concentrations; methylpyrazol

Dies legt nahe, dass aromatische Amine und alkylierte Polyzyklen aus dem Zigarettenrauch einen Kausalfaktor dieser Entartung darstellen. Zusammen mit der Abteilung Epidemiologie beobachteten wir eine Assoziation zwischen dem langsamen SULT1A1-Genotyp und dem Auftreten von Adipositas, dieser Einfluss könnte durch zentrale Neurotransmitter oder Schilddrüsenhormone vermittelt sein, die beide Substrate für die SULT1A1 sind.

[illegible]
Lan Ma, Heli Hollnagel

Lebensmittel enthalten eine Vielzahl von Inhaltsstoffen, über die Interaktionen bei schädigender Wirkung ist noch wenig bekannt. Mit unseren In-vitro-Systemen konnten wir zeigen, dass zahlreiche allylische und benzylische Alkohole durch SULT zu Mutagenen umgesetzt werden. Nach Verabreichung eines Vertreters dieser Substanzklasse, 1-Hydroxymethylpyren (HMP), an Ratten stellten wir eine starke DNA-Schädigung (Addukte) in Geweben fest. Eine Analyse der im Harn ausgeschiedenen Metaboliten und Untersuchungen mit Gewebeproben zeigten aber, dass HMP vor allem über einen unproblematischen Weg metabolisiert wurde, nämlich durch Dehydrogenasen zu Carbonsäuren. Hemmung dieses Weges durch Gabe des konkurrierenden Substrates Ethanol oder des Alkoholdehydrogenase-Hemmstoffes 4-Methylpyrazol (Antidot bei Methanol- und Glycol-Vergiftungen) führte zu einer dramatischen - bis 200fachen Zunahme - von DNA-Addukten durch HMP in Rattengeweben. Obwohl Ethanol im Tierversuch nicht kanzerogen ist, stellt Alkoholkonsum beim Menschen einen Risikofaktor für bestimmte Tumoren dar (besonders bei Rauchern). Es liegt deshalb nahe, indirekte Wirkmechanismen zu postulieren, wie der von uns entdeckte, starke Synergismus von Ethanol mit HMP (einem Metaboliten eines Inhaltsstoffes des Tabakrauches). Dieser Synergismus dürfte nicht auf HMP beschränkt sein, zumal der Mensch vielen Stoffen ausgesetzt ist, die durch Dehydrogenasen inaktiviert und durch SULT toxifiziert werden können.

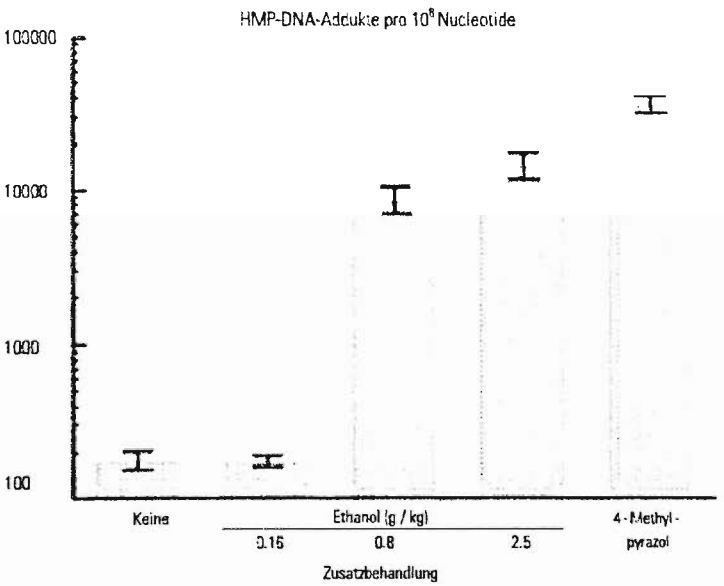


Abbildung 3
Einfluss einer gleichzeitigen Gabe von Ethanol und 4-Methylpyrazol, einem klinisch genutzten Alkoholdehydrogenase-Hemmstoff, auf die DNA-schädigende Wirkung (kovalente Bildung) von 1-Hydroxymethylpyren (HMP) in der Niere der Ratte *in vivo* (5 Tiere pro Gruppe).

Biomarker für die Bildung und Inaktivierung reaktiver Metaboliten
Heiko Schneider, Wolfram Engst, Lan Ma

Akut toxische Stoffe kommen in den industrialisierten Ländern nur noch selten in relevanter Konzentration in Nahrungsmitteln vor. Dagegen ist der Organismus mit vielen natürlichen und anthropogenen Stoffen konfrontiert, die ein Potenzial haben, Krebs zu verursachen.

Da eine völlige Vermeidung dieser Stoffe unmöglich ist, muss abgeschätzt werden, welche Belastungen ein reales Risiko beinhalten. Wir versuchen, aus bestimmten Klassen von ausgeschiedenen Substanzen auf die Art, Menge und Prozessierung der gebildeten reaktiven Metabolite und auf die Wirksamkeit von Schutzmaßnahmen zu schließen. Reaktive Metaboliten können entweder metabolisch detoxifiziert werden oder unkontrolliert mit Zellstrukturen reagieren. Von herausragender Bedeutung für die Detoxifizierung ist die Konjugation mit Glutathion. Die Konjugate werden vorwiegend zu Mercaptursäuren prozessiert und als solche ausgeschieden. Wichtige Zielstrukturen für toxikologische Wirkungen sind die Basen der DNA.

Geschädigte Basen werden bei der DNA-Reparatur oder beim Zelltod als Basen- oder Nucleosid-Addukte freigesetzt. Aus der Struktur der ausgeschiedenen Mercaptursäuren und Basen/Nucleosid-Addukte kann auf die Struktur des reaktiven Metaboliten geschlossen werden. Das Verhältnis der Mengen an Mercaptursäuren zu Basen-Nucleosid-Addukten aus einem reaktiven Metaboliten in einem Individuum stellt ein Maß für seine aktuelle Detoxifizierungskapazität dar. Die erwähnte Interaktion von Ethanol und 4-Methylpyrazol mit der Aktivierung von HMP in der Ratte manifestierte sich nicht nur in den DNA-Addukten, sondern auch an einem nicht-invasiven Biomarker, der Ausscheidung einer aus dem aktiven HMP-Metaboliten gebildeten Mercaptursäure.

The mercapturic acids and base/nucleoside adducts contain much structural information from which the nature of the reactive species may be inferred. Furthermore the ratio of the levels of mercapturic acids versus base/nucleoside adducts from a specific reactive metabolite in a subject represents a measure of its actual detoxification capacity.

The above mentioned interaction of ethanol and 4 methylpyrazole with the activation of HMP in the rat was manifested not only in the levels of DNA adducts, but also in a non invasive biomarker, the urinary excretion of a HMP derived mercapturic acid.

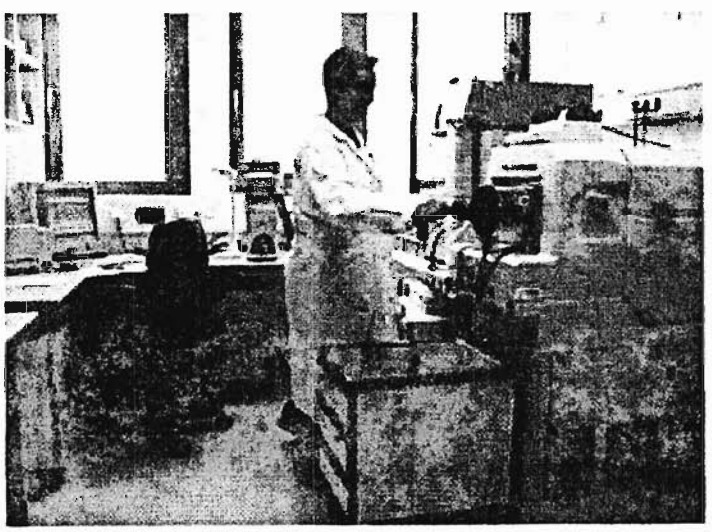


Abbildung 4
LC-MS-MS (Hochdruck-Flüssigkeitschromatographie mit Tandem-Massenspektrometrie) zur Bestimmung von Mercaptursäuren und Basen-/Nucleosid-Addukten.

Figure 4
LC-MS-MS (liquid chromatography and tandem mass spectrometry) for the determination of mercapturic acids and base/nucleoside adducts.

Drittmittelprojekte
External Funding

Titel: HC Amine: Heterozyklische aromatische Amine in gekochten Lebensmitteln - Rolle in der menschlichen Gesundheit/HC Amines: Heterocyclic amines in cooked food - role in human health
Finanzierung: EU
Laufzeit: 02/00-01/02

Titel: Untersuchung des Einflusses von Östrogen-Sulfamaten auf die Konjugation von Östradiol durch die verschiedenen Formen der humanen Sulfotransferasen
Finanzierung: Jenapharm
Laufzeit: 05/02-06/03

Titel: Die Nutzung hepatischer Funktionen für in vitro-Verfahren zur Prüfung von Stoffen mit dem Ziel der Einsparung von Tierversuchen
Finanzierung: BMBF
Laufzeit: 01/99-02/01

Ausgewählte Publikationen
Selected Publications

Originalarbeiten/Original Papers

Arlt, V. M., Glatt, H., Muckel, E., Pabel, U., Sorg, B. L., Schmeiser, H. H., Phillips, D. H.: Metabolic activation of the environmental contaminant 3-nitrobenzanthrone by human acetyltransferases and sulfotransferases. Carcinogenesis 23, 1937-1945 (2002)

Engst, W., Pabel, U., Glatt, H.: Conjugation of 4-nitrophenol and 4-hydroxylonazolac in V79-derived cells expressing individual forms of human sulphotransferases. Environ. Toxicol Pharmacol. 11, 243-250 (2002)

Glatt, H., Boeing, H., Engelke, C. E., Ma, L., Kuhlow, A., Pabel, U., Pomplun, D., Teubner, W., Meinl, W.: Human cytosolic sulphotransferases: genetics, characteristics, toxicological aspects. Mutat. Res. 482, 27-40 (2001)

Glatt, H., Pabel, U., Muckel, E., Meinl, W.: Activation of polycyclic aromatic compounds by cDNA-expressed phase I and phase II enzymes. Polycyclic Aromat. Comp. 22, 955-967 (2002).

Henschler, R., Appel, K. E., Heyworth, C. M., Glatt, H.: Proliferation and differentiation of murine haemopoietic progenitor cells in stroma-free culture in the presence of metabolites of chlorinated pesticides. Toxicol in Vitro 15, 31-37 (2001).

Kester, M. H. A., Bulduk, S., van Toor, H., Tibboel, D., Meinl, W., Glatt, H., Falany, C. N., Coughtrie, M. W. H., Schuur, A. G., Brouwer, A., Visser, T. J.: Potent inhibition of estrogen sulfotransferase by hydroxylated metabolites of polyhalogenated aromatic hydrocarbons reveals alternative mechanism for estrogenic activity of endocrine disrupters. J. Clin. Endocrinol. Metab. 87, 1142-1150 (2002).

Luch, A., Greim, H., Buters, J. T. M., Mahadevan, B., Baird, W. M., Doehmer, J., Seidel, A., Glatt, H.: The role of cytochrome P450 1B1 in dibenzo[*a,l*]pyrene-induced carcinogenesis. Polycyclic Aromat. Comp. 22, 781-789 (2002).

Ma, L., Kuhlow, A., Glatt, H.: Ethanol enhances the activation of 1-hydroxymethylpyrene to DNA adduct-forming species in the rat. Polycyclic Aromat. Comp. 22, 933-946 (2002)

Meinl, W., Glatt, H.: Structure and localization of the human SULT1B1 gene: neighborhood to SULT1E1 and a SULT1D pseudogene. Biochem. Biophys. Res. Commun. 288, 855-862 (2001)

Meinl, W., Meerman, J. H., Glatt, H.: Differential activation of promutagens by allozymes of human sulfotransferase 1A2 expressed in *Salmonella typhimurium*. Pharmacogenetics 12, 677-689 (2002)

Muckel, E., Frandsen, H., Glatt, H.: Heterologous expression of human *N*-acetyltransferases 1 and 2 and sulfotransferase 1A1 in *Salmonella typhimurium* for mutagenicity testing of heterocyclic amines. Food Chem. Toxicol. 40, 1063-1068 (2002).

Teubner, W., Meinl, W., Glatt, H.: Stable expression of rat sulfotransferase 1B1 in V79 cells: activation of benzylic alcohols to mutagens. Carcinogenesis 23, 1877-1884 (2002)

Williams, J. A., Stone, E. M., Fakis, G., Johnson, N., Cordell, J. A., Meinl, W., Glatt, H., Sim, E., Phillips, D. H.: *N*-Acetyltransferases, sulfotransferases and heterocyclic amine activation in the breast. Pharmacogenetics 11, 373-388 (2001)

Buchbeiträge/Book Articles

Glatt, H.: Genetische Polymorphismen von Sulfotransferasen als Suszeptibilitätsparameter. In: Biological Monitoring. Angerer, J. (Hrsg.). DFG, Wiley-VCH, Weinheim, pp 86-97 (2001).

Glatt, H.: Sulphotransferases. In: Ioannides, C. (ed.) Enzyme systems that metabolise drugs and other xenobiotics. John Wiley & Sons Ltd., pp 353-439 (2002)

Leitung: Prof. Dr. Michael Blaut

Die Abteilung Gastrointestinale Mikrobiologie hat das Ziel, die im Darm lebenden Mikroorganismen zu charakterisieren und deren physiologische und pathophysiologische Bedeutung aufzuzeigen. Zu den Erkrankungen, die mit Störungen in der Zusammensetzung und Aktivität der Darmflora in Verbindung gebracht werden, gehören unter anderem Kolonkrebs und entzündliche Darmerkrankungen. Ein erheblicher Anteil der dominanten bakteriellen Spezies im Darm des Menschen ist noch unbekannt. Durch Erweiterung des Spektrums verfügbarer Oligonukleotid-Sonden, mit deren Hilfe Bakterienzellen kultivierungsunabhängig mittels Fluoreszenz-in-situ-Hybridisierung nachgewiesen werden können, sind die im humanen Darm vorkommenden Bakterien mit größerer Präzision und geringerem Zeitaufwand als jemals zuvor erfassbar. Untersucht wird auch der Einfluss und der Abbau von Inhaltsstoffen von Nahrungsmitteln, zum Beispiel Flavonoiden, auf die Darmbakterien und die direkten und indirekten Auswirkungen auf den Wirtsorganismus Mensch.

Anreicherungsmedien ausgewählte Oligo- und Polysaccharide und sekundäre Pflanzenstoffe, die in Lebensmitteln vorkommen, als Substrate eingesetzt. Obwohl die meisten Isolate bekannten Taxa zugeordnet werden konnten, gelang es, eine Reihe neuer Bakterien zu isolieren wie z. B. *Dorea longicatena*, *Anaerostipes caccae*, und *Ruminococcus luti*. Andere Isolate werden noch charakterisiert. Die Analyse der 16S-ribosomalen RNA-Sequenz von Darmbakterien ermöglicht nicht nur die phylogenetische Zuordnung von Isolaten, sondern auch die Konstruktion von Oligonukleotid-Sonden, mit deren Hilfe Bakterienzellen kultivierungsunabhängig mittels Fluoreszenz-in-situ-Hybridisierung nachgewiesen werden können. Acht neue Oligonukleotid-Sonden wurden konstruiert und ihre Spezifität bezüglich der Zielorganismen verifiziert (Validierung). Um die Validierung der Sonden zu beschleunigen, wurde eine Methode entwickelt, die es ermöglicht, die Spezifität einer Sonde mit bis zu 768 Referenz-Stämmen zu überprüfen.

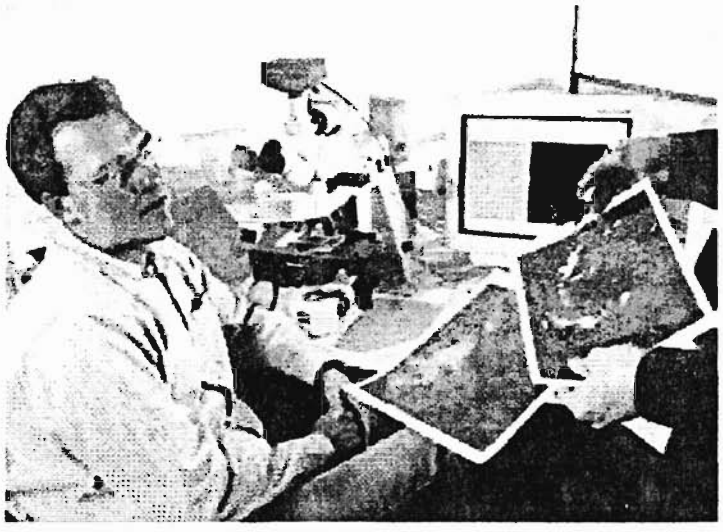


Abbildung 1
Automatisierte Erfassung fluoreszenzmarkierter Bakterienzellen

Diversität der Darmbakterien und ihre automatisierte Erfassung

Andreas Schwiertz, Ralph Thiel, Pawel Namsolleck

Aus humanen Stuhlproben wurden strikt und fakultativ anaerobe Bakterien angereichert mit dem Ziel, unbekannte Bakterien mit Relevanz für die Physiologie des humanen Darms zu identifizieren. Dazu wurden in den Verbessert wurde auch die Geschwindigkeit, Präzision und der Bedienungskomfort der automatisierten mikroskopischen Erkennung und quantitativen Erfassung Fluoreszenzmarkierter Bakterienzellen in Proben (Abb.1). Die automatische Steuerung des Analyseprozesses sowie die Algorithmen für die Bakterienerkennung wurden weiter optimiert.

Department of Gastrointestinal Microbiology

Head: Prof. Dr. Michael Blaut

It is the goal of the Department of Gastrointestinal Microbiology to characterize microorganisms of the gastrointestinal tract and to demonstrate their physiological and pathophysiological roles. Diseases that have been associated with disorders in relation to composition and activity of intestinal flora include e.g. cancer of the colon and inflammatory bowel diseases. A considerable portion of the dominant bacterial species in the human gut have yet to be identified. A broadening of the spectrum of available oligonucleotide probes, with which bacterial cells can be detected via fluorescence in situ hybridization and without cultivation, has made possible the more precise and time saving identification of bacteria located in the human intestine than ever before. Food ingredients, for example flavonoids, are being studied in relation to their influence on and degradation by intestinal bacteria as well as their direct and indirect effects on the human host.

Diversity of gut microorganisms and their automated detection

Recent culture independent studies have shown that approximately 70 % of the dominant human gut microorganisms have not yet been isolated and described. Therefore, efforts have been made to isolate and characterize previously undetected gut bacteria. Strictly anaerobic and facultative anaerobic bacteria exhibiting catalytic activities relevant to human physiology have been enriched and isolated from human feces. Food ingredients such as dietary oligo- and polysaccharides as well as dietary secondary plant metabolites were used as substrates for bacterial enrichments. Although the majority of the isolates obtained by these enrichments turned out to be known species, several new organisms could be isolated, some of which have been characterized and validly described, and included *Dorea longicatena*, *Anaerostipes caccae*, and *Ruminococcus luti*. Other isolates are still under characterization.

Analysis of the 16S rRNA sequence of human gut microorganisms facilitates the recognition of unknown bacterial species and has been used to design 16S rRNA targeted oligonucleotide probes for their culture-independent detection. Eight new 16S rRNA targeting oligonucleotide probes have been designed and validated for use in conjunction with fluorescence in-situ hybridization. The designed probes target several hitherto unknown or newly described bacterial species. Five of the eight probes are species specific while three are group-specific probes. To speed up specificity testing of newly designed probes an array based method was developed. This method can afford the simultaneous testing of up to 768 reference strains.

Figure 1
Automated detection of fluorescently labeled bacterial cells

To improve the speed, accuracy, and ease of use of the automated microscopic detection and enumeration of fluorescence labeled bacterial cells in fecal samples, the automatic control of the analysis and the algorithms for cell recognition have been optimized and the total throughput and the reproducibility has thereby been highly improved.

Metabolism of dietary flavonoids by intestinal microorganisms

In recent years, there has been a growing scientific interest in isoflavonoids such as genistein and daidzein because of their possible role in the prevention of hormone related cancers, atherosclerosis, and osteoporosis as well as alleviation of menopausal symptoms and the reduction in serum cholesterol levels.
Previous work had indicated that *Eubacterium ramulus*, a strictly anaerobic bacterium found to be present in the gastrointestinal tract of essentially every human subject tested, cleaves the ring system of several flavonols and flavones, giving rise to the corresponding hydroxyphenylacetic and hydroxyphenylpropionic acids, respectively, as well as acetate and butyrate. More detailed analyses in resting cells and cell free extracts of *E. ramulus* showed that the degradation of quercetin was accompanied by the formation of two transient intermediates, one of which was identified as taxifolin. The second unknown intermediate was subsequently purified, subjected to ^{1}H and ^{13}C NMR analysis and unambiguously identified as alphitonin (2-[3,4-dihydroxybenzyl]-2,4,6-trihydroxybenzofuran-3-one). These results were taken as evidence that the breakdown of quercetin by *E. ramulus* to phloroglucinol and 3,4-dihydroxyphenylacetic acid follows the pathway depicted in Fig. 1.

The conversion of the flavone luteolin by *E. ramulus* was studied in more detail. Resting cells of *E. ramulus* catalyzed the reduction of luteolin to eriodictyol, a reaction that is analogous to the reduction of quercetin to taxifolin. However, a structure analogous to alphitonin could not be detected in the luteolin degradation experiments. It was therefore postulated that the C-ring cleavage of eriodictyol involves the intracellular formation of a chalcone which is subsequently reduced to the corresponding dihydrochalcone (Fig. 2).

To further corroborate the proposed flavone-degradation pathway, phloretin was used as a model substance because it only differs from the dihydrochalcone postulated to be an intermediate in the pathway of eriodictyol breakdown by the absence of the 3' hydroxyl group in the B ring (Fig. 2).

Figure 2
Pathway of quercetin degradation as catalyzed by Eubacterium ramulus

Annett Braune, Lilian Schoefer, Claudia Herles, Ruchika Mohan

Seit einigen Jahren gibt es ein zunehmendes wissenschaftliches Interesse an Isoflavonen wie Genistein und Daidzein, da Berichten zufolge diese Verbindungen gegenüber hormonabhängigen Krebsarten, Atherosklerose und Osteoporose präventiv wirksam sein und eine Linderung menopausaler Symptome sowie eine Reduktion des Serum-Cholesterin-Spiegels bewirken konnten.
Frühere Untersuchungen hatten darauf hingewiesen, dass *Eubacterium ramulus*, ein strikt anaerobes Bakterium, das im Gastrointestinaltrakt nahezu aller bislang darauf hin untersuchten Menschen gefunden wurde, das Ringsystem verschiedener Flavonole und Flavone spaltet und daraus die entsprechenden Hydroxyphenylessigsäuren bzw. Hydroxyphenylpropionsäuren sowie Acetat und Butyrat bildet.
Detailliertere Untersuchungen an ruhenden Zellen und Zellextrakten von *E. ramulus* zeigten, dass der Abbau des Flavonols Quercetin mit der Bildung von zwei Intermediaten einhergeht: Taxifolin und ein von uns mittels ^{1}H und ^{13}C NMR Analyse als Alphitonin (2-[3,4-Dihydroxybenzyl]-2,4,6-trihydroxybenzofuran-3-on) identifiziertes Intermediat (Abb. 1).

Auch die Umsetzung des Flavons Luteolin durch *E. ramulus* wurde näher charakterisiert. Ruhende Zellen von *E. ramulus* katalysieren die Reduktion von Luteolin zu Eriodictyol. Diese Reaktion erfolgt analog zu der Reduktion von Quercetin zu Taxifolin. Allerdings wurde in den Experimenten mit Luteolin kein Produkt gefunden, das dem beim Quercetinabbau auftretenden Alphitonin analog ist. Daher wurde postuliert, dass die Spaltung des C-Ringes von Eriodictyol zur intrazellulären Bildung eines Chalkons führt, das anschließend zu dem entsprechenden Dihydrochalkon reduziert wird (Abb. 2). Um den vorgeschlagenen Abbauweg für Flavone weiter zu erhärten, wurde Phloretin als Modellsubstanz eingesetzt, da es sich von dem postulierten Dihydrochalkon-Intermediat des Eriodictyol-Abbauweges lediglich durch das Fehlen der 3'-Hydroxyl-Gruppe im B-Ring unterscheidet (Abb. 2).

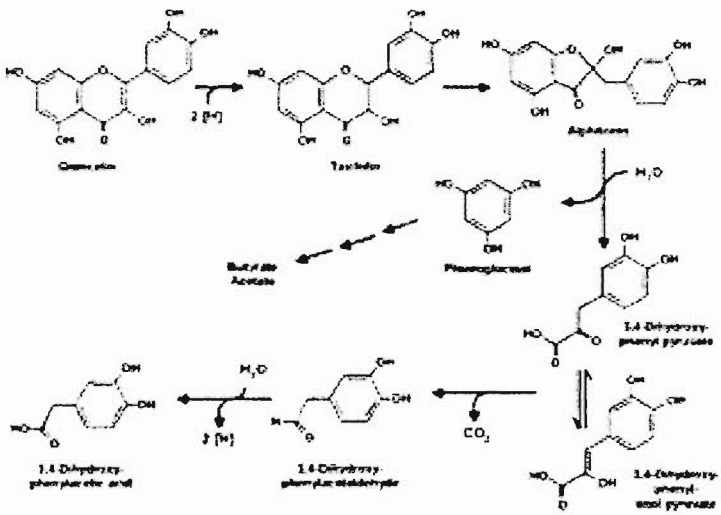


Abbildung 2
Weg des *Quercetin*-Abbaus durch *Eubacterium ramulus*

Mit Hilfe eines neu entwickelten Testsystems zum Aktivitätsscreening gelang es, das Phloretin-Hydrolase-Gen *(phy)* in *Escherichia coli* zu klonieren und zu überexprimieren. Das rekombinante Protein wurde gereinigt und charakterisiert. Das gereinigte Enzym katalysierte die Hydrolyse von Phloretin zu Phloroglucin und 3-(4-Hydroxyphenyl)-propionsäure. Einige bakteriell gebildete Derivate pflanzlicher Isoflavone wie z. B. Equol üben stärkere Effekte aus als die ursprünglich

Entzündungsreaktionen der TNBS-Kolitis (eine durch Trinitrobenzolsulfonsäure experimentell erzeugte Kolitis) mit einer Suppremierung der TFF-Expression assoziiert ist. Trefoilfaktorpeptide (TFF1, TFF2, TFF3) werden als protektive, heilende und tumor-supressive Faktoren von mucinbildenden Becherzellen des Darmepithels sekretiert.
Reportergenassays mit TFF-Promotorkonstrukten in HT-29-Zellen und Realtime-PCR-Analytik belegen eine TNF-α induzierte, NFκB-vermittelte Silencer-Funktion des TFF-Promotors.

Inhibierung des Proteasoms durch Flavonoide

Synthetische Proteasominhibitoren werden derzeit in klinischen Studien zur Krebstherapie getestet, da sie selektiv die Proliferation entarteter Zellen inhibieren und die Apoptose stimulieren. Einige natürliche Substanzen, isoliert aus grünem Tee oder aus anderen pflanzlichen Nahrungsmitteln, vermitteln ähnliche Wirkungen. Im Verlauf eines Modulatorscreenings identifizierten wir polyphenolische Verbindungen aus Tee, die in vitro selektiv die chymotrypsinähnliche Aktivität des 20S-Proteasoms inhibieren (Abb. 3). Zusätzlich wurde die Stabilisierung klassischer Proteinsubstrate des Proteasoms und deren polyubiquitinylierten Ubiquitinkonjugaten in HT-29-Zellen beobachtet.

Im Mittelpunkt dieses Projektes stehen mechanistische Studien, die auf die differentiellen Modulatoreigenschaften von Flavonoiden in Bezug auf verschiedene Proteasom-Subtypen und katalytische Aktivitäten gerichtet sind.

Gastrointestinale Mikrobiota als Modulatoren der Inflammationsantwort

Kommensale gastrointestinale Bakterien wirken als Effektoren der Epithelzellen des Kolons. Nicht-pathogene Bakterien können über proteasomvermittelte Regulationsmechanismen mit der inflammationsspezifischen Signaltransduktion in Epithelzellen interferieren. In Kooperation mit der Abteilung Gastrointestinale Mikrobiologie wurde ein experimentelles Konzept entwickelt, welches die Identifizierung anaerober, entzündungsmodulierender Bakterienspezies sowie die Aufklärung der entsprechenden Wirkungsmechanismen zum Ziel hat. In Epithelzelllinien wurden TNF-α abhängige Signaltransduktionswege stimuliert und modulatorische Einflüsse verschiedener anaerober gastrointestinaler Bakterienstämme in Kokultur gemessen.

Epi-Gallocatechin-Gallate inhibits the 20S Proteasome

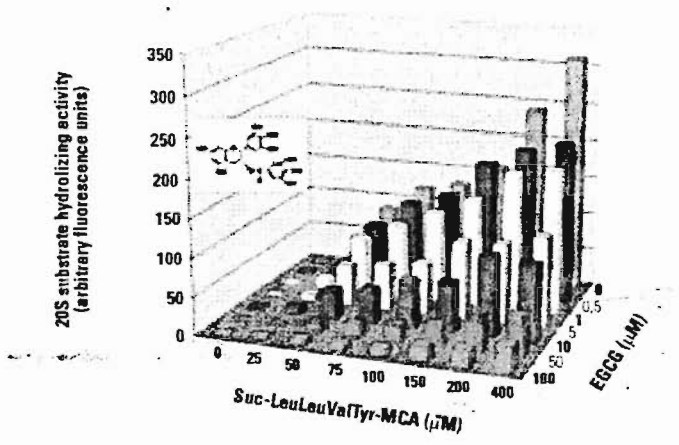


Abbildung 3:
Inhibierung von Peptidaseaktivitäten des 20S-Proteasoms durch Teepolyphenole
Während eines Screenings potentieller Proteasominhibitoren aus der Gruppe der Flavonoide, identifizierten wir Epigallocatechingallat als eine effektive Komponente, die ein natürlicher Bestandteil von grünem und schwarzem Tee ist. Das Diagramm zeigt die Abhängigkeit zwischen Substrat-(Suc-LLVY-AMC)- und Inhibitor-(EGCG)-Konzentration und der Chymotrypsin-ähnlichen Aktivität des 20S-Proteasoms, aufgereinigt aus Leber der Maus.

During the course of inflammatory bowel diseases, the transcription of several genes relevant with respect to therapy or prevention is regulated via the TNFα NFκB signal transduction pathway.
The expression of peptides displaying protective functions towards the gut epithelium, so called trefoil factor peptides, has been examined in collaboration with the University of Tübingen (M. Baus Loncar, N. Blin) and the Free University of Berlin (M. Kruschewski/H.J. Buhr).
Trefoil factor peptides (TFF1, TFF2, TFF3) are considered to be protective, healing and tumor suppressive factors secreted by mucin-producing goblet cells of the intestinal epithelium.
In cooperation with the University of Tübingen (M. Baus, Prof. N. Blin; Institute of Anthropology and Human Genetics), we could show that the epithelial activation of NFκB during chronical inflammation of TNBS colitis is associated with suppression of TFF3 expression (Figure 4).
Reporter gene assays performed using TFF promoter constructs transfected into HT 29 cells and real-time RT-PCR analysis revealed a TNFα induced and NFκB mediated silencer function of TFF promoter elements.

Proteasome inhibition by flavonoids

At present, synthetic proteasome inhibitors are under clinical investigation in cancer therapy, since they inhibit proliferation and stimulate apoptosis of transformed cells selectively. Several natural substances isolated from green tea or other plant-derived foods mediate similar effects. During the course of a modulator screening, we identified polyphenolic compounds of tea, which in vitro selectively inhibited the chymotrypsin-like peptidase activity of the 20S proteasome (Figure 3).
Additionally, the stabilization of classical proteasome substrates as well as of poly ubiquitinylated substrate conjugates was observed in HT-29 cells.
Mechanistic studies addressing the differential modulator qualities of flavonoids with respect to distinct proteasome subtypes and catalytic activities are the main focus of this project.

Gastrointestinal microbiota as modulators of inflammatory response

Commensal gastrointestinal bacteria act as effectors of epithelial cells in the colon. Non-pathogenic bacteria can interfere with inflammation specific signal transduction events via proteasome-mediated mechanisms. Hence, an experimental concept has been established in colaboration with the department GAMI that is aimed at identifying anaerobic bacterial modulators of inflammation and to unravel the corresponding mechanisms.

Figure 3:
Inhibition of peptidase activities of the 20S proteasome by tea polyphenoles. During a screening of potential proteasome inhibitors among flavonoids, we identified epi-gallocatechin-gallate as an effective compound, which naturally occurs in green and black teas. The diagram shows the relationship between substrate (Suc-LLVY-AMC) and inhibitor (EGCG) concentrations and the chymotrypsin-like peptidase activity of the 20S proteasome purified from mouse liver.

TNF dependent signal transduction pathways were stimulated in epithelial cell lines and modulatory influences of various species of anaerobic gastrointestinal bacteria have been studied in co culture

Physiological characterization of fruit juices enriched in dietary fiber and flavonoids and of dietary fiber-rich cereals

Physiological effects of complex fruit juice ingredients were studied in vitro, in rats, and in humans. Fruit and vegetable juices rich in dietary fiber and flavonoids were produced by our cooperative partners (Research Institute Geisenheim) using an enzymatic two-step process. The characterization of juices included the qualitative and quantitative analysis of compounds (flavonoids, dietary fiber) with respect to their functional relevance in vivo, the measurement of physiological parameters influenced by the consumption of such juice products, and research on the metabolism of flavonoids and dietary fiber in animal and human studies. The fermentation activity of intestinal microflora was tested, and butyrate was determined. Produced juice products of high physiological quality showed detectable effects on cholesterol metabolism. Consumption of apple dietary fiber present in apple juices resulted in a significant increase in the excretion of total bile acids.

In an additional project the production as well as the physiological effects of extrudates based on oatmeal and rich in dietary fiber β glucan and resistant starch) were investigated in vitro. Digested extrudates were able to interact with glyco-conjugated bile acids (BA). This is a prerequisite for the transport of BA into colon and their subsequent excretion. Interactions between BA and extrudates were enhanced at lower pH values and were influenced by the structure of BA and dietary fiber present in extrudates as well. The composition of dietary fiber in extrudates affected the production of short-chain fatty acids (including butyrate) during in vitro fermentation of extrudates with human faecal flora.

Figure 4:
Renal excretion of flavonoids and metabolites
HPLC analysis of flavonoids and metabolites from 24-h urine has been selected as example of a physiological study of metabolization of flavonoids. During the course of the human study, ten volunteers consumed 1.4 l of flavonoid and dietary-fiber-enriched apple juice daily (79 µmol quercetin/-glycoside). In parallel, physiological parameters were measured to examine chemopreventive features of the optimized apple beverage.

Sabine Sembries, Barbora Drziková, Gerhard Dongowski

Die physiologischen Wirkungen komplexer Inhaltsstoffe von Fruchtsäften wurden in vitro, an Ratten und am Menschen untersucht. Unter Verwendung eines enzymatischen Zwei-Stufen-Verfahrens wurden durch unsere Kooperationspartner (Forschungsanstalt Geisenheim) mit Ballaststoffen und Flavonoiden angereicherte Frucht- und Gemüsesäfte (Apfel, Traube, Rote Bete) hergestellt. Die Charakterisierung der Säfte beinhaltete die qualitative und quantitative Analyse der unter funktionellen Aspekten relevanten Verbindungen (Flavonoide, Ballaststoffe), zeigten nachweisbare Effekte auf den Cholesterolstoffwechsel. So war durch die Anwesenheit von Apfelballaststoffen im Apfelsaft die Ausscheidung an Gesamtgallensäuren signifikant höher.

In einem weiteren Projekt wurden die Herstellung und physiologischen Wirkungen von Extrudaten aus Hafer-Vollkornprodukten in vitro untersucht, die reich an Ballaststoffen (wie β-Glucan und resistenter Stärke) sind. Die verdauten Extrudate waren in der Lage, Wechselwirkungen mit glykokonjugierten Gallensäuren (GS) einzugehen.

Das ist eine Voraussetzung für den weiteren Transport der GS in den Dickdarm und damit für ihre Ausscheidung. Die Wechselwirkungen waren größer bei niedrigeren pH-Werten und wurden sowohl von der Struktur der GS als auch der der Ballaststoffe in den Extrudaten beeinflusst.

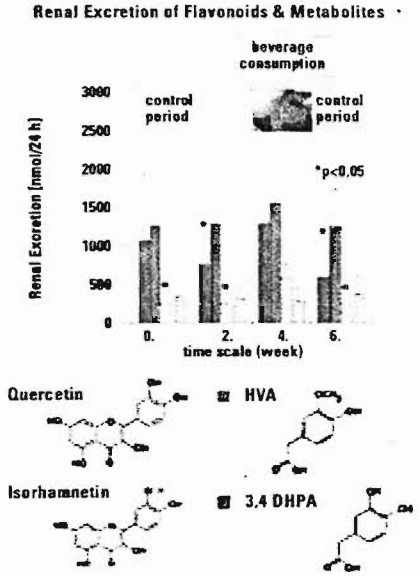


Abbildung 4:
Renale Exkretion von Flavonoiden und Metaboliten
Die HPLC-Analyse von Flavonoiden und Metaboliten aus dem 24h-Urin wurde als Beispiel einer physiologischen Studie zur Metabolisierung von Flavonoiden ausgewählt. Im Verlauf der Humanstudie konsumierten 10 Probanden täglich 1,4 l eines Flavonoid-und Ballaststoff-angereicherten Apfelsaftes (79 µmol Quercetin/-Glykoside). Gleichzeitig wurden physiologische Parameter gemessen, um potentiell chemopräventive Eigenschaften des optimierten Apfelsaftgetränks zu untersuchen.

die Messung physiologischer Parameter (z.B. Körpergewicht, Gallensäureausscheidung, Cholesterolspiegel), die durch den Verzehr der Fruchtsaftprodukte beeinflusst werden, sowie die Untersuchung des Metabolismus von Flavonoiden (Abb 4) und Ballaststoffen in Tier- und Humanstudien. Die Fermentationsaktivität der intestinalen Mikroflora wurde geprüft und der Butyratanteil bestimmt. Die hergestellten physiologisch qualitativ hochwertigen Saftprodukte

Die Zusammensetzung der Ballaststoffe wirkte sich auch auf die Bildung von kurzkettigen Fettsäuren (einschließlich Butyrat) während der Fermentation der Extrudate mit Faecesflora vom Menschen aus.

Technische Mitarbeiter:
Elke Chudoba, Bärbel Kunkel, Monika Niehaus, Katrin Warnke, Horst Maischack, Bärbel König, Karin Richter

verzehrter Isoflavone. Daher wurde untersucht, ob *E. ramulus* in der Lage ist, Genistein und Daidzein umzusetzen. *E. ramulus* setzt Genistein zu 2-(4-Hydroxy-phenyl)-propionsäure um, wobei im Zuge dieser Reaktion 5'-Hydroxy-O-desmethylangolensin als Intermediat gebildet wird. Im Gegensatz dazu wird Daidzein zu O-Desmethylangolensin als einzigem Produkt umgesetzt (Abb 4). Diese Ergebnisse zeigen, dass *E. ramulus* nicht in der Lage ist, aus den eingesetzten Isoflavonen Equol zu bilden und dass die Bakterien, die im humanen Darm Equol bilden, noch identifiziert werden müssen.

Brigitta Kleeßen, Andreas Schwiertz

Es gibt Hinweise darauf, dass Darmbakterien bei der Entstehung und beim Verlauf entzündlicher Darmerkrankungen (IBD) wie Colitis ulcerosa (UC) und Morbus Crohn (CD) eine wichtige Rolle spielen.

Um mögliche Unterschiede im Auftreten dominanter bakterieller Populationsgruppen auf der Mukosa-Oberfläche oder in der Mukosa zu finden, wurden aus Resektionspräparaten (12 Patienten mit aktiver UC, 12 Patienten mit aktiver CD) Gewebeschnitte des terminalen Ileums und des Kolons untersucht. Die Bakterienbesiedlung wurde mittels Fluoreszenz-in-situ-Hybridisierung quantitativ untersucht und mit Gewebeproben von Kontrollpatienten ohne entzündliche Darmerkrankungen verglichen. Die Studie wurde in Kooperation mit J. Kroeser und H. J. Buhr vom Universitätsklinikum Benjamin Franklin in Berlin durchgeführt. Auf der Mukosa-Oberfläche von Gewebe, das von Kontrollpersonen stammte, waren generell weniger Bakterien nachweisbar als in den IBD-Patienten ($p < 0.05$). Ein Eindringen von Bakterien in die Mukosa wurde bei UC-Patienten in 83 % der Kolon-Gewebeproben, sowie bei CD-Patienten in 55,6 % der Gewebeproben aus dem Ileum und in 25 % der Kolon-Gewebeproben gefunden. In Gewebeproben aus der Kontrollgruppe konnten keine eingedrungenen Bakterien beobachtet werden. Das Kolon-Gewebe der UC-Patienten war mit Bakterien besiedelt, die zur Gamma-Gruppe der *Proteobacteria, Enterobacteriaceae*, des *Bacteroides-Prevotella*-Clusters, der *Clostridium histolyticum-Clostridium lituseburense*-Gruppe, des *Clostridium coccoides-Eubacterium rectale* Clusters und zu den Sulfat-Reduzierern gehören.

Im Gegensatz dazu wiesen entsprechende Gewebeproben von CD-Patienten hauptsächlich Bakterien aus den ersten drei Gruppen auf. Diese Beobachtungen legten den Schluss nahe, dass sich die durch CD ausgelösten Veränderungen in der Zusammensetzung der mit der Mukosa assoziierten mikrobiellen Lebensgemeinschaften im Kolon und Caecum von denen unterscheiden, die durch UC ausgelöst werden.

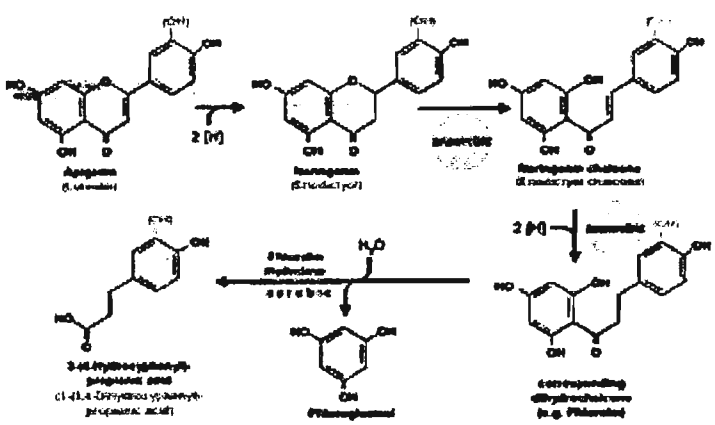


Abbildung 3
Vorgeschlagener Abbauweg für die Flavone *Apigenin* und *Luteolin*. Die Abbildung zeigt auch, welche Reaktionsschritte unter aeroben Bedingungen nachweisbar sind und welche sauerstoffempfindlich sind. Das *Dihydrochalkon*, dem die 3'-Hydroxylgruppe im B-Ring fehlt, wird *Phloretin* genannt.

Using a newly developed activity test, the phloretin hydrolase gene *(phy)* was cloned and overexpressed in *Escherichia coli*, the recombinant enzyme purified to homogeneity and biochemically characterized. The purified enzyme catalyzed the hydrolysis of phloretin to phloroglucinol and 3-(4-hydroxyphenyl) propionic acid.
Since some derivatives of dietary isoflavones such as equol, which are formed by unidentified intestinal bacteria, exhibit stronger physiological effects than the mother compound, *E. ramulus* was tested for its ability to convert genistein and daidzein. The organism catalyzed the cleavage of genistein to 6'-hydroxy-*O*-desmethylangolensin as an intermediate. While the latter was degraded further to 2-(4-hydroxyphenyl) propionic acid, the only product of daidzein breakdown, *O*-desmethylangolensin, was not degraded any further (Fig. 3). These results clearly indicate that *E. ramulus* is not capable of forming equol from daidzein and that the bacteria responsible for the formation of equol in the human intestinal tract still need to be identified.

Bacteria-host interactions

Intestinal bacteria have been implicated in playing a role in the onset and perpetuation of inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD). To identify possible differences in dominant bacterial population groups present on the mucosal surface or invading the mucosa, tissue sections from surgical resections from the terminal ileum and/or the colon from 12 patients with active UC and from 12 patients with active CD were studied by culture-independent methods and compared to tissues from 14 patients not suffering from IBD. The study was done in cooperation with J. Kroesen and H.J. Buhr, Benjamin Franklin Medical Center in Berlin.
The mucosal surface of tissue from control subjects harbored less bacteria than that of tissue from IBD patients ($p<0.05$). Bacterial invasion of the mucosa was evident in 83% of colonic specimens from the UC patients, and, in CD patients, in 55.6% of the ileal and in 25% of colonic specimens, while the corresponding tissues of the control subjects were devoid of bacteria. Colonic UC specimens were colonized by bacteria belonging to the gamma subdivision of *Proteobacteria, Enterobacteriaceae*, the *Bacteroides-Prevotella* cluster, the *Clostridium histolyticum-Clostridium lituseburense* group, the *Clostridium coccoides-Eubacterium rectale* cluster or sulfate-reducing bacteria. In contrast, samples from CD patients harbored mainly bacteria of the former three groups. These results led to the conclusion that the pathogenic events in IBD may result in alterations in the mucosal microbial communities of the ileum and cecum that differ in CD and UC.

Figure 3
Proposed pathway of flavone degradation using apigenin and luteolin as examples. The figure also indicates which steps in this pathway can be detected under aerobic conditions and which ones are oxygen-sensitive. The dihydrochalcone devoid of the 3'-hydroxyl group in the B-ring is called phloretin.

Colonization of the human intestinal tract starts during birth and continues thereafter. It has been hypothesized that an inappropriate colonization of the intestine of preterm infants may play a role in the development of neonatal necrotizing enterocolitis (NEC). To obtain information on the fecal bacterial community of premature infants in comparison to breast fed full term infants, the succession of the neonatal microbiota in the first weeks of life was characterized by analyzing the 16S rRNA gene variety in fecal samples with PCR denaturing gradient gel electrophoresis (PCR DGGE). Fecal samples from 29 preterm infants hospitalized in a neonatal intensive care unit were subjected to PCR DGGE analysis. DGGE profiles from all preterm infants obtained daily during the first four weeks of life were compared with the DGGE profiles of 15 full term breast fed infants and, in addition, a variety of clinical bacteria isolated from the intensive care unit. During the first days of life, the DGGE profiles were rather simple, but increased in complexity over time. Similarity values (C_s) increased in each preterm infant from 0 to 80%, while inter individual C_s increased from 18.1 to 57.4%, revealing the acquisition of a highly similar bacterial community in these infants. In contrast, C_s values obtained for full term breast fed infants were rather low (11.2%). *Escherichia coli, Enterococcus* spp., and *Klebsiella pneumoniae* were the bacteria most commonly found in all preterm infants. The inter individual bacterial composition in hospitalized preterm infants is more similar in comparison to full-term breast fed infants.

Eine Fehlbesiedlung des unreifen Darmes Frühgeborener wird mit der Entstehung der nekrotisierenden Enterocolitis (NEC) in ursächlichem Zusammenhang gesehen. Die Zusammensetzung der bakteriellen Populationsgemeinschaften im Darm Frühgeborener wurde mit der von gestillten reifen Säuglingen verglichen. Die Bakterienbesiedlung in den ersten Lebenswochen wurde durch Analyse der Variabilität des 16S-rRNA-Gens PCR-gestützt in Stuhlproben mittels PCR und nachfolgender denaturierender Gradienten-Gel-Elektrophorese (PCR-DGGE) verfolgt. Insgesamt wurden 29 Frühgeborene einer Intensivstation sowie 15 reife Säuglinge in die Studie eingeschlossen. Über einen Zeitraum von 4 Wochen nach der Geburt wurden täglich Analysen durchgeführt. Zusätzlich zu den Stuhlproben wurden bakterielle Isolate von der Intensivstation in die Untersuchungen einbezogen.

Während der ersten Lebenstage waren die DGGE-Profile der Frühgeborenen relativ einfach, wurden aber mit zunehmenden Alter komplexer. Bei allen Frühgeborenen stieg der Ähnlichkeitskoeffizient (C_s) in dieser Zeit von 0 auf 80 % an, d. h. die mikrobielle Lebensgemeinschaft stabilisierte sich. Im selben Zeitraum verringerten sich die anfänglich zwischen den Säuglingen bestehenden großen Unterschiede in der Bakterienzusammensetzung, was dafür spricht, dass diese durch einen ähnlichen Satz von Bakterien besiedelt wurden. Im Gegensatz dazu waren die Kinder in der Kontrollgruppe untereinander nur wenig Ähnlichkeit in der Bakterienzusammensetzung auf. *Escherichia coli, Enterococcus spp.* und *Klebsiella pneumoniae* wurden in den Frühgeborenen am häufigsten nachgewiesen. Demnach weisen Frühgeborene auf einer Intensivstation geringere interindividuelle Unterschiede in der Bakterienzusammensetzung auf als reife gestillte Säuglinge.

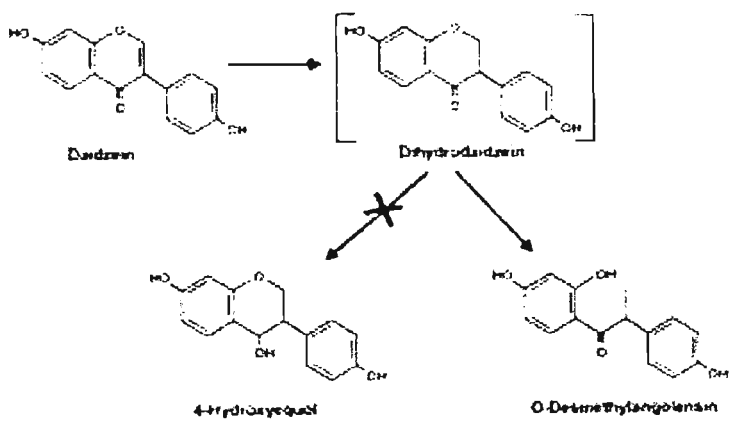


Abbildung 4
Postulierter Weg für die Transformation von *Daidzein* durch *Eubacterium ramulus*

Figure 4
Postulated transformation pathway for daidzein as catalyzed by Eubacterium ramulus

Ausgewählte Publikationen
Selected Publications

Originalarbeiten/Original Papers

Braune, A., Gütschow, M., Engst, W., Blaut, M.: Degradation of quercetin and luteolin by *Eubacterium ramulus*. Appl. Environ. Microbiol. 67, 5558-5567 (2001).

Henes, C., Braune, A., Blaut, M.: Purification and characterization of an NADH oxidase from *Eubacterium ramulus*. Arch. Microbiol. 178, 71-74 (2002).

Kleessen, B., Hartmann, L., Blaut, M.: Oligofructose and long-chain inulin: influence on the gut microbial ecology of rats associated with a human faecal flora. Br. J. Nutr. 86, 291-300 (2001).

Kleessen, B., Kroesen, A. J., Buhr, H. J., Blaut, M.: Mucosal and invading bacteria in patients with inflammatory bowel disease compared with controls. Scand. J. Gastroenterol. 37, 1034-1041 (2002).

Scharek, L., Hartmann, L., Heinevetter, L., Blaut, M.: *Bifidobacterium adolescentis* modulates the specific immune response to another human gut bacterium, *Bacteroides thetaiotaomicron*, in gnotobiotic rats. Immunobiology 202, 429-441 (2001).

Schoefer, L., Braune, A., Blaut, M.: A fluorescence quenching test for the detection of flavonoid transformation. FEMS Microbiol. Lett. 204, 277-280 (2001).

Schoefer, L., Mohan, R., Braune, A., Birringer, M., Blaut, M.: Anaerobic C-ring cleavage of genistein and daidzein by *Eubacterium ramulus*. FEMS Microbiol. Lett. 208, 197-202 (2002).

Schwiertz, A., Hold, G. L., Duncan, S. H., Gruhl, B., Collins, M. D., Lawson, P. A., Flint, H. J., Blaut, M.: *Anaerostipes caccae* gen. nov., sp. nov., a new saccharolytic, acetate-utilising, butyrate-producing bacterium from human faeces. System. Appl. Microbiol. 25, 46-51 (2002).

Schwiertz, A., Lehmann, U., Jacobasch, G., Blaut, M.: Influence of resistant starch on the SCFA production and cell counts of butyrate-producing *Eubacterium* spp. in the human intestine. J. Appl. Microbiol. 93, 157-162 (2002).

Simmering, R., Pforte, H., Jacobasch, G., Blaut, M.: The growth of the flavonoid-degrading intestinal bacterium *Eubacterium ramulus* is stimulated by dietary flavonoids in vivo. FEMS Microbiol. Ecol. 40, 243-248 (2002).

Simmering, R., Taras, D., Schwiertz, A., Le Blay, G., Gruhl, B., Lawson, P.A., Collins, M. D., Blaut, M.: *Ruminococcus luti* sp. nov., isolated from a human faecal sample. System. Appl. Microbiol. 25, 189-193 (2002).

Taras, D., Simmering, R., Collins, M. D., Lawson, P. A., Blaut, M.: Reclassification of *Eubacterium formicigenerans* Holdeman and Moore 1974 as *Dorea formicigenerans* gen. nov., comb. nov., and description of *Dorea longicatena* sp. nov., isolated from human faeces. Int. J. Syst. Evol. Microbiol. 52, 423-428 (2002).

Übersichtsarbeiten/Reviews

Blaut, M., Collins, M. D., Welling, G. W., Doré, J., van Loo, J., de Vos, W.: Molecular biological methods for studying the gut microbiota: the EU human gut flora project. Br. J. Nutr. 87 (Suppl. 2), S203-S211 (2002).

Mattila-Sandholm, T., Blaut, M., Daly, C., De Vuyst, L., Doré, J., Gibson, G., Goossens, H., Knorr, D., Lucas, J., Lähteenmaki, L., Mercenier, A., Saarela, M., Shanahan, F., de Vos, W. M.: Food, GI-tract functionality and human health cluster: PROEUHEALTH. Microbial Ecol. Health Dis. 14, 65-74, (2002).

Simmering, R., Blaut, M.: Pro- and prebiotics - the tasty guardian angels? Appl. Microbiol. Biotechnol. 55, 19-28 (2001).

Drittmittelprojekte
External Funding

Titel: Untersuchung der gesundheitlichen Aspekte der intestinalen Isomalttermentation bei gesunden Probanden
Finanzierung: Südzucker
Laufzeit: 03/01-02/02

Titel: MICROBE DIAGNOSTICS: Development and application of high throughput molecular methods for studying the human gut microbiota in relation to diet and health (QLRT 2000 00108).
Projektkoordinator
Finanzierung: EU
Laufzeit: 02/01-01/05

Titel: CROWNALIFE Functional Food, Gut Microflora and Healthy Ageing (QLRT 2000 00067)
Finanzierung: EU
Laufzeit: 01/01-12/03

Titel: Untersuchung der Beeinflussung der intestinalen Mikroflora durch definierte Diät
Finanzierung: Zuckerinstitut e.V.
Laufzeit: 01/01-04/01

Department of Vitamines and Atherosclerosis

Head: Prof. Dr. Regina Brigelius-Flohé

How important vitamins and trace elements are for the human organism can only be understood when we have an exact knowledge of their biological functions. In this connection, our attention is focused on the antioxidative effect of vitamin E and on the essential trace element selenium.
An involvement of reactive oxygen species in the development of diseases, e.g., atherosclerosis, (auto)immune diseases, cancer, or accelerated ageing processes, has been under discussion for a long time. Clinical studies investigating whether these diseases can be prevented by antioxidants have delivered mostly negative or at least contradictory results. In addition, there is mounting evidence for physiological functions of reactive oxygen species, e.g., in signal transduction and in the regulation of cell growth and differentiation.
For a long time, vitamin E was considered to be only an antioxidant, but this fact cannot explain why it is essentialy. New investigations point to important functions of vitamin E in cellular signal transduction and in the regulation of gene activities; these are more in line with its essential function.
Selenium is a component of many proteins, of which the glutathione peroxidases (GPx) certainly belong to the antioxidative arsenal of the cell but also have functions that go beyond the mere defense against oxidative stress.
The aim of the research of the Department of Vitamins and Atherosclerosis is thus to understand the roles of selenium and vitamin E beyond their postulated antioxidative functions in physiological and pathophysiological processes.

In the ***Selenium*** Project, the tissue-specific expression of the glutathione peroxidases and their role in the cellular signal-transduction pathways is investigated, with interleukin-1 (IL-1) signaling as an example. In the ***Atherosclerosis*** Project, IL-1- and oxidation-mediated events in atherogenesis are main topics. Thus, both projects complement each other. The metabolism and novel functions of vitamin E are studied in the ***Vitamin E*** Project.

The Selenium Project

Glutathione peroxidases

The four selenium-dependent glutathione peroxidases (GPx), cytosolic GPx (cGPx, GPx-1), phospholipid hydroperoxide GPx (PHGPx, GPx-4), plasma GPx (pGPx, GPx-3), and the gastrointestinal GPx (GI-GPx, GPx-2) are expressed in a tissue-specific manner. Whereas cGPx is found ubiquitously, PHGPx prevails in brain and testes, pGPx in the plasma, and GI-GPx in the gastrointestinal system. Diversified tissue and substrate specificities reveal that these enzymes have different functions that are not restricted to hydroperoxide detoxification. Even the subcellular distribution is different.

Figure 1:
Localization of GI-GPx in the endoplasmic reticulum. Colon tissue of a patient with colorectal adenocarcinoma was stained with primary antibodies against GI-GPx- and calnexin (as a marker for endoplasmic reticulum). The secondary antibody was Cy3- (red, GI-GPx) or Cy2 (green, calnexin)-labeled. The mixed colour (yellow) in the overlay reveals perfect colocalization.

Leitung: Prof. Dr. Regina Brigelius-Flohé

Wie wichtig Vitamine und Spurenelemente für den menschlichen Organismus sind, ist nur über eine genaue Kenntnis ihrer biologischen Funktion zu erfassen. In diesem Zusammenhang gilt unsere Aufmerksamkeit dem antioxidativ wirkenden Vitamin E und dem essentiellen Spurenelement Selen.

Eine Beteiligung von reaktiven Sauerstoffspezies an der Entstehung von Krankheiten wie Atherosklerose, (Autoimmun)krankheiten, Krebs oder beschleunigten Alterungsprozessen wird seit langem diskutiert. Klinische Studien, die zeigen sollten, dass diese Krankheiten durch Antioxidantien zu verhindern sind, erbrachten meist negative, zumindest widersprüchliche Resultate. Auch mehren sich die Hinweise auf physiologische Funktionen von reaktiven Sauerstoffspezies, z.B. im Signaltransfer und bei der Regulierung von Zellwachstum und -differenzierung.

Vitamin E wurde lange lediglich als Antioxidans betrachtet, was aber seine Essentialität nicht allein erklären kann. Neue Untersuchungen weisen auf wichtige Funktionen von Vitamin E im zellulären Signaltransfer und bei der Regulation von Genaktivitäten hin, die eher mit seiner vitalen Funktion in Einklang zu bringen sind. Selen ist Bestandteil verschiedener Proteine, von denen die Glutathionperoxidasen (GPx) zwar zum antioxidativen Arsenal von Zellen gehören, aber auch Funktionen haben, die über eine bloße Abwehr des oxidativen Stresses hinausgehen. Das Forschungsziel der Abteilung ist deshalb die Aufklärung der Rolle von Selen und Vitamin E jenseits ihrer postulierten antioxidativen Funktionen in physiologischen und pathophysiologischen Prozessen.
Im *Selen*-Projekt wird die gewebsspezifische Expression von Glutathionperoxidasen und deren Bedeutung für die Regulation zellulärer Signalwege am Beispiel der Signaltransduktion von Interleukin-1 (IL-1) untersucht.
Im *Atherosklerose*-Projekt stehen IL-1- bzw. oxidationsvermittelte Atherosklerose-relevante Ereignisse und ihre Beeinflussung durch selenabhängige Glutathionperoxidasen im Vordergrund, sodass beide Projekte einander ergänzen. Das Vitamin E-Projekt behandelt den Metabolismus und neue Funktionen von Vitamin E und setzt somit die langjährige Tradition des DIfE in der Vitaminforschung fort.

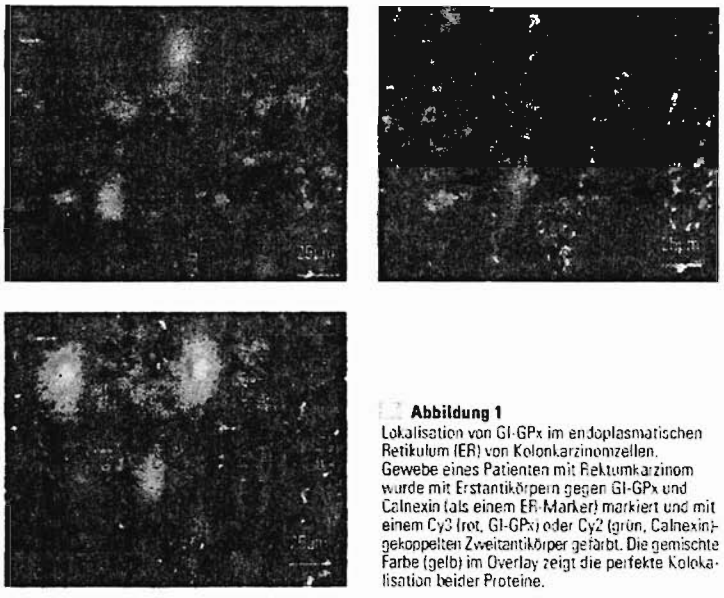


Abbildung 1
Lokalisation von GI-GPx im endoplasmatischen Retikulum (ER) von Kolonkarzinomzellen. Gewebe eines Patienten mit Rektumkarzinom wurde mit Erstantikörpern gegen GI-GPx und Calnexin (als einem ER-Marker) markiert und mit einem Cy3 (rot, GI-GPx) oder Cy2 (grün, Calnexin)-gekoppelten Zweitantikörper gefärbt. Die gemischte Farbe (gelb) im Overlay zeigt die perfekte Kolokalisation beider Proteine.

Gaby-Fleur Böl, Cordula Müller, Dagmar Kupper, Nadine Jurrmann

Glutathionperoxidasen

Die 4 selenabhängigen Glutathionperoxidasen (GPx), die cytosolische GPx (cGPx, GPx-1), die Phospholipidhydroperoxid GPx (PHGPx, GPx-4), die Plasma GPx (pGPx, GPx-3) und die gastrointestinale GPx (GI-GPx, GPx-2) werden gewebsspezifisch exprimiert. Während die

cGPx ubiquitär vorkommt, überwiegt PHGPx in Gehirn und Testes, pGPx im Plasma und GI-GPx im Gastrointestinaltrakt. Unterschiedliche Gewebs- und Substratspezifitäten legen unterschiedliche Funktionen nahe, die nicht auf Hydroperoxid-Entgiftung beschränkt sind. Auch ihre subzelluläre Verteilung ist unterschiedlich. Die cGPx findet man in fast allen zellulären Kompartimenten, die PHGPx in Cytosol, Mitochondrien und Kern. Die GI-GPx findet man je nach Darmabschnitt in Panethzellen (Ileum), oder in kernnahen Strukturen auf der luminalen Seite (Kolon), die als endoplasmatisches Retikulum identifiziert werden konnten (Abb.1).
Bei Selenmangel ändern sich die Spiegel der GPxs entsprechend ihrer Stellung in der Hierarchie der Selenoproteine. Während GI-GPx und PHGPx relativ stabil bleiben, verlieren pGPx und cGPx schnell an Aktivität durch Abbau ihrer mRNA. Die RNA von Selenoproteinen enthält in der 3'-nicht-translatierten Region (3'-UTR) eine charakteristische Sekundärstruktur, die sog. SECIS, für *Selenocysteine-inserting sequence*

codierender Sequenz von cGPx, PHGPx und GI-GPx als Beispiele unterschiedlich stabiler Selenoproteine wurde diese Hypothese überprüft. Die 3'UTRs von GI-GPx und PHGPx konnten ohne Verlust der mRNA-Stabilität im Selenmangel ausgetauscht werden. Die GI-GPx und PHGPx 3'UTRs konnten aber nicht die mRNA der cGPx im Selenmangel stabilisieren. Fusion der cGPx 3'UTR an die codierende Sequenz von PHGPx oder GI-GPx machte beide mRNAs instabil. Somit ist die 3'UTR nötig aber nicht ausreichend für die Stabilisierung der mRNA von PHGPx und GI-GPx. Es bleibt die Möglichkeit, dass zusätzliche Sequenzen in der codierenden Region zur Stabilisierung erforderlich sind.

BHK-Zellen, die keine endogene GI-GPx bilden, exprimierten GI-GPx unter der Kontrolle des CMV-Promoters und hielten die zugehörige mRNA im Selenmangel stabil.
Somit ist die gewebsspezifische Expression der GI-GPx eher über spezifische Transkriptionsfaktoren, die im Promoter binden, als über 3'UTR-bindende Proteine reguliert

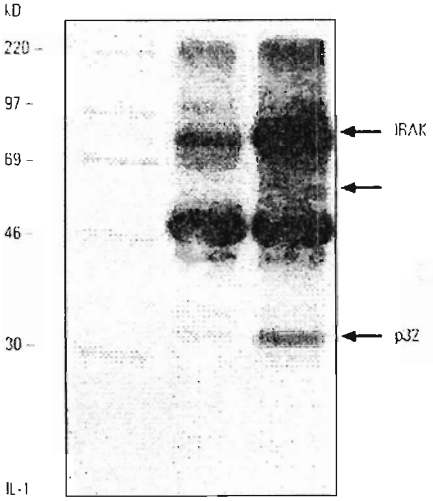


Abbildung 2
Rekrutierung von Proteinen mit freien Thiolgruppen an den IL-1R durch IL-1. IL-1R Immunpräzipitate von EL4 Zellen mit (+) und ohne (-) IL-1 Stimulierung wurden mit ^{125}I-IAIT (Iodo-Acetyl-[^{125}I]-Iodotyrosine) markiert. Die kopräzipitierten Proteine wurden auf SDS-PAGE getrennt und autoradiographiert. Die ca. 80 kDa Bande wurde als IL-1R-assoziierte Kinase (IRAK) identifiziert.

Diese ist unerlässlich für die Erkennung des Selenocystein-Codons TGA. Ein SECIS-bindendes Protein (SBP-2) bindet während der Translation an die SECIS, interagiert mit an die mit Selenocystein beladene tRNA assoziiertem SelB und stabilisiert den für Selenoproteine typischen Translationskomplex. Andererseits könnten 3'-UTR-bindende Proteine auch die Stabilität der mRNA von Selenoproteinen bestimmen. Durch wechselseitigen Austausch von 3'UTR und

Interleukin-1 ist ein pro-inflammatorisches Cytokin, das seine Funktion über einen membranständigen Rezeptor ausübt und dessen Signaltransduktion redox-reguliert ist.
Nach Bindung von IL-1 werden verschiedene Proteine an den IL-1-Rezeptor (IL-1R) rekrutiert, zu denen u. a. die IL-1R-assoziierte Kinase (IRAK) gehört. Mindestens 3 dieser Proteine enthalten freie SH-Gruppen (Abb.2).

cGPx occurs in almost all cellular compartments; PHGPx is found in cytosol, mitochondria, and nucleus. Depending on the intestinal area, GI-GPx is expressed in Paneth cells (ileum) or concentrated in structures capping the nucleus at the luminal side of colonic cells. These structures were identified as endoplasmic reticulum (Fig.1).
In selenium deficiency, levels of GPxs vary according to their ranking in the hierarchy of selenoproteins. Whereas GI-GPx and PHGPx remain relatively stable, pGPx and cGPx rapidly lose activity due to the degradation of the respective mRNA.

The mRNA of selenoproteins contains a characteristic secondary structure in the 3 non translated region (3 UTR), called SECIS for selenocysteine-inserting sequence, which is indispensable to recognize TGA as selenocysteine codon. During translation, a SECIS binding protein (SBP 2) binds to the SECIS and interacts with mSelB, which is associated with the selenocysteyl-loaded tRNA, thus stabilizing the translation complex typical for selenoproteins. The 3'UTRs may have additional functions, e.g., stabilizing mRNA by binding either SBPs or alternative proteins. This hypothesis was tested by mutually exchanging 3 UTRs and coding regions of cGPx, PHGPx and GI-GPx as examples for differently stable selenoproteins. The 3 UTRs of GI-GPx and PHGPx could be mutually exchanged without loss of mRNA stability in selenium deficiency. They could, however, not confer stability to cGPx. Fusion of the cGPx 3 UTR to the coding regions of PHGPx or GI-GPx made both mRNAs instable. Thus, 3 UTR is necessary but not sufficient for the stabilization of PHGPx and GI GPx mRNAs, leaving the possibility that additional sequences most probably within the coding region are required.
BHK cells, which do not express endogenous GI GPx, expressed GI GPx under the control of the CMV promoter. Also, GI-GPx mRNA produced this way remained stable in selenium deficiency. Expression of GI-GPx, therefore, depends on tissue-specific transcription factors targeting the 5'UTR, while factors binding to and stabilizing RNA appear to be less tissue-specific.

IL-1 signaling
IL-1 is a pro-inflammatory cytokine which exerts its signals via the IL-1-receptor (IL-1-R). Its signaling cascade is redox-regulated. Upon binding of IL-1, various proteins are recruited to the receptor, among them the IL-1R associated kinase-1 (IRAK-1). At least 3 of these proteins contain free SH groups (Fig.2).

Figure 2:
IL-1 initiates recruitment of proteins with free thiols to the IL-1 receptor. IL-1R immunoprecipitates from EL4 cells with (+) or without (-) IL-1 stimulation were labelled with ^{125}I-IAIT (Iodo-acetyl-[^{125}I]-iodotyrosine). Coprecipitated proteins were separated by SDS-PAGE and visualized by autoradiography. The approx. 80 kDa band was identified as IRAK.

Thiol modifying agents such as diamide, menadione, and phenylarsine oxide (PAO) inhibited the IL-1 mediated phosphorylation of an endogenous substrate within the IL-1R complex and also the recruitment of IRAK to the receptor. Recruitment correlated with the availability of thiol groups in IRAK for the thiol marker ^{125}I-IAIT. Thus, one of the proteins in the IL-1R complex was identified as IRAK. IL-1 signaling is mediated by reactive oxygen species. These can either function as signals by themselves or modify proteins involved in signaling, the latter should be enzyme regulated for specificity. The most likely candidate for such a regulation is PHGPx. It is the only GPx able to react with hydroperoxides in complex lipids within membranes and with protein thiols. Glutathione and overexpressed PHGPx reversed the inhibition of the association of the IRAK to the receptor by menadione but not by PAO in line with the reaction mechanism of these compounds. It is concluded that the formation of the IL-1R complex is regulated by the glutathione system, a reduced status being a prerequisite for an appropriate IL-1 response.

The Atherosclerosis Project

Many events in atherogenesis are initiated by oxidative stress or are redox regulated, e.g., the expression of cell adhesion molecules (CAM). Rabbit aortic smooth muscle cells stably transfected with either PHGPx or 15-lipoxygenase provide a model to investigate the role of hydroperoxide-reducing (PHGPx) and hydroperoxide-producing (15-LOX) in processes relevant to atherogenesis. To study the expression of VCAM-1 (vascular cell adhesion molecule 1), mRNA for rabbit VCAM-1 had first to be cloned and sequenced. Basal VCAM-1 expression was inhibited by approximately 50% in SMC^{PHGPx} and almost 100% in SMC^{LOX}. VCAM-1 inducibility by IL-1 was maintained in SMC^{PHGPx} while IL-1 did not induce VCAM-1 in SMC^{LOX} (Fig. 3).
All RNA data were confirmed by immunocytochemistry. PHGPx effects support the view that a moderately oxidized status facilitates IL-1 signaling and, in consequence, VCAM-1 expression. In contrast, a highly oxidized status, evoked by 15-LOX, appears to make cells refractory for an IL-1 stimulus.

The Vitamin-E Project

The long lasting DIfE tradition in vitamin-E research has been continued. The postulated chain degradation of tocopherols and tocotrienols via a β-oxidation pathway has been confirmed by the identification of almost all possible intermediates by GC/MS (Fig. 4 A/B).

Figure 3:
Inhibition of VCAM-1 expression by PHGPx and 15-LOX.
SMC, SMC^{PHGPx} or SMC^{LOX} were grown with or without selenite and stimulated with IL-1. GAPDH-normalized VCAM-1 mRNA was quantified by real time PCR by the comparative method with VCAM-1 mRNA from selenium-deficient SMC as calibrator.

Thiolmodifizierende Agenzien wie Diamid, Menadion und Phenylarsinoxid (PAO) hemmten die Phosphorylierung im IL-1R-Komplex enthaltener endogener Substrate sowie die Rekrutierung von IRAK. Die Rekrutierung der IRAK korrelierte mit ihrer Zugänglichkeit für den SH-Gruppen Marker ^{125}I-IAIT. Eines der 3 Proteine wurde somit als IRAK identifiziert. Zu den am IL-1-Signaltransfer beteiligten Mediatoren gehören auch reaktive Sauerstoffspezies.
Diese haben entweder selbst Signalfunktion oder modifizieren an der Signalkaskade beteiligte Proteine, ein Prozess der, um spezifisch abzulaufen, Enzymreguliert sein sollte. Ein Kandidat hierfür ist die PHGPx, da diese als einzige GPx sowohl mit membranständigen Lipidhydroperoxiden als auch mit Proteinthiolen reagieren kann. Die Hemmung der Assoziierung von IRAK an den Rezeptor durch Menadion war durch GSH und durch PHGPx-Überexpression reversibel. Dagegen war der PAO-Effekt irreversibel, was im Einklang mit den Mechanismen der Thiolmodifizierung beider Substanzen steht.

Kerstin Schnurr, Antje Banning

Eine Vielzahl von Prozessen in der Atherogenese wird durch oxidativen Stress initiiert oder ist redox-reguliert. Hierzu gehören z.B. die Expression von Zell-Adhasions-Molekülen (CAM). Mit glatten Muskelzellen (SMC) aus Kaninchenaorten, die entweder PHGPx oder 15-Lipoxygenase (15-LOX) stabil überexprimieren, steht ein Modell zur Verfügung, mit dem die Rolle von Hydroperoxid-abbauenden (PHGPx) und Hydroperoxid-bildenden Systemen (15-LOX) in Atherosklerose-relevanten Prozessen überprüft werden kann.
Um die Expression von VCAM-1 (vascular cell adhesion molecule-1) zu untersuchen, wurde zunächst die cDNA für Kaninchen VCAM-1 aus SMC isoliert und sequenziert. Im Vergleich zu nicht transfizierten SMC war die basale VCAM-1 Expression in SMC^{PHGPx} um ca. 50%, in SMC^{LOX} fast vollständig gehemmt.
Die Induzierbarkeit von VCAM-1 durch IL-1 blieb in SMC^{PHGPx} erhalten. In SMC^{LOX} ließ sich VCAM-1 nicht durch IL-1 induzieren (Abb.3).

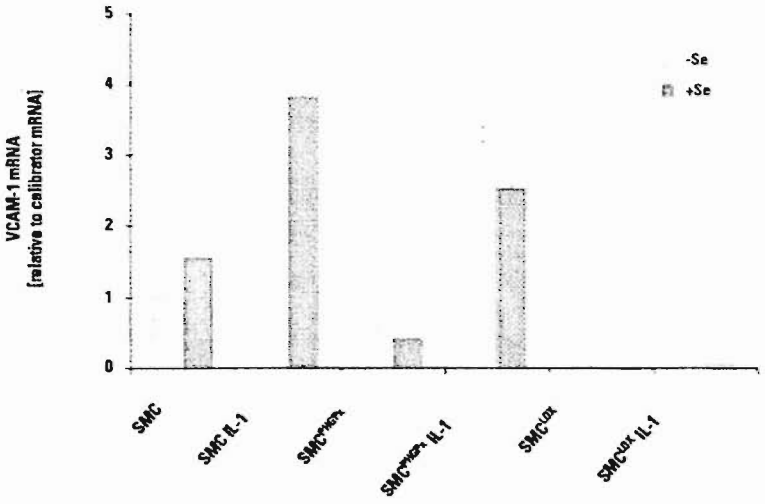


Abbildung 3
Hemmung der VCAM-1 Expression durch PHGPx und 15-Lipoxygenase. SMC, SMC^{PHGPx} oder SMC^{LOX} wurden mit und ohne Selenit kultiviert und mit IL-1 stimuliert. GAPDH-standardisierte VCAM-1 RNA wurde mit real time PCR über die komparative Methode quantifiziert. VCAM-1 von Selenarmen SMC diente als Kalibrator.

Hieraus ist ersichtlich, dass die Bildung des IL-1R-Komplexes über das Glutathionsystem reguliert wird. Ein reduzierter Status im IL-1R-Komplex enthaltener Proteine ist Vorbedingung für eine adäquate Antwort auf IL-1.

Alle RNA-Daten wurden in immunocytochemischen Untersuchungen bestätigt. Die PHGPx-Effekte unterstützen die Annahme, dass ein moderat oxidierter Status den IL-1 Signaltransfer und damit die VCAM-1 Expression erleichtert. Eine Überoxidation durch 15-LOX scheint dagegen Zellen gegen eine IL-1 Stimulierung refraktär zu machen.

Marc Birringer, Nico Landes, Paul Pfluger, Dirk Kluth

Der Mechanismus des Vitamin-E-Metabolismus wurde weitgehend aufgeklärt. Die über eine β-Oxidation verlaufende Verkürzung der Seitenketten von Tocopherolen und Tocotrienolen wurde durch Identifizierung fast aller Zwischenprodukte mittels GC/MS bestätigt (Abb 4 A/B).
Als das die ω-Hydroxylierung katalysierende Cytochrom P_{450} war nach Hemm- und Induktionsstudien CYP3A4 vorgeschlagen. Wahrscheinlicher ist jetzt eine ω-Hydroxylierung durch CYP4F2 (Sontag & Parker, 2002). Auf jeden Fall werden alle Vitamin-E-Formen über ein Enzymsystem metabolisiert, das auch für den Abbau von Arzneimitteln und Fremdstoffen verantwortlich ist.

Viele Fremdstoffe induzieren die sie metabolisierenden CYPs über die Aktivierung des Pregnan-X-Rezeptors (PXR). In HepG2-Zellen, die mit dem humanen PXR und einem Chloramphenicol Acetyltransferase (CAT) Reportergen unter Kontrolle von PXR transfiziert waren, wurde die CAT-Aktivität auch von Vitamin E stimuliert. α- und γ-Tocotrienol stimulierten stärker als Rifampicin, während δ-, α- und γ-Tocopherol weniger effektiv waren. γ-Tocotrienol induzierte endogenes CYP3A4 und CYP3A5 im gleichen Ausmaß wie Rifampicin. Es ist also möglich, dass individuelle Vitamin E Formen (1) genregulatorisch wirken und (2) mit dem Arzneimittelstoffwechsel interferieren. Den einzigartigen essentiellen Funktionen von α-Tocopherol in der Reproduktion und in der neuromuskularen Koordination könnten daher auch andere als antioxidative Mechanismen zugrunde liegen.

Inhibition and induction studies suggested that CYP3A4 catalyze the initial ω hydroxylation. Recently, CYP4F2 was shown to be the more likely candidate (Sontag & Parker, 2002). In any case, all forms of vitamin E are degraded via an enzymatic system which is responsible for the metabolism of drugs and xenobiotics. Many xenobiotics induce cytochromes catalyzing their metabolism via the activation of the pregnane X receptor (PXR).
In HepG2 cells transfected with the human PXR and the chloramphenicol acetyl transferase (CAT) gene linked to PXR responsive elements, CAT activity was most strongly induced by α- and γ tocotrienol followed by rifampicin, δ-, α- and γ tocopherol. γ Tocotrienol induced endogenous CYP3A4 and CYP3A5 mRNA as efficiently as rifampicin. It is thus possible that individual forms of vitamin E (1) have gene regulatory functions and (2) interfere with drug metabolism.
Other mechanisms than antioxidative ones might also determine the unique and essential functions of α tocopherol in reproduction and neuromuscular coordination.

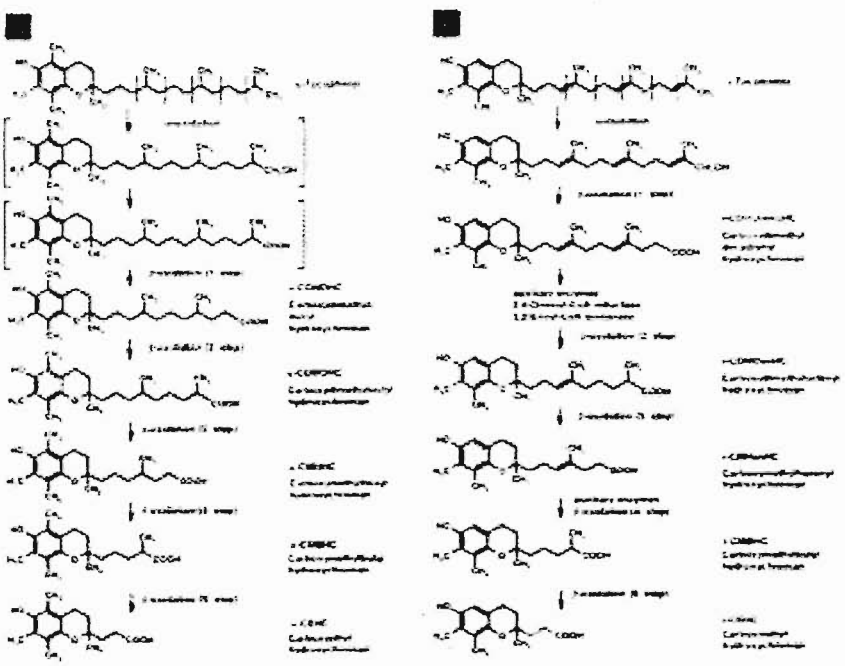


Abbildung 4
A. Seitenkettenabbau von Tocopherolen (Beispiel α-Tocopherol). Nach initialer ω-Hydroxylierung wird die Seitenkette in Analogie zur β-Oxidation verzweigtkettiger Fettsäuren verkürzt. Der Mechanismus wurde durch Identifizierung der aufgeführten Intermediate durch GC/MS bestätigt. Metabolite in Klammern wurden bisher nur für γ-Tocopherol gezeigt.

B. Seitenkettenabbau von Tocotrienolen (Beispiel γ-Tocotrienol). Nach initialer ω-Hydroxylierung wird die Seitenkette in Analogie zur β-Oxidation ungesättigter Fettsäuren verkürzt. Die in Schritt 2 und 4 gebildeten konjugierten Doppelbindungen werden von der Enoyl-Hydratase nicht akzeptiert und müssen durch Hilfsenzyme reduziert und isomerisiert werden. Die Zwischenprodukte γ-CDMOenHC und γ-CMBHC, die nicht die Doppelbindungen der Vorstufen enthalten, bestätigen dies.

Figure 4:
A. Side chain degradation of tocopherols (e.g. α-tocopherol).
Upon initial ω-hydroxylation, the side chain is shortened via β-oxidation in analogy to branched-chain fatty acid. Identification of the indicated intermediates by GC/MS confirmed the mechanism. Metabolites in brackets, have, so far, only been identified from γ-tocopherol.

B. Side chain degradation of tocotrienol (e.g. γ-tocotrienol). Upon initial ω-hydroxylation, the side chain is shortened via β-oxidation in analogy to that of unsaturated fatty acids. Conjugated double bonds as formed in step 2 and 4 are not accepted by enoyl-CoA-hydratase and therefore have to be reduced and isomerized by auxiliary enzymes. Identification of γ-CDMOenHC and γ-CMBHC not containing the double-bonds present in their precursors, confirms the pathway.

Drittmittelprojekte
External Funding

Titel: Gewebsspezifische Regulation der Expression individueller Glutathionperoxidasen/Tissue specific regulation of the expression of individual glutathione peroxidases
Finanzierung: DFG
Laufzeit: 01/01-01/02 und 07/02-06/04

Titel: Mechanismus und Funktion der Seitenkettenverkürzung von Tocopherolen
Finanzierung: DFG
Laufzeit: 01/01-12/02

Titel: Identifizierung redoxregulierter Proteine bei Interleukin-1 vermittelten Signalen
Finanzierung: DFG
Laufzeit: 01/01-12/02

Titel: Redoxprozesse in der Expression von Adhäsionsmolekülen. Rolle der Phospholipidhydroperoxid Glutathionperoxidase (PHGPx) und der 15 Lipoxygenase
Finanzierung: DFG
Laufzeit: 05/01-04/03

Titel: VITAGE: Fat-soluble vitamin status and metabolism during ageing. Functional and nutritional consequences
Finanzierung: EU
Laufzeit: 01/00-12/03

Ausgewählte Publikationen
Selected Publications

Originalarbeiten/Original Papers

Birringer, M., Drogan, D., Brigelius-Flohé, R.: Tocopherols are metabolized in HepG2 cells by side chain omega-oxidation and consecutive beta-oxidation. Free Radic. Biol. Med. 31, 226-232 (2001).

Birringer, M., Pfluger, P., Kluth, D., Landes, N., Brigelius-Flohe, R.: Identities and differences in the metabolism of tocotrienols and tocopherols in HepG2 cells. J. Nutr. 132, 3113-3118 (2002).

Brigelius-Flohé, R., Müller, C., Menard, J., Florian, S., Schmehl, K., Wingler, K.: Functions of GI-GPx: Lessons from se enium-dependent expression and intracellular localization. BioFactors 14, 101-108 (2001).

Brigelius-Flohe, R., Wingler, K., Müller, C.: Estimation of individual types of glutathione peroxidases. Methods Enzymol. 347, 101-112 (2002).

Florian, S., Wingler, K., Schmehl, K., Jacobasch, G., Kreuzer, O.J., Meyerhof, W., Brigelius-Flohe, R.: Cellular and subcellular localization of gastrointestinal glutathione peroxidase in normal and malignant human intestinal tissue. Free Radic. Res. 35, 655-663 (2001).

Lehmann, C., Wollenberger, U., Brigelius-Flohé, R., Scheller, F.W.: Modified gold electrodes for electrochemical studies of the reaction of phospholipic hydroperoxide glutathione peroxidase with glutathione and glutathione disulfide. Electroanalysis 13, 364-369 (2001).

Lisdat, F., Utepbergenov, D., Haseloff, R.F., Blasig, I.E., Stocklein, W., Scheller, F.W., Brigelius-Flohé, R.: An optical method for the detection of oxidative stress using protein-RNA interaction. Anal. Chem. 73, 957-962 (2001).

Müller, C., Friedrichs, B., Wingler, K., Brigelius-Flohé, R.: Perturbation of lipid metabolism by linoleic acid hydroperoxide in CaCo-2 cells. Biol. Chem. 383, 637-648 (2002).

Traber, M.G., Winklhofer-Roob, B.M., Roob, J.M., Khoschsorur, G., Aigner, R., Cross, C., Ramakrishnan, R., Brigelius-Flohé, R.: Vitamin E kinetics in smokers and nonsmokers. Free Radic. Biol. Med. 31, 1368-1374 (2001).

Wingler, K., Müller, C., Brigelius-Flohé, R.: Stability of gastrointestinal glutathione peroxidase mRNA in selenium deficiency depends on its 3'UTR. BioFactors 14, 43-50 (2001).

Übersichtsarbeiten/Reviews

Birringer, M., Pilawa, S., Flohe, L.: Trends in selenium biochemistry. Nat. Prod. Rep. 19, 693-718 (2002).

Brigelius-Flohé, R., Kelly, F.J., Salonen, J.T., Neuzil, J., Zingg, J.-M., Azzi, A.: The European perspective on vitamin E: current knowledge and future research. Am. J. Clin. Nutr. 76, 703-716 (2002).

Brigelius-Flohé, R., Maiorino, M., Ursini, F., Flohé, L.: Selenium: An Antioxdant? In: Cadenas, E., Packer, L. (eds.) Handbook of Antioxidants. Marcel Dekker, New York Basel, pp 633-664 (2001).

Buchbeiträge/Articles in books

Bol, G.-F., Brigelius-Flohé: IL-1-induced trafficking of interleukin-1 receptor-associated proteins. In: Heilmeyer, L., Friedrich, P. (eds.) Protein Modules in Cellular signalling. NATO ASI A/316, IOS Press, Amsterdam, pp 288-297 (2001).

Brigelius-Flohé, R.: Arteriosklerose. In: Biesalski, H.K., Köhrle, J., Schumann, K. (eds.) Vitamine, Spurenelemente und Mineralstoffe. Prävention und Therapie mit Mikronährstoffen. Thieme Verlag, Stuttgart, New York, pp 437-449 (2002).

Wegen der komplexen Zusammensetzung der Nahrung ist es schwierig, für entzündliche und neoplastische Darmerkrankungen die Zusammenhänge zwischen Erkrankungen und Ernährung aufzuzeigen. Der polymorphe genetische Hintergrund und die Stoffwechselfähigkeit des Organismus und seiner Darmbakterien ist bisher nur unzureichend untersucht worden. Durch Flavonoide, kurzkettige Fettsäuren und gastrointestinale Mikrobiota ausgelöste Mechanismen könnten chemopräventiv hinsichtlich neoplastischer Darmerkrankungen sein. Ziel unserer Arbeiten ist es, die physiologischen, zellbiologischen und molekularen Grundlagen protektiver und schädlicher Wirkungen von Nahrungsinhaltsstoffen aufzuklären. Mechanismen des intrazellulären Proteinabbaus stehen im Vordergrund, da wir eine Interferenz von Nahrungsfaktoren mit molekularen Komponenten des Ubiquitin-Proteasom-Weges nachweisen konnten.

Zellen aufgewendet, um ubiquitinmarkierte Proteine über diesen Proteolyseweg abzubauen. Ubiquitin, ein konserviertes Protein aus 76 Aminosäuren, wird enzymatisch unter ATP-Verbrauch aktiviert und über seinen carboxyterminalen Glycinrest mit Lysinresten in Substratproteinen kovalent verknüpft. Das 26S-Proteasom erkennt, bindet, entfaltet und verdaut ubiquitinylierte Proteine und generiert dabei MHC-Klasse-I-restringierte Peptide für die Antigenpräsentation. Das 20S-Proteasom, ein Subkomplex des 26S-Proteasoms, ist eine kompartimentierte multikatalytische Threonin-Nukleophil-Protease. Ihre sechs aktiven katalytischen Zentren mit multiplen Schnittpräferenzen (Peptidaseaktivitäten) liegen im Inneren eines 20S-Proteinkomplexes, der aus 14 verschiedenen Untereinheiten besteht. Proteasom-Aktivatoren regulieren den Zugang von Proteinen/Peptiden zum inneren

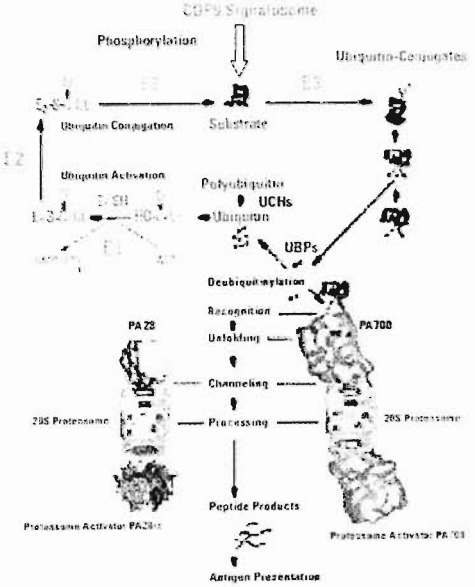


Abbildung 1:
Der Signalosom-Ubiquitin-Proteasome Pathway (SUPP): ein Überblick. Die enzymatische Maschinerie zur Ubiquitinylierung von Proteinsubstraten (E1-, E2-, E3-Enzyme) sowie die erforderlichen Aktivitäten zur Stabilisierung des monomeren Ubiquitin-Pools (Carboxyterminale Ubiquitinhydrolasen, UCHs und Ubiquitin-spezifische Processing-Proteasen, UBPs) sind im oberen Teil der Abbildung dargestellt. Der untere Abschnitt zeigt das 20S Proteasom und einige seiner assoziierten Modulatoren. Funktionen der Proteasomaktivatoren PA700 and PA28 wurden spezifiziert. Unterschiedliche Peptidaseaktivitäten der aktiven Zentren der 20S-Protease sind durch rote, grüne und blaue Sternchen symbolisiert.

Der Ubiquitin-Proteasom-Weg

Basisfunktionen eukaryontischer Zellen wie Proliferation, Differenzierung und Zelltod werden über die Stabilität molekularer Schaltermoleküle reguliert. Der Abbau der meisten dieser Proteine erfolgt über den Ubiquitin-Proteasom-Weg (Abb. 1). Er stellt ein intrazelluläres, cytosolisch/nukleär lokalisiertes Netzwerk aus Proteasen und ubiquitinylierenden Enzymen dar. Etwa 20 % des verfügbaren ATPs werden von eukaryontischen

katalytischen Reaktionsraum. Da das 26S-Proteasom fehlerhafte Proteine konstitutiv abbaut und der Abbau regulatorischer Schalterproteine nach Induktion durch kovalente Modifikation (Dephosphorylierung oder Phosphorylierung, Ubiquitinylierung) erfolgt, ist ein hoher Grad an Selektivität der Proteinsubstraterkennung erforderlich. Diese Selektivität wird durch eine spezifische Erkennung der individuellen Substrate durch Hunderte korrespondierender Ubiquitin-Protein-Ligasen,

Research Group Food Chemistry and Preventive Nutrition

Head: PD Dr. Ralf Stahlwasser

Due to the complex composition of food, it is difficult to demonstrate the relation between disease and nutrition for inflammatory and neoplastic diseases of the gut. The polymorphic genetic background and the metabolizing capability of the organism and its gastrointestinal microbiota have been insufficiently unrevealed so far. The protective mechanisms triggered by flavonoids, short chain fatty acids, and gastrointestinal microbiota could have a chemopreventive effect as to neoplastic diseases of the gut. Our scientific work is aimed at elucidating the physiological, cell biological, and molecular basis of protective or detrimental effects of food compounds. The intracellular protein degradation is the main focus of our research, since we were able to demonstrate that food factors interfere with molecular targets within the ubiquitin-proteasome pathway.

The ubiquitin-proteasome pathway

Basic cellular functions of eukaryotes such as proliferation, differentiation, and cell death are regulated via controlling the stability of molecular-switch molecules. The degradation of most of these proteins is performed by the ubiquitin-proteasome pathway (Figure 1), which is an intracellular, cytosolic/nuclear localized network of proteases and ubiquitinylating enzymes. Approximately 20% of the available ATP is used by eukaryotic cells to degrade ubiquitinylated proteins via this pathway of proteolysis. Ubiquitin, a conserved protein composed of 76 amino acids, is enzymatically activated with ATP consumption and covalently linked to lysine residues of substrate proteins by its carboxy-terminal glycine residue. The 26S proteasome recognizes, binds, unfolds, and degrades ubiquitinylated proteins, thereby generating MHC-class I restricted peptides for antigen presentation. The 20S proteasome, a subcomplex of the 26S proteasome, is a compartmentalized multicatalytic threonine nucleophile protease. Its six active catalytic sites representing multiple cleavage preferences (peptidase activities) are hidden in the internal cavities of the barrel shaped 20S protein complex, composed of 14 different subunits. Proteasome activators regulate the access of proteins/peptides to the inner catalytic chamber. Since the 26S proteasome degrades defective proteins constitutively and the degradation of regulatory switch proteins occurs in an induced manner after covalent modification (dephosphorylation or phosphorylation, ubiquitinylation), a high degree of selectivity of protein-substrate recognition is necessary.

Figure 1:
The signalosome-ubiquitin-proteasome pathway (SUPP): an overview.
The upper panel summarizes the enzymatic machinery for ubiquitinylation of protein substrates (E1, E2, E3 enzymes) and required activities stabilizing the pool of monomeric ubiquitin (ubiquitin carboxy-terminal hydrolases, UCHs and ubiquitin-specific processing proteases, UBPs). The lower panel depicts the 20S proteasome and some of its associated modulators. Functions of proteasome activators PA700 and PA28 are specified. Distinct peptidase activites of the catalytic 20S core protease are indicated by red, blue, and green asterisks.

This selectivity is ensured by specific recognition of individual substrates by hundreds of corresponding ubiquitin-protein ligases, E2 Ub conjugating enzymes, and ubiquitin hydrolases. Unlimited proliferation of malignantly transformed cells is partially based on deregulated proteolysis. In aggressively growing colon tumors, proteasome specific degradation of the p27 tumor suppressor protein is increased and accompanied by significant overexpression of the p27 specific F box protein, responsible for p27 substrate recognition by the corresponding ubiquitinylating E3 ubiquitin protein ligase. This example underlines that basic cellular processes and the detrimental pathological deregulation under disease conditions require regulation/deregulation of intracellular proteolysis via the ubiquitin-proteasome pathway. Chemopreventive food factors on the other hand might interfere with deregulated compounds and therefore be beneficial from a therapeutic or preventive perspective.

Modulation of basic cellular functions by non-nutritive food factors

Cellular metabolization of flavonoids

Considering the examination of molecular changes that are induced by flavonoids in epithelial cell lines of the gastrointestinal tract, the metabolization capacity of those cells (methylation, glucuronidation, sulfatation) should not be neglected, since modulatory effects of bioactive flavonoids might be extended by effects caused by these metabolites. We could demonstrate that HT 29 cells sulfate, glucuronidate, and methylate quercetin, thereby generating the corresponding metabolites.

Modulation of gene expression during inflammation and tumorgenesis

Based on our working hypothesis that non-nutritive food factors might modulate the mRNA expression of gene products participating in the ubiquitin-proteasome pathway, and thereby might modulate intracellular protein breakdown, we performed oligonucleotide microarray and Northern hybridization experiments to analyze differential gene expression in HT 29 adenocarcinoma cells (Fig.2). Some of the modulators tested revealed not only inhibition of proliferation and stimulation of apoptosis but also influenced the expression of genes associated with the ubiquitin proteasome pathway in a time- and concentration-dependent manner.

Figure 2:
Modulation of gene expression by flavonoids. Oligonucleotide microarray (left panel) and Northern analysis of gene expression in HT-29 cells. Cy3 (green) and Cy5 (red) labeled cDNA probes generated from reverse-transcribed total RNA of modulator-treated or solvent-treated cells were hybridized on glass slides. The custom-made pilot array covers 33 genes related to intracellular proteolysis. Each gene is represented by three individual 50-mer oligonucleotides and spotted in triplicates. The differential expression of SUPP-related genes detected by microarray analysis has been confirmed by Northern analysis (right panel) during time-course experiments as demonstrated here for the modulator-dependent suppression of the PA28γ-mRNA.

E2-Ub-konjugierende Enzyme und Ubiquitin-Hydrolasen gewährleistet. Eine unbegrenzte Proliferation maligne entarteter Zellen basiert teilweise auf einer deregulierten Proteolyse. In aggressiv wachsenden Kolontumoren ist der proteasomspezifische Abbau des Tumorsuppressorproteins p27 erhöht und von einer signifikanten Überexpression des p27-spezifischen F-box-Proteins begleitet, das für die p27-Substraterkennung durch die entsprechende ubiquitinylierende E3-Ubiquitin-Proteinligase verantwortlich ist. Dieses Beispiel unterstreicht, dass zelluläre Basisprozesse und die schädliche pathologische Deregulation unter Krankheitsbedingungen die Regulation/Deregulation der intrazellulären Proteolyse über den Ubiquitin-Proteasom-Weg erfordern. Chemopräventive Nahrungsfaktoren könnten andererseits mit deregulierten Verbindungen interferieren und deshalb aus therapeutischer oder präventiver Perspektive nützlich sein.

dieser Zellen (Methylierung, Glukuronidierung, Sulfatierung) nicht vernachlässigt werden, da modulatorische Effekte bioaktiver Flavonoide möglicherweise durch Wirkungen dieser Metabolite erweitert werden. Wir konnten nachweisen, dass HT-29-Zellen Quercetin sulfatieren, glucuronidieren und methylieren, wobei die entsprechenden Metabolite entstehen.

Modulation der Genexpression bei Entzündung und Tumorentstehung

Ausgehend von unserer Arbeitshypothese, dass nicht-nutritive Nahrungsfaktoren die mRNA-Expression von Genprodukten, die am Ubiquitin-Proteasom-Weg beteiligt sind, und somit den intrazellulären Proteinabbau modulieren, wurden Oligonukleotid-microarray- und Northern-Hybridisierungen zur Analyse der differentiellen Genexpression in HT29-Adenokarzinomazellen eingesetzt (Abb 2).

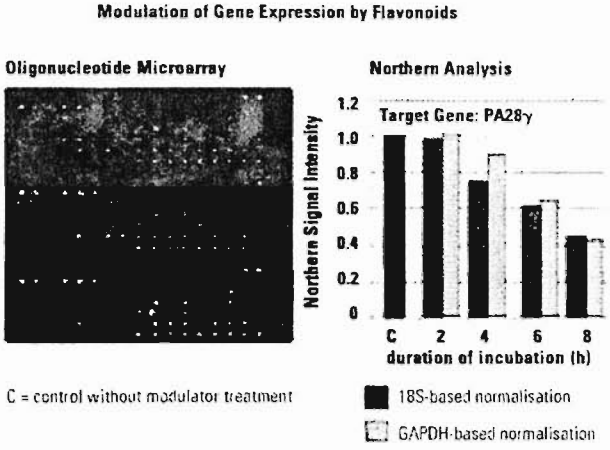


Abbildung 2:
Modulation der Genexpression durch Flavonoide
Oligonukleotid-Microarray-(links) und Northern-Analyse der Genexpression in HT-29 Zellen. Cy3 (grün) und Cy5 (rot) markierte cDNA-Proben wurden durch reverse Transkription von Gesamt-RNA modulator- oder lösungsmittel-behandelter Zellen synthetisiert und auf Glasobjektträger hybridisiert. Der nach unseren Vorgaben spezialangefertigte Pilotarray repräsentiert 33 Gene der intrazellulären Proteolyse. Für jedes Gen wurden drei verschiedene 50-mer-Oligonucleotide als Triplikate auf den Objektträger aufgebracht. Die differentielle Expression von SUPP-relevanten Genen, nachgewiesen durch Microarrayanalyse, wurde durch Northernhybridisierungen in Zeitabhängigkeitsexperimenten, wie hier für die modulatorabhängige Supprimierung der PA28γ-mRNA-Expression gezeigt (rechts), bestätigt.

Barbara Raab, Undine Lehmann, Hella Jürgens, Markus Glaubitz, Morana Marinovic, Ralf Stohwasser

Zelluläre Metabolisierung von Flavonoiden

Bei der Untersuchung molekularer Veränderungen, die durch Flavonoide in epithelialen Zelllinien des Gastrointestinaltraktes induziert werden, darf das Metabolisierungspotential

Einige der getesteten Modulatoren erwiesen sich nicht nur als proliferationshemmend und apoptosefördernd, sondern beeinflussten konzentrations- und zeitabhängig auch die Expression von Genen, die mit Funktionen des Ubiquitin-Proteasomweges assoziiert sind. In Kooperation mit der Universität Tübingen (M. Baus, Abt. Prof. N. Blin; Institut für Anthropologie und Humangenetik) konnten wir zeigen, dass die epitheliale NFκB-Aktivierung während der chronischen

Ausgewählte Publikationen
Selected Publications

Originalarbeiten/Original Papers

Aust, L., Dongowski, G., Frenz, U., Taufel, A., Noack, R.: Estimation of available energy of dietary fibres by indirect calorimetry in rats. Eur. J. Nutr. 40, 23-29 (2001)

Brigelius-Flohé, R., Muller, C., Menard, J., Florian, S., Schmehl, K., Wingler, K.: Functions of GI-GPx: Lessons from selenium-dependent expression and intracellular localization. BioFactors 14, 101-106 (2001)

Dongowski, G., Huth, M., Gebhardt, E., Flamme, W.: Dietary fiber-rich barley products beneficially affect the intestinal tract of rats. J. Nutr. 132, 3704-3714 (2002).

Dongowski, G., Lorenz, A., Prol, J.: The degree of methylation influences the degradation of pectin in the intestinal tract of rats and in vitro. J. Nutr. 132, 1935-1944 (2002)

Dongowski, G., Sembries, S.: Effects of commercial pectolytic and cellulolytic enzyme preparations on the apple cell wall. J. Agric. Food Chem. 49, 4236-4242 (2001)

Dongowski, G., Sembries, S., Bauckhage, K., Will, F., Dietrich, H.: Degradation of apple cell wall material by commercial enzyme preparations. Nahrung/Food 46, 105-111 (2002).

Florian*, S., Wingler, K., Schmehl, K., Jacobasch, G., Kreuzer, O. J., Meyerhof, W., Brigelius-Flohé, R.: Cellular and subcellular localization of gastrointestinal glutathione peroxidase in normal and malignant human intestinal tissue. Free Radic. Res. 35, 655-663 (2001)

Förster*, S., Dongowski, G., Kunzek, H.: Structure, Physicochemical properties and in vitro fermentation of enzymatically degraded cell wall materials from apples. Nahrung/Food 46, 158-166 (2002)

Gebhardt, E., Dongowski, G., Huth, M., Rabe, E.: Resistente Stärke - Bestandteil in bifunktionellen ballaststoffreichen Extrudaten. Ernährung/Nutrition 25, 202-208 (2001).

Graefe, E. U., Wittig, J., Mueller, S., Riethling, A.-K., Uehleke, B., Drewelow, B., Pforte*, H., Jacobasch, G., Derendorf, H., Veit, M.: Pharmacokinetics and bioavailability of quercetin glycosides in humans. J. Clin. Pharmacol. 41, 492-499 (2001)

Hohl, U., Neubert, B., Pforte*, H., Schonhof, I., Böhm, H.: Flavonoid concentrations in the inner leaves of head lettuce genotypes. Eur. Food Res. Technol. 213, 205-211 (2001).

Lehmann*, U., Jacobasch, G., Schmiedl, D.: Characterization of resistant starch type I from banana (Musa acuminata). J. Agric. Food Chem. 50, 5238-5240 (2002)

Mehrländer, K., Dietrich, H., Sembries, S., Dongowski, G., Will, F.: Structural characterization of oligosaccharides and polysaccharides from apple juices produced by enzymatic pomace liquefaction. J. Agric. Food Chem. 50, 1230-1236 (2002)

Rössler*, C., Jacobasch, G., Schmiedl, D., Gebhardt, E.: Resistante Stärke Typ 3 aus Roggenstärke, ein funktionelles Lebensmittel. Ernährung/Nutrition 26, 297-305 (2002).

Schwertz, A., Lehmann*, U., Jacobasch, G., Blaut, M.: Influence of resistant starch on the SCFA production and cell counts of butyrate-producing Eubacterium spp. in the human intestine. J. Appl. Microbiol. 93, 157-162 (2002)

Simmering, R., Pforte*, H., Jacobasch, G., Blaut, M.: The growth of the flavonoid-degrading intestinal bacterium, Eubacterium ramulus, is stimulated by dietary flavonoids in vivo. FEMS Microbiol. Ecol. 40, 243-248 (2002).

* Im Berichtszeitraum veröffentlichte Publikationen aus Diplom- und Doktorarbeiten ehemaliger Doktoranden.

Drittmittelprojekte
External Funding

Titel: Wertstoffgewinnung durch zweistufige enzymatische Behandlung von Obst- und Gemüsemaische - Analyse und physiologische Charakterisierung der gewonnenen Produkte
Finanzierung: AiF
Laufzeit: 03/01-12/03

Titel: Verfahrenstechnische und ernährungsphysiologische Aspekte zur Bildung von resistenter Stärke in Hafer durch Extrusion
Finanzierung: AiF
Laufzeit: 12/00-08/03

* Publications during the reporting period which are from dissertations by former doctoral students

Ernährungsberatungszentrum

Leitung: Dr. Christiana Ehrig

Center of Nutritional Counseling

Head: Dr. Christiana Ehrig

The Center of Nutritional Counseling is engaged in the development and evaluation of counseling strategies aimed at initiating and encouraging the long term realization of scientifically based dietary recommendations by the general public.
Studies indicate that most obese people are able to lose weight but unable to maintain it. Therefore, new concepts of counseling are required to develop strategies for weight reduction and maintenance. It is important to promote the participants' ability to control their weight and to maintain a healthy lifestyle (good diet, increased physical activity), also after completion of a weight loss program.
A personal focused concept was developed with strategies related to nutrition (diet with a moderate caloric deficit, fat reduction and modification, foods rich in starch and dietary fiber), behavior, and physical activity.
In order to examine the effectiveness of the concept, a pilot study has been started with 85 participants, 21-65 years of age with a mean body mass index of 33.6 kg/m². The program includes a long term training program for nearly 15 months, with a very intensive counseling phase (10 meetings in 2 1/2 months) followed by a phase with fewer group meetings (8 meetings in 12 months). In order to evaluate the effectiveness of the program, 1, 2, and 3 years after the end of the course, dietary behavior and physical activity, anthropometric data (e.g. weight, waist and hip measurements, body fat), and clinical biochemical parameters (blood glucose, cholesterol fractions etc.) will be determined.
Criteria of the Institute of Medicine of the National Academy of Science (USA) are in use as indicators of successful weight reduction. Accordingly, 35% of those who started the program were able to achieve and maintain a successful weight loss of at least 5% of initial body weight during the long term counseling period. 25% of the participants did not fulfill this criterion (intention to treat analysis).
Following the pilot study, the concept will be evaluated by a controlled intervention study.
Furthermore, the Center of Nutritional Counseling continues to be a well known scientific contact address for the regional and national public with regard to nutritional questions (Fig.1). In the last 2 years, 365 individuals with nutrition related diseases were advised. Most questions concerned obesity and associated comorbidities (dyslipidemia, diabetes mellitus etc.). Also, celiac disease and lactose intolerance were discussed.
More than 1400 inquiries (by telephone or letter) from people of the Potsdam/Berlin area and from the whole Federal Republic of Germany were answered. Also, numerous interviews on current topics of nutrition were given on radio and television and, moreover, in the press.

Figure 1
Christine Hofmann, staff member of the Center of Nutritional Counseling, "Day of Nutrition Research", DIfE, 2002

Das Ernährungsberatungszentrum beschäftigt sich mit der Entwicklung und Evaluation von Beratungsstrategien zur Initiierung und Unterstützung der langfristigen Umsetzung von aktuellen Ernährungsempfehlungen in der Bevölkerung. Studien zeigen, dass nicht die kurzfristige Gewichtsreduktion, sondern deren Stabilisierung für Übergewichtige bzw. Adipöse problematisch ist. Ein Wiederanstieg des Körpergewichts ist nach abgeschlossenen Maßnahmen zur Gewichtsreduktion und der damit wegfallenden Anleitung in aller Regel zu beobachten. Neue Konzepte der Beratung müssen sich darauf richten, diesem Wiederanstieg entgegen zu wirken. Die Selbstkompetenz der Betroffenen gilt es derart zu stärken, dass sie eigenständig die Entwicklung ihres Körpergewichts beurteilen und die optimalen Maßnahmen (günstige Kostwahl, adäquate körperliche Aktivität) flexibel ergreifen können.
Aufgrund dieser Erkenntnis wurde eine personenzentrierte Strategie entwickelt, bei der die Eigenverantwortung und die Selbstkompetenz der Betroffenen im Vordergrund stehen. Zur Effektivitäts- und Effizienzabschätzung der Konzeption läuft derzeit ein Pilotprojekt mit 85 Teilnehmern/innen im Alter von 21-65 Jahren und einem mittleren BMI von 33.6 kg/m².
Das Programm beinhaltet einen Trainingsprozess über annähernd 15 Monate mit einer intensiven Betreuungsphase (10 Kurstreffen in 2,5 Monaten) und einer anschließenden Phase mit nur noch 8 Treffen in 12 Monaten.

Die Beratungsschwerpunkte konzentrieren sich auf:
- Informationsvermittlung zu einer ausgewogenen Mischkost mit mäßigem kalorischen Defizit, Fettreduktion, -modifikation, Bevorzugung stärke- und ballaststoffreicher Lebensmittel, Steigerung der Alltags- und Freizeitbewegung
- Selbstkritische Beobachtung der Lebensmittelauswahl, des Ess- und Bewegungsverhaltens
- Selbstentwicklung und Integration persönlicher situationsgerechter Strategien zur Bewältigung ungünstigen Ess- und Bewegungsverhaltens
- Training des Selbstmanagements und der Eigenverantwortung für einen gesundheitsförderlichen Lebensstil.

Abbildung 1 Ernährungsberaterin Christine Hofmann am "Tag der Ernährungsforschung" im DIfE 2002

Zur Evaluierung der Konzeption werden 1, 2 und 3 Jahre nach Kursende folgende Zielgrößen erhoben:
- Anthropometrische Messgrößen (Körpergewicht, Taillenumfang, Körperfett usw.)
- Klinisch-biochemische Parameter (z.B. Blutzucker, Cholesterinfraktionen, Triglyceride)
- Protokoll- und Fragebogendaten zum Ess- und Bewegungsverhalten.

Als Indikatoren für eine erfolgreiche Gewichtsreduktion gelten die Kriterien des Institute of Medicine der National Academy of Sciences der USA. Hiernach konnten 35 % der Teilnehmer, die das Programm beendet hatten, eine Gewichtsreduktion von mindestens 5 % ihres Ausgangsgewichts erreichen und über den langen Kurszeitraum stabilisieren. 25 % der Teilnehmer erfüllten dieses Erfolgskriterium nicht (Intention-to-Treat-Analyse). Im Anschluss an das Pilotprojekt soll die Konzeption in einer kontrollierten Interventionsstudie überprüft werden.
Des weiteren gilt das Ernährungsberatungszentrum als regionaler und überregionaler wissenschaftlicher Ansprechpartner (Abb 1). In den letzten 2 Jahren wurden 365 Personen mit ernährungsabhängigen Erkrankungen beraten. Der Beratungsanlass war zumeist Übergewicht, verknüpft mit Folgeerkrankungen (Fettstoffwechselstörungen, Diabetes mellitus usw.). Auch Osteoporose, Zöliakie und Lactoseintoleranz zählten u.a. zum Themenkreis. Mehr als 1400 telefonische und schriftliche Anfragen aus der Region Potsdam/Berlin sowie aus der gesamten Bundesrepublik wurden beantwortet und Interviews zu Ernährungsthemen in Presse und Fernsehen gegeben.

Max-Rubner-Laboratorium (MRL)

Leitung: Dr. Christa Thöne-Reineke

Tierexperimentelle Versuchsvorhaben führen nur dann zu klaren, wissenschaftlich unanfechtbaren Ergebnissen, wenn die eingesetzten Tiere gesund und bestens betreut sind. Das Max-Rubner-Laboratorium beherbergt die zentrale Versuchstierhaltung des DIfE. Der 1996 in Betrieb genommene Neubau entspricht den modernsten Standards der Versuchstierhaltung. In gut ausgestatteten Tierhaltungs- und Funktionsräumen können Tiere optimal gehalten und physiologisch charakterisiert werden. Das MRL ist mit spezieller technischer Ausrüstung versehen, um die Haltungsbedingungen für die jeweiligen Versuchstiere unter höchstem Standard zu realisieren. Ein streng kontrolliertes Hygieneregime schützt Tiere und Menschen. Die Umsetzung der Ziele der modernen Versuchstierkunde hat im MRL oberste Priorität. So werden Versuche soweit als möglich durch Alternativmethoden ersetzt (**R**eplacement). Die Zahl der Tierversuche wird auf ein für die jeweilige wissenschaftliche Fragestellung unerlässliches Maß reduziert (**R**educement). Die Belastung der Tiere wird durch eine Verfeinerung der Methoden und Messtechniken sowie bei der Auswertung immer weiter verringert. Es gilt, mehr Informationen aus einem einzelnen Versuch (**R**efinement) zu gewinnen. Diese als Drei-R-Regel bezeichnete Maxime der Engländer *W.M.S. Russel* und *R.L.Burch* ist im MRL maßgebend.

Die Beratung der tierexperimentell tätigen Wissenschaftler in versuchstierkundlichen Fragen sowie die fachkundige Unterstützung in der Antragstellung und im Experiment erfolgt durch die Tierschutzbeauftragten, die Pflege und Betreuung der Tiere durch ein erfahrenes Team aus Fachtierärzten und Fachwissenschaftlern für Versuchstierkunde, Versuchstierpflegern und Biologielaboranten, die mit hohem Engagement und fürsorglicher Zuwendung zum Tier wesentlich zum Gelingen der Versuche beitragen.

Neben den Standardmethoden werden am MRL in Zusammenarbeit mit den wissenschaftlichen Abteilungen die in der Tabelle dargestellten besonderen Untersuchungsmethoden und Techniken verwendet.

Spezielle Techniken am DIfE

- Stoffwechseluntersuchungen in speziellen Stoffwechselkäfigen für Maus, Ratte, Hamster
- Energieumsatz- und Substratumsatzmessung (indirekte Kalorimetrie) in Maus, Ratte und Hamster mit und ohne Kälteprovokation
- Quantitative und zeitliche Erfassung der Nahrungsaufnahme (Feeding Monitor) und Nährstoffpräferenzen, Herstellung von speziellen Diäten
- Bestimmung der Körperzusammensetzung am lebenden Tier mittels Dexa-Messung und Kernspin (NMR) in der Maus als angewandtes Refinement
- Infusionen, Perfusionen, akute und chronische Katheter, ICV-Implantierung, Mikropumpenimplantation
- Transponderimplantation (kontinuierliche Temperatur- u. Aktivitätsmessungen)
- Blutdruckmessungen blutig und unblutig in Maus und Ratte, Operation zur Stenosierung der Nierenarterie (2K1C) bei der Ratte als Hochdruckmodell
- Zucht und Haltung von keimfreien Ratten und Generierung von gnotobiotischen Ratten
- Zucht und genetische Überwachung von Versuchstierstämmen
- Oozytenpräparation aus Xenopus laevis

In addition to the standard methods of laboratory animal science and welfare, special techniques are in use in the MRL in cooperation with the other departments of the DIfE.

Table 1: Special techniques

- Examination of metabolism of rats, mice, and hamsters by using metabolic cages
- Indirect calorimetric measurements to calculate energy expenditure and respiratory quotients in rodents under different temperature conditions
- Analysis of body composition by dual-energy X-ray absorptiometry (Dexa) and quantitative nuclear magnetic resonance (NMR) in living mice
- Monitoring of food intake by a feeding monitoring system using choice diets to analyze food preference
- Infusion, perfusion, acute and chronic catheter implantation (i.c.v.- implantation and implantation of osmotic pumps)
- Transponder implantation (continuous measurement of locomotive activity and body temperature).
- Intravascular blood-pressure measurement and non-invasive measurement using a tail cuff. Operative stenosis (two kidneys, one clip; 2K1C) as a model of renal hypertension.
- Processing, breeding, and maintenance of germfree rats
- Breeding and genetic monitoring of different laboratory animal strains
- Preparation of oocytes from Xenopus laevis

Max Rubner Laboratory (MRL)

Head: Dr. Christa Thöne-Reineke

Experimental research with animals can only provide sound, scientifically incontestable results when the animals used are healthy and cared for well. The Max Rubner Laboratory houses DIfE's central facilities for experimental animals. The new building, which went into operation in 1996, meets the most modern standards for keeping experimental animals. The animals can be kept under optimal conditions in well equipped animal keeping and functional rooms and physiologically characterized.

The MRL is especially equipped technically to realize the highest standards of care for the particular experimental animals. A strictly controlled hygiene regime protects both animals and humans. The realization of the goals of modern experimental-animal science has the highest priority at the MRL. Whenever possible, experiments are replaced by alternative methods (Replacement). The number of animal experiments is reduced to an essential level for the scientific question in mind (Reduction). Stress of the animals is even further reduced by using more refined analytical methods and measurement techniques. It is a matter of obtaining more data from every single experiment (Refinement). This so called 3 R rule of the Englishmen W.M.S. Russel and R.L.Burch is important at the MRL.

An animal protection official gives the scientists involved in animal experiments advice as to questions of animal science and expert support in filing of applications and during experiments. An experienced team of specialized veterinarians, experimental animal scientists and keepers, as well as biological laboratory assistants, who are all highly motivated and considerate, care for and treat the animals. They thus contribute substantially to the success of the experiments.

Forschungspreise und Ehrungen / Awards and Honors

Christian A. Barth
Ehrenmitgliedschaft der Deutschen Gesellschaft für Ernährung (DGE)

Matthias Tschop (Abt. Pharmakologie)
Young Investigator Award for Clinical Research 2002 of the
European Neuroendocrine Association

Hans-Georg Joost (Abt. Pharmakologie)
Jühling-Vorlesung und Jühling-Medaille 2002
"Molekulare Grundlagen von Adipositas und Typ-2-Diabetes
in Mausmodellen mit Metabolischem Syndrom"

Poster- und sonstige Preise / Poster and other Awards

M. Osterhoff, J. Schumacher, M. Möhlig, A. Kutz und A. Pfeiffer
Forschungspreis 2001 des Fachbereichs Humanmedizin
am Universitätsklinikum Benjamin Franklin der FU Berlin
Epinephrine potentiates glucose stimulated insulin secretion via a Ca2+/calmodulin-dependent protein kinase II δ_2 deficient rat insulinoma cell line INS-1(-/-) 2

C. Einig, C. Hofmann, K. Hoffmann, D. Johnsen, W. Luder und C. Barth
Posterpreis, 17. Jahrestagung der Deutschen Adipositas-Gesellschaft
Bremen 18.-20.10.2001
Erste Ergebnisse eines ambulanten langfristigen Gewichtsmanagementkonzepts

Gaby-Fleur Böl
Posterpreis, 5th Joint meeting 'Signal Transduction - Receptors, Mediators and Genes'
Weimar 8.-10.11.2001
Intracellular transport of PI3 kinase and interleukin-1 receptor associated kinase, IRAK, into the nucleus

L. Ma, A. Kuhlow und H.-R. Glatt
Aventis-Preis für den besten Kurzvortrag der Sektion Toxikologie vorgestellt
auf der Frühjahrstagung der Deutschen Gesellschaft für experimentelle und
klinische Pharmakologie und Toxikologie, Mainz 12.-14.3. 2002
Enhancement of the bioactivation of 1-hydroxymethylpyrene in rats treated with ethanol: studies on alcohol and aldehyde dehydrogenases. Naunyn-Schmiedeberg's Arch. Pharmacol. 365 (2002), P 79

Michael Ristow
Posterpreis, Jahrestagung der Deutschen Diabetes Gesellschaft, Dresden 8. - 11.5.2002
Beta-cell-specific inactivation of the Frataxin-Gene in mice results in impaired insulin secretion and manifest Diabetes mellitus and in vitro reversible Superoxid-Detoxification

Joachim Spranger
Posterpreis, Jahrestagung der Deutschen Diabetes Gesellschaft, Dresden 8. - 11.5.2002
Interleukin-6 ist ein unabhängiges Prädiktor für Typ 2 Diabetes mellitus: Ergebnisse der prospektiven populationsbasierten Kohortenstudie EPIC Potsdam

N. Landes, P. Pfluger und D. Kluth
Young investigator award, XIth Biennal Meeting of the International Society for
Free Radical Research (SFRR-I), Paris 16.-20.7.2002
Vitamin E activates hPXR-mediated gene expression in HepG2 cells

Patente 2002

Verfahren zur Expressionsveränderung von Polypeptiden
in rekombinanten Expressionssystemen (Abt. Ernährungstoxikologie)

Bitter taste receptors (Abt. Molekulare Genetik)

Ausgründung 2001

Peptides & elephants, Systeme zur Peptidsynthese

Veranstaltungen / Lectures and Conferences

2001

Rehbrücker Kolloquien / Rehbrücker Lectures

05.01.2001	Walter Hieber, Bad Honnef	Wissenschaft im Dialog 2001 - Das Jahr der Lebenswissenschaften
24.01.2001	Jens Brüning, Köln	Konditionale Mutagenese des Insulin-Rezeptors
02.04.2001	Elio Riboli, Lyon, F	The role of the EPIC study in elucidating the link between diet and cancer
04.04.2001	Michael Stumvoll, Tübingen	Ernährungseinflüsse auf die Insulin-Sekretion und -Resistenz
18.04.2001	Jürgen Kurths, Universität Potsdam	Nonlinear analysis of physiological data
09.05.2001	Angela D. Liese, University of South Carolina, USA	Diabetes surveillance in youth - the Richland-Lexington County child and adolescent diabetes registry
16.05.2001	Thomas Mothes, Leipzig	Wechselwirkung zwischen Getreideproteinen und dem Darm-assoziierten Lymphgewebe bei Zöliakiepatienten
07.06.2001	Michel Eichelbaum, Stuttgart	Absorption, metabolism and transport of drugs by the gastrointestinal tract
13.06.2001	Pierre Fafournoux, Clermont-Ferrand, F	Amino acid regulation of gene expression
27.06.2001	Pierre Brandtzaeg, Oslo, N	How does the intestinal immune system counterbalance the antigenic impact from food and bacteria?
02.07.2001	Maris Lazdins, Riga, LT	Mikrosatelliten und ihre Verwendung in genetischen Analysen und pränataler Diagnostik
09.07.2001	Lynda Williams, Aberdeen, UK	Body weight regulation and the melatonin signal
16.07.2001	M. D. Breyer, Vanderbilt University, USA	Investigating the role of renal prostanoid receptors in blood pressure control
18.07.2001	V. Bähr, Universitätsklinikum B. Franklin, Berlin	Interaktion des Steroid-Stoffwechsels mit dem Metabolischen Syndrom
27.07.2001	W.G. Bessler, Freiburg	Immunadjuvanzien bakterieller Herkunft
10.10.2001	Markus Brielmeier, Neuherberg	PHGPx-Knockouts: From induction of lymphoma to spermatogenesis
24.10.2001	Ellen Blaak, Maastricht University, NL	Changes in fat metabolism in diabetes and with age
24.10.2001	Patrick Schrauwen, Maastricht University, NL	The Role of UCP in energy and substrate metabolism
01.11.2001	Heike Englert, University of Illinois, USA	Community transformation by lifestyle medicine - challenge and chance?
07.11.2001	Jaap Keijer, Wageningen, NL	Analysis of functional effects of dietary components by DNA microarray technology
20.11.2001	Hindrik Mulder, Lund, S	Lipids and stimulus-secretion coupling in pancreatic beta cells - novel insights from the hormone-sensitive lipase null mouse
28.11.2001	Sigrun Korsching, Universität Köln	Information processing in the olfactory nervous system: Dissecting the network
03.12.2001	Eva Arrigoni, ETH Zürich, CH	In vitro Fermentierbarkeit unverdaulicher Polysaccharide
06.12.2001	Ulrich Hammerling, New York, USA	Vitamin A: New life for an old molecule, vitamin A meets zinc
12.12.2001	Gerd Hamscher, Hannover	Human- und Tierarzneimittel als neue resistente Umweltkontaminanten

Symposien, Workshops, andere Veranstaltungen / Symposia, Workshops, other Events

11.01.2001	Ringvorlesung: "Biotechnologie und Ernährung" Hans-Rudolf Glatt, DIfE	Bewertung von gentechnisch veränderten Lebensmitteln aus toxikologischer Sicht
23.01.2001	Ringvorlesung "Biotechnologie und Ernährung" Michael Blaut, DIfE	Warum bevölkern Bakterien unseren Darm?
25.01.2001	Ringvorlesung "Biotechnologie und Ernährung" Ernst Heinz, Universität Hamburg, Inst. für Allgemeine Botanik	Bessere Speiseöle aus transgenen Ölsaaten: Realisierung einer Provokation

01.02.2001	Ringvorlesung "Biotechnologie und Ernährung" Lothar Willmitzer, Max-Planck-Institut für Molekulare Pflanzenphysiologie, Golm	Biotechnologie bei Pflanzen - Grundlagen und Anwendungen
05.04.2001	DIfE Symposium	Pathogenesis and prevention of overweight - future perspectives
11.07.2001	DIfE-Wissenschaftstag	Ernährung und maligne Entartung
Juli 2001	Summer School, Abt. Epidemiologie	Grundlagen der Ernährungsepidemiologie
25.10.2001	Round table discussion	The future of nutrition research
15./16.11.2001	Internationaler Workshop	Physical activity (in Verbindung mit der EPIC-Studie), Organisation: Abt. Epidemiologie
14./15.12.2001	Internationaler Workshop	The future of biotechnology in the food market

2002

Rehbrücker Kolloquien / Rehbrücker Lectures

10.01.2002	Martina Pyrski, Baltimore, USA	The OMP-lacZ transgene mimics the unusual expression pattern of OR-Z6, a new odorant receptor gene on mouse chromosome 6 Implication for locus-dependent gene expression
16.01.2002	Arne Astrup, Fredriksberg, DK	The optimal diet composition for body weight regulation - where do we stand in 2002?
30.01.2002	Sheila Bingham, Cambridge, UK	Endogeneous N-nitrosation in the human colon
06.02.2002	Laszlo Nagy, Debrecen, H	Roles for nuclear hormone receptors in myeloid cell differentiation and function
13.02.2002	Harry Flint, Aberdeen, UK	Microbial ecology of the mammalian gut
20.02.2002	Hadi Al-Hasani, Köln	Targeting of the insulin-responsive glucose transporter GLUT4 in adipocytes
04.03.2002	Matthias Tschöp, Eli Lilly, Indianapolis, USA	Ghrelin – ein neues Hormon mit pleiotroper Wirkung
20.03.2002	Walter Becker, Aachen	Signaltransduktion der Leptinrezeptor-Spleißvarianten (LEPRa und LEPRb)
21.03.2002	Bette Phimister, Nature Genetics, New York, USA	Publication of research articles and peer review
24.04.2002	Andreas Rechner, London, UK	The metabolic fate of dietary polyphenols in humans - The colon as an important metabolic site
15.05.2002	Martin Klingenspor, Marburg	Molecular physiology of energy balance
27.05.2002	Shanta Persaud, London, UK	Pancreatic beta cell interactions and insulin secretion
29.05.2002	Gerd Kobal, Philip Morris, Richmond, USA	Neue Methoden zur Visualisierung olfaktorischer Aktivitäten im ZN
29.05.2002	Helmut Jungermann, Berlin	Risiko Wahrnehmung und Kommunikation
12.06.2002	Ingrid Boekhoff, Stuttgart	Signaltransduktionsprozesse chemosensorischer Neurone
19.06.2002	Thomas Kietzmann, Göttingen	Sauerstoff und Glucose als Regulatoren der Genexpression. Zwei Signale, ein Ziel?
26.06.2002	Gordon Birch, Reading, GB	The role of water in sweet taste chemo reception
10.07.2002	Ibrahim Elmadfa, Wien, A	ß-Carotin Aktuelles über Bioverfügbarkeit und gesundheitsrelevante Aspekte
21.08.2002	Annick Mercenier, Lausanne, CH	Lactic acid bacteria a new type of mucosal biotherapeutic agents
18.09.2002	Wolfgang Dubiel, Berlin	The COP9 signalosome and the regulation of ubiquitin-dependent proteolysis
27.09.2002	Gersh Zvi Taicher, Houston, USA	NMR methods for in-vitro and in-vivo analysis of bone tissue and whole body composition
09.10.2002	Ulf B. Göbel, Berlin	Diversity and specificity Oral microbiota and the initiation of chronic periodontal infections
10.10.2002	Moshe J. Weimann, Haifa, IL	Fructose and glycation Evidences and consequences in various systems

11.12.2002	Burkhard Viell, Berlin	Funktionelle Lebensmittel und gesundheitliche Aussagen auf Lebensmittel Möglichkeiten, Perspektiven, Grenzen
11.12.2002	Johannes Hebebrand, Marburg	Molecular-genetic aspects of obesity
19.12.2002	Robert F. Margolskee, New York, USA	Making sense of taste

Symposien, Workshops, andere Veranstaltungen / Symposia, Workshops, other Events

09.01.2002	Workshop, Organisation: A. Pfeiffer, Abt. Klinische Ernährung + UKBF	Aktueller Stand der Risikovorhersage häufiger Erkrankungen der Inneren Medizin durch Gendiagnostik. Wer will seine Genetik wissen und wofür?
23.01.2002	Workshop, Organisation: A. Pfeiffer, Abt. Klinische Ernährung + UKBF	Diabetische Retinopathie und Maculopathie – eine interdisziplinäre Herausforderung
28.02.2002	Workshop, Organisation: H. Boeing, Abt. Epidemiologie	Epidemiologisches Langzeitmonitoring des Lebensmittelverzehrs zum Nachweis gesundheitsrelevanter Wirkungen funktioneller Lebensmittel
06.03.2002	Workshop, Organisation: A. Pfeiffer, Abt. Klinische Ernährung + UKBF	UPDATE Endokrinologie
08.03.2002	Arbeitstagung der Gesellschaft für Anthropologie, Organisation: Abt. Interventionsstudien	Traditionelle und moderne Methoden zur Bestimmung der Körperzusammensetzung
16./17.03.2002	Meeting mit EU-Partnern, Organisation: W. Meyerhof, Abt. Molekulare Genetik	Somatostatin and its receptors in brain function and dysfunction
14./15.03.2002	International Workshop, Organisation: H. Boeing, Abt. Epidemiologie	Scientific evidence and public health nutrition: how to derive dietary recommendations
22.07.-02.08.2002	Summer School	Grundlagen der Ernährungsepidemiologie
01.09.2002	Workshop mit EU-Partnern, Organisation: Hans-Joachim Zunft, Abt. Interventionsstudien	Eurolive
06.09.-07.09.2002	Workshop mit EU-Partnern, Organisation: Hans-Joachim Zunft, Abt. Interventionsstudien	Crownalife
19.09.2002	Tag der offenen Tür	
26.09.2002	Workshop mit EU-Partnern, Organisation: Hans-Joachim Zunft, Abt. Interventionsstudien	Body Life
31.10.-02.11.2002	Internationales DIfE-Symposium	Frontiers in nutrition research
01.11.-02.11.2002	Workshop mit EU-Partnern, Organisation: Susanne Klaus, AG Energiestoffwechsel	Adipose tissue and its function in obesity (COST)

Presse- und Öffentlichkeitsarbeit 2001/2002

Mitarbeiter des DIfE sind begehrte Ansprechpartner für die Medien. Knapp 200 mal standen die Wissenschaftler und Wissenschaftlerinnen im Berichtszeitraum in Fernsehen und Radio im Mittelpunkt von Beiträgen (Tab.1, S.66) in der Regionalpresse, aber auch überregional wurden DIfE-Mitarbeiter als Gesprächspartner zu zahlreichen Themen rund um die Ernährungsforschung befragt und gaben gerne Auskunft. Angeregt durch die wissenschaftlichen Forschungsergebnisse aus dem DIfE konnten Journalisten z.B. für die Themen Geruch- und Geschmack, Adipositas oder Diabetes über neue Aspekte berichten. Zahlreiche Anfragen von Einrichtungen und auch Bürgern wurden vom Referat Öffentlichkeitsarbeit oder dem Ernährungsberatungszentrum beantwortet.

2001

Das Jahr 2001 präsentierte sich als das "Jahr der Lebenswissenschaften"; für das DIfE willkommener Anlass, sich bei zahlreichen Gelegenheiten zu engagieren und öffentlich in Erscheinung zu treten.

So fand vom 7.-9. Juni das DAAD-BioForum Berlin "Grenzenlos forschen" statt. Bei einer Veranstaltung an der Humboldt-Universität zu Berlin trat das DIfE zusammen mit der Technischen Universität Berlin auf. Anhand eines Modells des Verdauungstraktes konnten die Besucher nach "innen blicken", die angebotenen Informationen reichten von den DIfE-Forschungsprojekten bis zur Zusammensetzung von Lebensmitteln. Auch der Verein zur Förderung der Nutrigenomforschung wurde vorgestellt.

Höhepunkt war ein Forschungsmarkt vom 12.-17. September in den Arkaden des Potsdamer Platzes zum Ausklang des Wissenschaftssommers in Berlin, der von der Technischen Universität Berlin (Wissenstransfer Berlin) organisiert wurde. 46 Aussteller beteiligten sich, das DIfE war mit einem Stand im Rahmen des Gemeinschaftsprojektes "Die lebende Zelle" vertreten. Auf Postern wurde zum Thema "Ballaststoffe in der Ernährung" informiert, ein Mikroskop bot Einblicke in kleinste Welten. Der Stand war jeden Tag durch zwei Betreuer besetzt, im Rahmen der „Langen Nacht der Wissenschaft" sogar bis 24 Uhr. Dieser Einsatz war nur dadurch möglich, dass zahlreiche DIfE-Abteilungen mithalfen und die Mitarbeiter "Schichtdienst" leisteten.

Das "Jahr der Lebenswissenschaften" nahm eine Gruppe Berliner und Brandenburger Forschungsinstitutionen zum Anlass, eine Einführung in das "Buch des Lebens" zu geben. In der Berliner Urania fanden vom 12.-15. November Informationsveranstaltungen dazu statt. Durch einen Vortrag von Prof. Dr. Susanne Klaus zum Thema Hunger- und Sättigungsregulation wurde das Thema "My Food" wissenschaftlich begleitet.

2002

Über 200 mittelständische Unternehmen und Forschungseinrichtungen aus ganz Deutschland präsentierten am 3. Juni auf dem 9. Innovationstag der Arbeitsgemeinschaft industrieller Forschungsvereinigungen "Otto von Guericke" (AiF) neue Produkte und Ideen. Das DIfE beteiligte sich mit zwei Projekten, die von Barbora Drzikova und Dr. Gerhard Dongowski betreut wurden: "Herstellung, funktionelle Eigenschaften und ernährungsphysiologische Wirkungen ballaststoffhaltiger Produkte aus Gerste" und "Zusammensetzung und physiologische Wirkungen eines Apfelsaftproduktes, hergestellt durch enzymatische Tresterbehandlung".

Am 27. Juni fand auf dem Potsdamer Telegrafenberg der 4. Tag der Wissenschaft im Land Brandenburg statt. In der Themengruppe Ernährungswissenschaften, Gesundheit, Sport hielt Ute Nöthlings, Abteilung Epidemiologie, einen Vortrag mit dem Titel "Was können wir mit Fragebögen zur Ernährung erfassen?"

Nach einer baubedingten Pause konnte das DIfE am Tag der offenen Tür am 19. September mehr als 500 interessierte Besucher begrüßen. Die begleitende Ausstellung "55 Jahre Ernährungsforschung in Rehbrücke" schöpfte aus dem Fundus des Instituts, Vergangenheit und Gegenwart der modernen Ernährungsforschung konnten bestaunt werden. Neben den Abteilungen des DIfE war auch das Institut für Ernährungswissenschaft der Universität Potsdam vertreten.

Am 20. Oktober fand im Stern-Center der 8. Gesundheitsmarkt der Stadt Potsdam statt. Das DIfE war durch das Ernährungsberatungszentrum mit Frau Dr. Christiana Eimg und Frau Christine Hofmann vertreten. Zahlreiche Fragen zur Ernährung wurden kompetent beantwortet. Mit dem BIA-Gerät (unter Betreuung von Katja Ruttkowski und Christa Scholz aus der Abteilung Interventionsstudien) zur Bestimmung des Körperfettanteils konnten interessierte ihren kritischen Wert erfahren und gleich besprechen, was zu tun wäre.

Großes Echo in den Medien fand das vom 31. Oktober - 2. November veranstaltete Internationale Symposium "Frontiers in Nutrition Research" mit 200 Teilnehmern. Anlässlich einer Feierstunde zum 10jährigen Bestehen des DIfE sagte Prof. Johanna Wanka, Ministerin für Bildung, Forschung und Kultur des Landes Brandenburg: "Das DIfE hat viel getan, um zur ersten Adresse in der deutschen Ernährungsforschung zu werden."
Der wissenschaftliche Direktor des DIfE, Prof. Hans-Georg Joost, eröffnete den wissenschaftlichen Teil der Veranstaltung mit einer Einführung in die Fragestellungen der modernen Ernährungsforschung z.B. auf den Gebieten Krebs und Diabetes. Prof. Hannes Stähelin aus Basel bezog Stellung zu Functional-Food- und Gen-Food-Forschung.

DIfE beim Blind Date: Initiiert durch den Brandenburgischen Kunstverein Potsdam e.V. haben sich fünf Institute des Potsdamer Raums mit je einem Künstler zusammen getan und den Versuch unternommen, den Dialog zwischen Wissenschaft und Kunst aufzunehmen. Das DIfE kooperierte mit dem Künstler Malte Brekenfeld, das gemeinsame Projekt lief im Kutschstall in Potsdam bis zum 1. Dezember 2002.

Press and public relations in 2001 and 2002

Members of the DIfE staff are very much in demand as contacts for the media – on nearly 200 occasions within these two years, scientists of the institute were the focus of attention on radio and television. Personnel of the DIfE were interviewed on numerous topics centering around nutritional research and they were glad to provide information, not only for the regional press but also at a supraregional level. Stimulated by the results of nutritional research at the DIfE, journalists were able to report on new aspects of topics such as smell and taste, obesity, or diabetes. Many inquiries by institutions or individuals were answered by the Public Relations Department or the Center of Nutritional Counseling.

2001

Since the year 2001 had been declared "Year of Life Sciences," it was a good reason for DIfE to get involved in the activities and to appear in public. Thus, the CAAC Bioforum "Boundless Research" took place in Berlin. Together with the Berlin Technische Universität, DIfE made its appearance at an event at the Humboldt University of Berlin. A model of the gastrointestinal tract allowed visitors to get an "inside look." The information presented covered a spectrum reaching from DIfE's research projects to the composition of foods. In addition, the Society to Promote Nutrigenome Research was presented.

The research market on September 12-17 was a highlight at the commercial center of Potsdam Plaza (Berlin), to close the "Science Summer," which had been organized by the Technische Universität (Wissenstransfer Berlin). Forty-six exhibitors took part and DIfE was represented at a stand within the framework of a joint project, "The living cell." Posters with information on "Fiber in Foods" were shown, and a microscope gave visitors the opportunity to "take a peek." Two people were in charge of the stand every day, even until midnight on the "Long Night of Science." This was made possible by the many staff members who helped out and worked in shifts.

A group of Berlin and Brandenburg research institutions used the "Year of Life Sciences" as an opportunity to introduce the "Book of Life." At the Urania in Berlin, informational events took place (November 12-15). A lecture by Prof. Dr. Susanne Klaus on the regulation of hunger and satiety accompanied the theme "My Food" from a scientific standpoint.

2002

On June 3, more than 200 medium-sized companies and research institutions from all of Germany presented new products and ideas at the 9th Innovation Day of the Working Group of Industrial Research Associations "Otto von Guericke." DIfE participated with two projects, of which Barbora Drzikova and Dr. Gerhard Dongowski were in charge. "Production, functional characteristics, and nutritional-physiological effects of fiber-containing products made from barley" and "Composition and physiological effects of an apple-juice product made by enzymatic treatment of pomace."

On June 27, the 4th Day of Science took place in Brandenburg at the Potsdam Telegrafenberg. Within the topics "nutritional science, health, and sports", Ute Nöthlings, Department of Epidemiologie, held a talk with the title "What can we find out with questionnaires on nutrition?".

After a break due to construction work, DIfE was able to greet more than 500 interested guests at its "Open House" on September 19. This was accompanied by an exhibit "55 Years of Nutritional Research in Rehbrücke", which included items with relation to nutrition research from the past and from the present to amaze the visitors. The Institute of Nutritional Science of the Universität Potsdam was also represented, in addition to the departments of the DIfE.

On October 26 at the Stern Center, the City of Potsdam had its 8th Health Market. DIfE was represented by Dr. Christiana Einig and Christine Hofmann of the Center of Nutritional Counseling. Interested individuals could have their relative body fat measured using the BIA apparatus (in charge were Katja Ruttkowski and Christa Scholz from the Department of Intervention Studies), find out their personal value, and discuss on the spot what they should do.

The media broadly reported about the international symposium "Frontiers in Nutrition Research" that took place from October 31 until November 2 and which attracted 200 participants. Celebrating DIfes 10th anniversary Brandenburgs ministery for Education, Science and Culture, Prof. Dr. Johanna Wanka, pointed out: "DIFE has done a lot to become the top address in German nutritional research." The Scientific Director of the institute, Prof. Hans-Georg Joost, opened the scientific program of the event with an introduction to the questions of modern nutrition research, for example, in the areas of cancer and diabetes. Prof. Hannes Stähelin from Basel, Switzerland, gave his views on research into functional foods and genetically altered foods.

DIfE on a blind date: each of five institutes in the Potsdam area worked together with an artist in an attempt to open a dialog between science and art. This was initiated by the Brandenburg Kunstverein (art association) Potsdam e.V. DIfE cooperated with the artist Malte Brekenfeld and the joint project was at the Kutschstall in Potsdam until December 1, 2002.

Das DIfE im Hörfunk und TV 2001/2002 – Auswahl aus über 190 Sendungen

DIfE in TV and radio programs – Selection from more than 190 interviews

ARD-Ratgeber Gesundheit Beitel	ARD/SFB	Dr. Christiana Ehrig
Wissenschaft: Epidemiologie	ARTE	Dr. Manuela Bergmann
Vegetarische Ernährung	n-tv	Prof. Dr. Andreas Pfeiffer
Ostern BodPod	ORB - Wissenschaftsmagazin	Dr. Dieter Johnsen
Tagung Krebs und Ernährung	TV Berlin	Prof. Dr. H.-R. Glatt
Logo BioProfile	NDR 4	Prof. Dr. Christian Barth
Trinken im Sommer	SAT1 - Nachrichten	Dr. Jörg Haseler
"Schlankheitsmittel" BMI 23	SAT1-Frühstücksfernsehen	Dr. Michael Boschmann
isotonische Getränke	MDR, Radio Sachsen-Anhalt	Dr. Jörg Haseler
Gesundheitssystem Deutschland	RAI (Italien)	Prof. Dr. Wolfgang Meyerhof
Quivive Diabetes	SFB	Prof. Dr. Andreas Pfeiffer
Geschmackssinne	WDR	Prof. Dr. Wolfgang Meyerhof
Abendjournal: Neuer Direktor	ORB - ORB3	Prof. Dr. Hans-Georg Joost
Expertenrunde	SFB - 88,8	Dr. Dieter Johnsen
Gesund abnehmen	MDR - Radio Sachsen-Anhalt	Prof. Dr. Susanne Klaus
Ernährungsphysiologie Fisch	HR1	Dr. Jörg Haseler
Dick sein macht krank	inforadio	Prof. Dr. Christian Barth
Butterverbrauch	SFB - B1	Dr. Jörg Haseler
Adipositas	infoRadio	Dr. Dieter Johnsen
Quivive. Übergewicht und was kann man dagegen tun	SFB - B1	Dr. Christiana Ehrig
Hauptsache gesund Gemüse	MDR3	PD Dr. Ralf Stohwasser
Wildpflanzen	ORB	Christiana Hamisch
Vitamine	Pro7	Dr. Gaby Bol
Vitamine	SAT1 - Nachrichten	Dr. Gaby Bol
Abnehmen	SAT1 - Vera am Mittag	Dr. Christiana Ehrig
Krebsstudie	Deutschlandfunk	PD Dr. Heiner Boeing
Nitrofen	n-tv Nachrichten	Prof. Dr. Dr. Hans-Georg Joost
Wochenmarkt: Lebensmittelskandale	SFB - B1	Prof. Dr. Dr. Hans-Georg Joost
Hauptsache gesund Geschmacksgedächtnis	MDR	Prof. Dr. Hans-Joachim Zunft
Appetitzügelndes Hormon PYY 3-36	MDR - Info	Dr. Matthias Tschöp
Essstörungen	SAT1-Frühstücksfernsehen	Dr. Christiana Ehrig
Fitness ohne Schwitzen - Hormone lassen die Pfunde auch von Unbeweglichen schwinden	Deutschlandfunk	Dr. Matthias Tschöp
Kochkunst Geruch und Geschmack	Arte	Prof. Dr. Wolfgang Meyerhof
Vegetarier	ORB	Prof. Dr. Dr. Hans-Georg Joost
Schwierigkeiten bei Empfehlungen - Ernährungsexperten ringen um allgemeinverständliche Ratschläge	Deutschlandradio	PD Dr. Heiner Boeing
Ghrelin	Deutschlandfunk	Dr. Matthias Tschöp
Westblick Entstehung von Krebs durch Ernährung	WDR - WDR5	PD Dr. Heiner Boeing
Sprechstunde: Adipositas und Diabetes	Deutschlandfunk	Dr. Matthias Tschöp, Dr. Michael Ristow

Besucher willkommen! Visitors welcome!

Das DIfE freut sich über Besucher, der Austausch vor Ort wird gewünscht und gesucht. Studentengruppen und Wissenschaftler sind ebenso willkommen wie Senioren oder Schulklassen im Rahmen des Tages der offenen Tür. Auch Politiker nahmen die Gelegenheit wahr, sich an Ort und Stelle über die Aktivitäten des Institutes zu informieren. Beispielhaft einige Ereignisse aus dem Berichtszeitraum, bildlich festgehalten.

The DIfE is pleased to have guests and encourages an on-site exchange of ideas. Scientists and groups of students are just as welcome as are senior citizens and classes of school students within the framework of DIfE's "open house" day. In addition, politicians took the opportunity to be informed on the spot about the activities of the institute. Examples of some of the events during the two-year period of this report, in the following photographs.

7. Juni 2002: Brandenburger SPD-Bundestagsabgeordnete besuchen das DIfE. Angelika Krüger-Leißner (SPD), Dr. Peter Wilhelm Danckert (SPD), Ernst Bahr (SPD), Dr. Babette Reipert, Dr. Emil Schnell (SPD) und Prof. Dr. Michael Brand (v.l.n.r.).

6. November 2002: Rund 200 Besucher sind zu Gast im DIfE beim Symposium "Frontiers in Nutrition Research", darunter die Brandenburgische Ministerin für Wissenschaft, Forschung und Kultur Frau Prof. Johanna Wanka.

12. November 2002: Prof. Dr. Hans-Olaf Henkel (2.v.r.) im Bild: spricht zum Thema "Forschungsstandort Deutschland: Probleme und Perspektiven unter Berücksichtigung der Umweltwissenschaften". Als weitere Gäste kamen Prof. Dr. Christian Barth, Dr. Wilhelm Seyfarth vom Zentrum für Agrarlandschafts- und Landnutzungsforschung (ZALF) in Müncheberg, Prof. Dr. Jürgen Zaske vom Institut für Agrartechnik (ATB) in Potsdam-Bornim, Dr. Manfred Stock vom Institut für Gemüse- und Zierpflanzenbau (IGZ) in Großbeeren, Dr. Eckhard George vom Institut für Klimafolgenforschung (PIK) und Hr. Heiner Kohlzus begrüßt werden. (Foto: Plantekt)

17. Mai 2002 Workshop Epidemiologie

8. März 2002 Arbeitstagung der AG "Angewandte Anthropologie" der Gesellschaft für Anthropologie e.V. (GfA). Die Organisatoren vor Ort hatten Dr. Dieter Johnsen (im Bild mit Frau Prof. Dr. Holle Greiß und Frau Dr. Ulrike Trippe).

Jeweils Januar bis April 2001/2002: Kurs Ernährungsmedizin der Ernst-von-Bergmann-Akademie für ärztliche Fortbildung der Ärztekammer Berlin, der Akademie für ärztliche Fortbildung der Landesärztekammer Brandenburg und des DIfE. Intensive Lern-Wochenenden für die Kursteilnehmer.

8. August 2002 Summer School Epidemiologie

Mitarbeiter 2001/2002 - Staff 2001/2002

Vorstand / Board

Barth, Christian, Prof. Dr
(bis 31.12.01)
Echtermeyer, Brigitte
Joost, Hans-Georg, Prof. Dr. Dr
(ab 01.01.02)
Kohlmann, Gudrun
Lammersmann, Monika
Schäfer, Judith, Dr
Schmidt, Anke, Dr
Schulz, Hartmut, Dr

Abteilung Molekulare Genetik
Department of Molecular Genetics

Behrens, Mark, Dr
Brune, Nicole, Dipl.-Biol.
Burk, Bernd, Dipl.-Biol.
Carlo, Anne-Sophie, Neurobiol.
Chirokova, Elena, Dipl.-Biophysikerin
Demgensky, Stefanie
Kocharyan, Ara, Dr
Krautwurst, Dietmar, Dr
Kretzschmar, Beate
Kreuzer, Oliver, Dipl.-Ing
Kuhn, Christina, Dipl.-Biol
Lerner, Ulrike
Meyerhof, Wolfgang, Prof. Dr.
Otas, Gisela, Dr
Pyrsk., Martina, Dr.
Robert, Christophe, Dr.
Rogers-Blaut, Lynne, Dr
Schmiedeberg, Kristin, Dipl.-Biol
Schöley-Pohl, Ellen
Silkeit, Monika
Stepanyan, Zaruhi, Medizinerin

Abteilung Pharmakologie
Department of Pharmacology

Al-Hasani, Hadi, Dr
Castañeda, Tamara, Dr.
Kluge, Reinhart, Dr
Pium, Leona, Dr
Rischke, Brigitte
Scheepers, Andrea, Dipl.-Biol
Schmolz, Katja, Dipl.-Biol.
Schürmann, Annette, Prof. Dr
Tschöp, Matthias, Dr.
Warnke, Kathrin
Zahn, Claudia, Dipl.-Biol

Arbeitsgruppe Physiologie des Energiestoffwechsels
Laboratory of Physiology of the Energy Metabolism

Adams, Frauke, Dipl.-Ern.-wiss.
Albrecht, Petra
Backes, Gunda, Dr.
Boeuf, Stéphane, Dr.
Boschmann, Michael, Dr
Brüning, Silke
Daenzer, Maren, Dipl.-Ern.-wiss
Klaus, Susanne, Prof. Dr
Metges, Cornelia C., PD Dr
Ortmann, Sylvia, Dr
Petzke, Klaus-Jürgen, Dr.
Plaue, Carola
Schaller, Karin
Sylvester, Antje
Ulbricht, Dorit
Wiedmer, Petra, Dipl.-Ern.-wiss

Abteilung Klinische Ernährung

Assert, Roland, Dr
Glaubitz, Markus, Dipl.-Oec.troph
Hannemann, Melanie
Jahr, Beate
Käding, Helga
Koske, Anke
Möhlig, Matthias, Dr.
Osterhoff, Martin, Dr.
Pfeiffer, Andreas F. H., Prof. Dr
Pomplun, Doreen, Dipl.-Ern.-wiss.
Richter, Susann
Richter, Susanne, Dipl.-Lebensmittelchem
Ristow, Michael, PD Dr.
Rucovich, Natalia, Dr.
Salami, Shojaie Farideh
Simon, Kristin
Spranger, Joachim, Dr.
Sprengel, Katrin
Stemmer, Kerstin, Dipl.-Biol.
Trölenberg, Heike
von Kraack, Wiltraut
Wegewitz, Uta, Dipl.-Ern.-wiss

Abteilung Interventionsstudien
Department of Intervention Studies

Hanisch, Christiana, Dipl.-Oec.troph.
Höhne, Antje, Dipl.-Ern.-wiss
Hoppe, Konrad, Dr
Hoyer, Stephan, Dipl.-Ern.-wiss
Johnsen, Dieter, Dr.
Koebnick, Corinna, Dr
Lorse, Martina
Machowetz, Anja, Dipl.-Oec.troph
Möseneder, Jutta, Dr
Nicolai, Karin, Dipl.-Agraring
Pester, Silvia
Reimann, Manja, Dipl.-Ern.-wiss.
Ruttkowski, Katja
Scholz, Christine
Schröder, Renate
Schultz, Stefanie
Seppelt, Brigitte, Dipl.-Math
Simchen, Ulrike, Dipl.-Ern.-wiss
Trippo, Ulrike, Dr
Ulbricht, Gottfried, Dr
Vulprecht, Dagmar
Wagner, Karen, Dipl.-Ern.-wiss
Zunft, Hans-Joachim Franz, Prof. Dr.

Abteilung Epidemiologie

Behling, Kay
Bergmann, Manuela, Dr
Bergmann, Wolfgang
Boeing, Heiner, PD Dr.
Dahl, Andreas, Dipl.-Ern.-wiss.
Drogan, Dagmar, Dipl.-Ern.-wiss
Falkosch, Sabine
Ficht, Wilfried
Fidanicke, Ulrike, Dipl.-Ern.-wiss
Fisher, Eva, Dr
Fleischhauer, Wolfgang, Mediziner
Franke, Andrea
Haftenberger, Marjolein, Dipl.-Ern.-wiss.
Heidemann, Christin, Dipl.-Ern.-wiss
Heinrich, Christine
Hoffmann, Kurt, Dr.
Knipstein-Grobusch, Kerstin, Dr
Kohlsdorf, Ellen
Kroke, Anja, Dr.
Kühn, Kathrein
Lahmann, Petra, Dr
Lebsa, Heiderose
Nöthlings, Ute, Dipl.-Ern.-wiss.
Schienkiewitz, Anja, Dipl.-Oec.troph
Schulz, Mandy, Dipl.-Ern.-wiss.
Schulze, Matthias, Dipl.-Ern.-wiss.
Walter, Dietmar, Dipl.-Oec.troph.
Weeske, Gabriele

Abteilung Ernährungstoxikologie
Department of Nutritional Toxicology

Braune, Sabine
Brüning, Silke
Engst, Wolfram, Dr
Glatt, Hans-Rudolf, Prof. Dr
Gumz, Christine
Hollnagel, Heli Miriam, Dipl.-Lebensmittelchem.
Katschak, Andrea
Knuth, Brigitte
Liu, Yungang, Dr
Ma, Lan, Dr.
Meinl, Walter, Dr.
Meyer, Elisabeth
Muckel, Eva, Dr
Pabel, Ulrike, Dipl.-Biol
Schneider, Heiko, Dr
Scholtyssek, Martina
Schwenk, Jutta
Sommer, Yasmin, Dipl.-Lebensmittelchem

Mitarbeiter* 2001/2002 - Staff** 2001/2002

Abteilung
Gastrointestinale Mikrobiologie
Department of Gastrointestinal Microbiology

Blaut, Michael, Prof. Dr
Braune, Annett, Dr.
Clavel, Thomas, Dipl.-Biol.
Grubl, Barbel
Herles, Claudia, Dr.
Herzog, Renate
Hollnagel, Heli Miriam, Dipl.-Lebensmittelchem.
Kleeßen, Brigitta, Dr.
Lachmann, Kathrin
Lehmann, Katrin, Dipl.-Ern.-wiss.
Mohan, Ruchika, Mikrobiologin (Master)
Müller, Susanne, Dipl.-Ern.-wiss.
Namsolleck, Pawel, Dipl.-Meeresbiol.
Schindler, Regine
Schmidt, Sabine
Schoefer, Lilian, Dr.
Schwiertz, Andreas, Dr.
Szczesny, Silke, Dr.
Taras, Davic, Dr.
Thiel, Ralph, Dipl.-Biol.
Urbich, Marion
Zimmermann, Sabine

Abteilung Vitamine und Atherosklerose
Department of Vitamins and Atherosclerosis

Aust, Gerlinde
Banning, Antje, Dipl.-Ern.-wiss.
Birringer, Marc, Dr.
Bittner, Jörg-Uwe
Böl, Gaby-Fleur, Dr.
Brigelius-Flohé, Regina, Prof. Dr.
Deubel, Stefanie
Gühler, Anke
Jurrmann, Nadine, Dipl.-Biol.
Kluth, Dirk, Dipl.-Biol.
Krohn, Elvira
Kuka, Daniela, Dipl.-Biol.
Kupper, Dagmar, Dr.
Landes, Nico, Dipl.-Ern.-wiss.
Müller, Cordula, Dr.
Müller-Schmehl, Katrin, Dr.
Pfluger, Paul, Dipl.-Biol.
Schindler, Regine
Schnurr, Kerstin, Dr.
Wendt, Edith

Arbeitsgruppe Präventiv-Medizinische Lebensmittelforschung
Laboratory of Food Chemistry and Preventive Nutrition

Chudoba, Elke
Dongowski, Gerhard, Dr.
Drzikova, Barbora, Dipl.-Ern.-wiss.
Florian, Simone, Dipl.-Oec.troph.
Jürgens, Hella, Dipl.-Oec.troph.
König, Bärbel
Kretzschmar, Beate
Kunkel, Bärbel
Lehmann, Undine, Dr.
Wolschack, Horst
Marinova, Mariana, Dipl.-Biochem.
Niehaus, Monika
Pforte, Holger, Dipl.-Lebensmittelchem.
Raab, Barbara, Dr.
Richter, Karin
Rössler, Christine, Dr.
Sembries, Sabine, Dr.
Stohwasser, Ralf, PD Dr.

Max-Rubner-Laboratorium
Max Rubner Laboratory

Bebert, Ines
Gaebel, Gerhard
Gruener, Ines
Güldenpenning, Siegfried
Hoffmeister, Katrin
König, Swetlana
Krause, Janet
Lehmann, Ute
Steinmeyer, Elvira
Thom, Elke
Thomas, Irmgard
Thöne-Reineke, Christa, Dr.
Weinert, Kerstin

Ernährungsberatungszentrum
Nutrition Consultation Center

Einig, Christiana, Dr.
Hofmann, Christine
Lüder, Wolfgang, Dr.

Presse- und Öffentlichkeitsarbeit
Press and Public Relations

Backes, Gunda, Dr.
Häseler, Jörg, Dr.
Johnsen, Dieter, Dr.

Bibliothek
Library

Heidmann, Regine
Kolihof, Dagmar

Informationstechnik
Information Technology

Kozerke, Wolfgang, Dr.
Lux, Wolfgang
Munzke, Michael
Taubert, Dieter

Pesonal- und Sozialwesen
Personnel Department

Goetzmann, Thekla
Ozierenski, Bärbel
Rödel, Heidrun
Zimmermann, Karin

Haushalts- und Rechnungswesen
Budgeting and Accounting Department

Döring, Ann-Cathrin
Draeger, Angelika
Friedrich, Helga
Hunkler-Wittleder, Ruth
Liebe, Erika
Wilke, Michaela

Technik/Betrieb
Engineering and Service Department

Backers, Hans-Gunter
Grindemann, Jürgen
Götsch, Horst
Gutsche, Christian
Kirchner, Reiner
Luckmann, Andreas
Reißmann, Andreas
Retusch, Michael
Roeder, Ralph-Thomas
Wolke, Horst
Wuthe, Ralf

Allgemeine Dienste / Beschaffung
General Services / Purchasing Department

Findeisen, Karin
Gartner, Helmut
Gräser, Marina
Heinrich, Helge
Hirsch, Friedhelm
Krause, Marion
Liebe, Edith
Osché, Elke

Auszubildende
Apprentices

Fähnrich, Thomas
Jäger, Ines
Karasinsky, Anne
Müller, Jens
Schmidt, Korinna
Schöttle, Doreen
Tragsdorf, Tobias

*ohne studentische Hilfskräfte und Aushilfen
**without students

Organigramm

Stand: 15.07.2003

Wissenschaftliches Komitee

Vorsitzender:
Prof. Dr. Dieter Häussinger, Düsseldorf

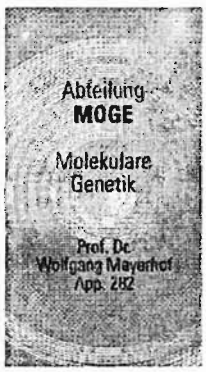
Wissenschaftlicher Rat

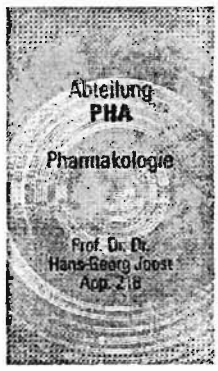
Vorsitzender: Prof. Dr. Michael Blaut, App. 470

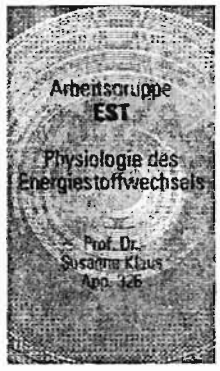
Personalrat

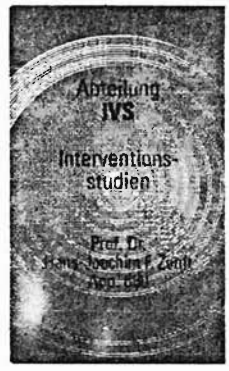
Vorsitzender: Dr. Klaus-Jürgen Petzke, App. 429

Forschende Abteilungen und Arbeitsgruppen









Zentrale Einrichtungen und Administration

Ernährungs-beratungszentrum	Max-Rubner-Laboratorium	Bibliothek	Informationstechnik
Dr. Christiana Einig App. 469	Dr. Christa Thöne-Reineke App. 615	Dagmar Kollhof App. 229	Wolfgang Lux App. 223

Deutsches Institut für
Ernährungsforschung
Potsdam-Rehbrücke

Arthur-Scheunert-Allee 114 - 116
14558 Bergholz-Rehbrücke

Telefon +49(0)33 200-88 0
Telefax +49(0)33 200-88 444
http://www.dife.de

Kuratorium

Vorsitzende: Frau Konstanze Pistor
Ministerium für Wissenschaft, Forschung und Kultur
des Landes Brandenburg

Stellvertretender Vorsitzender: MinR Dr. Peter Lange
Bundesministerium für Bildung und Forschung

Stiftungsvorstand

Wissenschaftliches Mitglied
Prof. Dr. Dr. Hans-Georg Joost
App. 216

Administratives Mitglied
Dr. Hartmut Schulz
App. 221

Vorstandsreferat

Dr. Anke Schmidt
App. 213

Presse- und Öffentlichkeitsarbeit

Dr. Susanne Schelosky
App. 335

Dr. Gunda Backes
App. 665 / 335

Forschende Abteilungen und Arbeitsgruppen

Abteilung **EPI**
Epidemiologie
PD Dr.
Heiner Boeing
App. 319

Abteilung **ETOX**
Ernährungs-
toxikologie
Prof. Dr.
Hans-Rudolf Glatt
App. 321

Abteilung **GAMI**
Gastrointestinale
Mikrobiologie
Prof. Dr.
Michael Blaut
App. 470

Abteilung **AVA**
Vitamine &
Atherosklerose
Prof.Dr.
Regina Brigelius-Flohé
App. 353

Arbeitsgruppe **PML**
Präventiv-Medizinische
Lebensmittelforschung
PD Dr.
Ralf Stohwasser
App. 543

Zentrale Einrichtungen und Administration

Förderangelegenheiten
Dr. Judith Schäfer
App. 292

Personal- und
Sozialwesen
Bärbel Ozierenski
App. 259

Haushalts- und
Rechnungswesen
Michaela Wilke
App. 233

Technik / Betrieb
Jürgen Glindemann
App. 512

Allgemeine Dienste /
Beschaffung
Marion Krause
App. 235

Organe des DIfE

Kuratorium / Advisory Board (Stand 15.07.2003)

Vorsitzende:
Konstanze Pistor, Potsdam

Stellvertr. Vorsitzende:
MinR Dr. Peter Lange, Bonn

Mitglieder:
Prof. Dr. Rudi Balling, Braunschweig
Prof. Dr. Helmut Erberscobler, Kiel
Prof. Dr. Dr. Ulf B. Göbel, Berlin
Prof. Dr. Dieter Haussinger, Düsseldorf
Prof. Dr. Heidrun Mühle, Leipzig
Prof. Dr. Martin Paul, Berlin
Dr. Astrid Petz, Bonn
Prof. Dr. Günter Schultz, Berlin
Prof. Dr. Bernd Walz, Potsdam
Prof. Dr. Walter Rosenthal, Berlin

Wissenschaftliches Komitee des Kuratoriums / Scientific Committee

Vorsitzender:
Prof. Dr. Dieter Haussinger, Düsseldorf

Mitglieder:
Prof. Dr. Rudi Balling, Braunschweig
Prof. Dr. Helmut F. Erberscobler, Kiel
Prof. Dr. Dr. Ulf B. Göbel, Berlin
Prof. Dr. Hans-Ulrich Haring, Tübingen
Prof. Dr. Heidrun Mühle, Leipzig
Prof. Dr. Peter Morgan, Aberdeen, GB
Prof. Dr. Walter Rosenthal, Berlin
Prof. Dr. Hans-Holger Rogers, Berlin
Prof. Dr. Günter Schultz, Berlin
Prof. Dr. Peter van Bladeren, Lausanne, CH

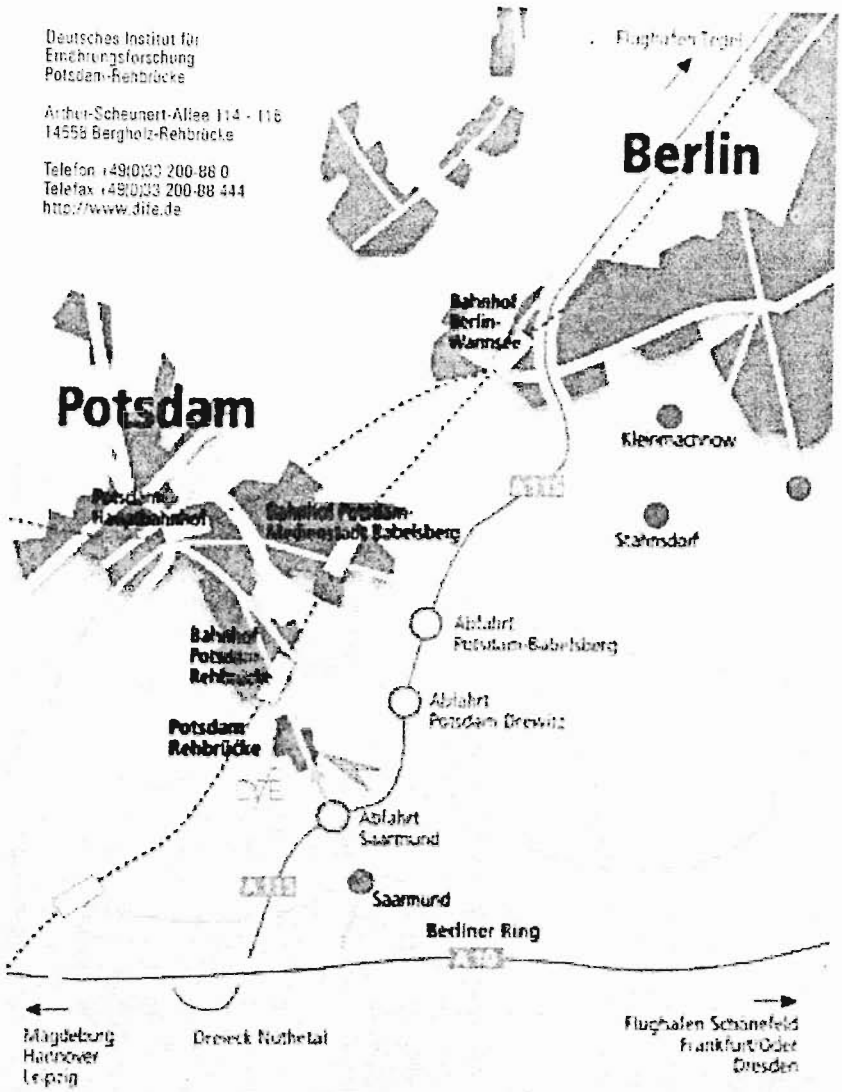
Stiftungsvorstand / Board of Directors

Wissenschaftlicher Vorstand / Scientific Director:
Prof. Dr. Dr. Hans-Georg Joost

Administrativer Vorstand / Administrative Director:
Dr. Hartmut Schulz

Personalrat / Staff Council

Dr. Klaus-Jürgen Petzke



Sie erreichen das DIfE

mit dem Auto

von Berlin: Heinrich-Mann-Allee Richtung Bergholz-Rehbrücke zur Arthur-Scheunert-Allee (Entfernung ab Stadtzentrum ca.7 km)

von Berlin:
A 115 (AVUS) Richtung Hannover bis Ausfahrt Saarmund, dann Richtung Potsdam, das DIfE befindet sich nach ca. 2 km auf der rechten Seite.

aus allen anderen Richtungen:
über den Berliner Ring, Abzweig Dreieck Nuthetal, die A 115 (AVUS) Richtung Berlin bis Ausfahrt Saarmund, folgen Sie dann dem Wegweiser Potsdam

mit öffentlichen Verkehrsmitteln:

von Potsdam:
mit dem Bus 611 ab 'Bassinplatz' oder Bahnhof 'Potsdam Hauptbahnhof' bis Haltestelle 'Ernährungsinstitut' (fährt i.d.R. stündlich), mit den Straßenbahnlinien X91 (fährt nur im Berufsverkehr), 90 oder 93 bis Endhaltestelle 'Potsdam-Rehbrücke', dann weiter mit dem Bus 611 bis Haltestelle 'Ernährungsinstitut' (fährt i.d.R. stündlich) oder zu Fuß ca. 1300 m auf der Arthur-Scheunert-Allee.

von Berlin:
mit der Regionalbahn ab Bahnhof 'Alexanderplatz', 'Friedrichstraße', 'Zoologischer Garten' in Richtung Belzig oder Dessau bis Bahnhof 'Potsdam-Rehbrücke', weiter wie von Potsdam

oder mit dem Regionalexpress ab Bahnhof 'Alexanderplatz', 'Friedrichstraße' oder 'Zoologischer Garten' in Richtung Brandenburg oder Magdeburg bis Bahnhof 'Potsdam-Hauptbahnhof', dann weiter mit den Straßenbahnlinien X91, weiter s. Potsdam

oder ab 'Wannsee' mit dem Regionalexpress in Richtung Belzig oder Dessau oder mit der Regionalbahn in Richtung Jüterbog oder Beelitz bis Bahnhof 'Potsdam-Rehbrücke', weiter s. Potsdam

aus allen anderen Richtungen:
mit der Bahn bis Bahnhof 'Berlin-Wannsee' oder 'Potsdam Stadt', mit dem Flugzeug bis Berlin-Tegel, -Schönefeld oder -Tempelhof.

Dann wie oben beschrieben weiter mit öffentlichen Verkehrsmitteln oder einem Mietwagen/Taxi

How to get to DIfE

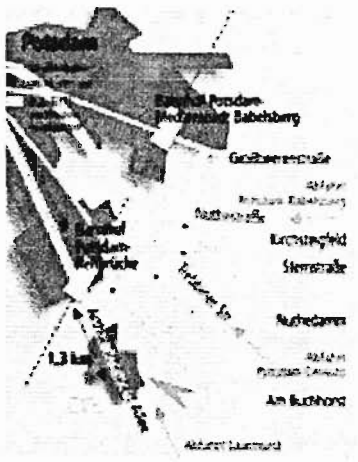
by air:
preferably to Berlin-Tegel airport, from there with bus lines X9 or 109 to train station 'Berlin-Zoologischer Garten' and further by train (direction of Belzig or Dessau) to station 'Potsdam-Rehbrücke'; continue along Arthur-Scheunert-Allee by bus line 611 (bus stop near the tram platform) to bus stop 'Ernährungsinstitut' or on foot in the same direction (about 15 minutes walking time, 1300m).
from 'Berlin-Zoologischer Garten' further by train (direction of Brandenburg or Magdeburg) to 'Potsdam-Hauptbahnhof'. From 'Potsdam-Hauptbahnhof' by tram line X91, 90 or 93 in direction of 'Bahnhof Rehbrücke' out of town to the end of the line, 'Bahnhof Rehbrücke'; continue along Arthur-Scheunert-Allee by bus line 611 (bus stop near the tram platform) to bus stop 'Ernährungsinstitut' or on foot in the same direction (about 15 minutes walking time, 1300m)

by train:
to 'Potsdam-Hauptbahnhof', from there by tram line X91, 90 or 93 direction 'Bahnhof Rehbrücke' out of town to the end of the line, 'Bahnhof Rehbrücke'; continue along Arthur-Scheunert-Allee by bus line 611 (bus stop near the tram platform) to bus stop 'Ernährungsinstitut' or on foot in the same direction (about 15 minutes walking time, 1300m).

to 'Berlin-Zoologischer Garten' and further by train (direction of Belzig or Dessau) to station 'Potsdam-Rehbrücke'; continue along Arthur-Scheunert-Allee by bus line 611 (bus stop near the tram platform) to bus stop 'Ernährungsinstitut' or on foot in the same direction (about 15 minutes walking time, 1300m).

to 'Berlin-Wannsee', from there by train to station 'Potsdam-Rehbrücke' (direction Belzig, Beelitz, Jüterbog or Dessau); continue along Arthur-Scheunert-Allee by bus line 611 (bus stop near the tram platform) to bus stop 'Ernährungsinstitut' or on foot in the same direction (about 15 minutes walking time, 1300m).

by car:
via the Berliner Ring (A 10) to the junction 'Dreieck Nuthetal', from there in the direction of Berlin to exit 'Saarmund', further on to the right and continue for 2 km. DIfE will be on the right.



BIOPRACT GmbH
Microbial products • Biotechnological processes
Soil remediation and water depollution

Methanol from Methane by Biological Oxidation

A. Blokesch, M. Gerhardt, M. Zittwitz, M. Wolf, M. Ringpfeil

Biotechnology becomes involved in climate-regarding product and process development [1]. The vision of biorefineries based on renewable resources is discussed [2, 3]. Roots of this idea can be found in complex production based on wood, grain, sugar cane or beets and oil plants [4].

Methanol production from renewable sources by chemical conversion has been investigated recently [5]. Biological pathways for oxidation of methane to methanol have been presented by Furuto et al [6] pursuing aerobic oxidation and Boetius [7, 8] elucidating anaerobic oxidation. Physical properties of methanol allow to apply advantageous fermentation conditions [9].

A continuously flowing source of methane fed by waste materials of various origin is available in the anaerobic sludge stabilization plants within municipal and industrial sewage works. BIOPRACT has successfully shown that their productivity can be raised by applying polysaccharolytic enzymes in the microbial degradation processes [10]. Methanol production will fit into such service plants opening the opportunity for additional profit.

Literature

[1] Committee on Biobased Industrial Products (Board on Biology, Commission on Life Sciences, National Research Council): *"Biobased Industrial Products (Priorities for Research and Commercialization)"*, National Academy Press, Washington D.C., 2000

[2] Innovationsforum BioRaffinerie/Grüne BioRaffinerie, 29.01.2001, Teltow - Seehof, Germany, www.biopos.de

[3] biorefinica 2001 International Symposium Green BioRefinery, 10. - 11. Oktober 2001, Teltow - Seehof, Germany, www.biopos.de

[4] M. Ringpfeil: *"Biobased Industrial Products and Biorefinery Systems -Industrielle Zukunft des 21. Jahrhunderts?"*, Innovationsforum BioRaffinerie/Grüne BioRaffinerie, 29.01.2001, Teltow - Seehof, www.biopract.de/news.htm (News, 21. März 2001)

[5] J.M. Norbeck, K. Johnson: *"Evaluation of a Process to Convert Biomass to Methanol Fuel"*, prepared for US Environmental Protection Agency, Office of Research and Development, Washington, D.C., www.methanol.org/methanol/report/epahynol.pdf

[6] T. Furuto, M. Takeguchi, I. Okura: *"Semicontinuous methanol biosynthesis by Methylosinus trichosporium OB3b"*, (1999) J. Molecul. Catalysis A: Chemical 144, 257-261

[7] A. Boetius, K. Ravenschlag, C.J. Schubert, D. Rickert, F. Widdel, A. Gieseke, R. Amann, B.B. Joergensen, U. Witte, O. Pfannekuche: *"A marine microbial consortium apparently mediating anaerobic oxidation of methane"*, (2000) Letters to Nature, Vol 407, 623-626

[8] A. Boetius: *"Tiefseeforschung: Anaerobe Oxidation von Methan durch eine mikrobielle Symbiose"*, BIOspektrum 6/01 - 7. Jahrgang (2001), 536 - 538

[9] M. Ringpfeil, A. Blokesch, M. Gerhardt, M. Wolf, M. Zittwitz: *"Verfahren zur biologischen Synthese von Methanol"*, Patent application DE 10132561.4, 9. Juli 2001

[10] M. Ringpfeil: *"Prospects for Industrial Enzymes in Environmental Protection"* BIO 2001, Industrial and Environmental Section, San Diego, June 25, 2001, www. biopract.de (News, 16. Juli 2001)

Contact:

Prof Manfred Ringpfeil and Dr-Ing Matthias Gerhardt
Tel.: +49(30) 6392-6205, Fax: +49(30) 6392-6206
Rudower Chaussee 29 • Entrance Kekuléstraße 7
12489 Berlin
e-mail: biopract@biopract.de
Internet: http://www.biopract.de

AIChE's 2002 Spring National Meeting
March 10-14, 2002 • Hilton New Orleans Riverside • New Orleans, LA

NBT
Newbiotechnic

Exploring genomic resources for sustainability

Propiedades antifúngicas de las enzimas líticas de *Trichoderma*

NEWBIOTECHNIC, S.A.

Los hongos del género *Trichoderma* son muy utilizados como agentes de control biológico por su capacidad antagonista frente a un elevado número de hongos fitopatógenos. Los mecanismos por los cuales *Trichoderma* spp. antagoniza a otros hongos son: (a) competición, por el espacio y/o los nutrientes (ejemplo, carbono, nitrógeno, microelementos); (b) antibiosis, entendida como la inhibición de un organismo por un producto metabólico de otro y (c) micoparasitismo, que consiste en un proceso complejo que requiere, entre otros, la producción de enzimas líticas (tales como proteasas, glucanasas, y quitinasas) para degradar la pared celular de los hongos fitopatógenos y así poder utilizar sus nutrientes. La pared celular de la mayoría de los hongos filamentosos esta constituida por polímeros estructurales de quitina y glucanos, de manera que las actividades quitinolíticas, y gluconolítica son cruciales durante la penetración. Los niveles de actividad enzimática producidos por *Trichoderma* spp. durante la interacción con el patógeno han demostrado tener una relación directa con el efecto antifúngico. Además de estos mecanismos directamente implicados en el antagonismo, se han descrito cepas de *Trichoderma* capaces de la inducción de los mecanismos de resistencia de la planta huésped y de potenciar su crecimiento. Este conjunto de actividades hacen que *Trichoderma sp.* sea una fuente potencial de aplicaciones agronómica de gran interés que están siendo investigadas y desarrolladas en la actualidad por un gran número de grupos de investigación de empresas e instituciones pública.

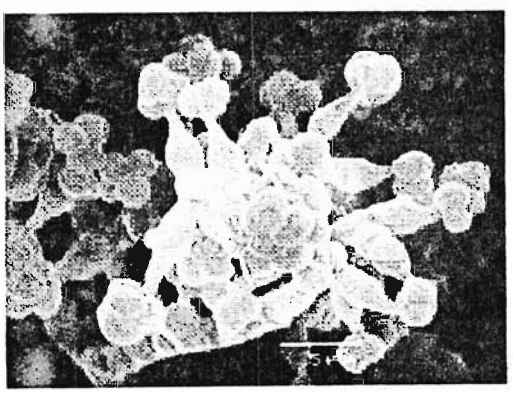
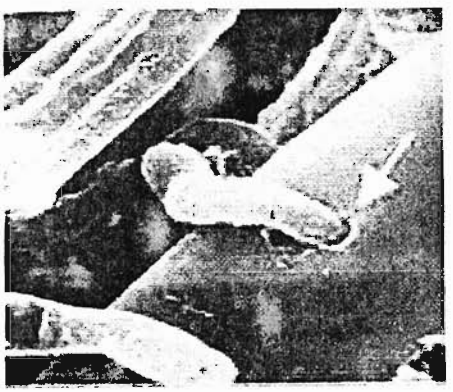


Imagen de *Trichoderma* al microscopio electrónico de barrido

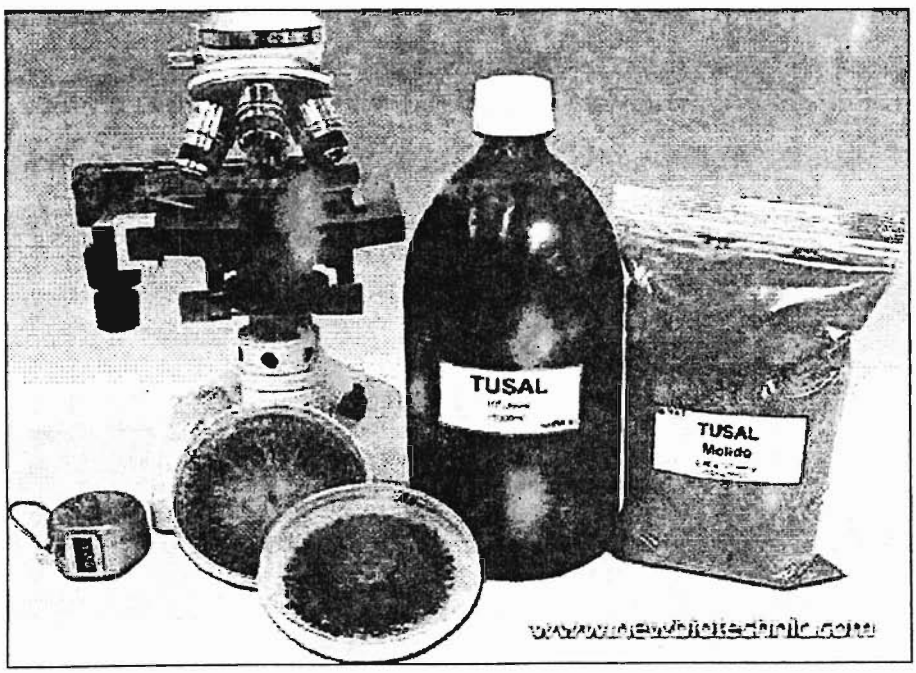


Efecto micoparásito de *Trichoderma* sobre hongos patógenos. Se observa la lesión sobre la pared celular, producida por enzimas líticas, a través de la cual *Trichoderma* penetra en el interior de la hifa del patógeno

Recientemente se han desarrollado estudios que indican que las enzimas líticas de *Trichoderma* spp., que actúan sobre componentes de la pared celular fúngica, también llamadas enzimas degradadoras de pared celular (EDPC), tienen un gran potencial en agricultura como componentes activos de nuevas formulaciones de fungicidas, debido a que: (a) las EDPC purificadas de distintas cepas de *Trichoderma* son muy efectivas como agentes inhibidores de la germinación de esporas y del crecimiento del micelio de un amplio grupo de hongos patógenos; (b) algunas de ellas como las quitinasas, las ß1,6- y a1,3-glucanasas, hidrolizan biopolímeros no presentes en los tejidos vegetales; (c) las EDPC han

mostrado tener efecto sinérgico con algunas proteínas PR de plantas, lo que posibilitaría potenciar los mecanismos de defensa de las plantas; (d) se ha demostrado una mayor actividad inhibitoria de diversos fungicidas químicos sobre el crecimiento de varios fitopatógenos por la adición de pequeñas cantidades de EDPC de *Trichoderma* y (e) por último, son inhibidores muy selectivos y por lo tanto no son perjudiciales para el hombre y los animales. Si bien son numerosos los estudios realizados con las EDPC, la mayoría de ellos se refieren fundamentalmente a estudios *in vitro* frente a diversos patógenos y además dichos estudios se basan, principalmente, en el uso de enzimas purificadas de distintas cepas de *Trichoderma*.

El estudio de la efectividad de proteínas líticas de *Trichodema* spp. en condiciones *in vivo*, permitirá impulsar una nueva estrategia de control de las enfermedades de las plantas. Dicho estudio se abordará en dos condiciones a) en cultivo: utilizando como modelo el patosistema fresa (*Fragaria* x *ananassa* D.)-*Botrytis cinerea* y b) en post-cosecha con los patosistemas: 1) naranja (*Citrus sinensis* (L.) Osbeck)-*Penicillium italicum*; y, 2) naranja (*Citrus sinensis* (L.) Osbeck)- *Penicillium digitatum*. En la actualidad, el control de estas enfermedades se realiza en la mayoría de los casos mediante fungicidas químicos. La progresiva pérdida de efectividad de los fungicidas debido a la selección de patógenos resistentes, el riesgo para el medio ambiente y la salud humana de los residuos de pesticidas en las frutas y hortalizas y las restricciones legislativas han promovido la búsqueda de métodos alternativos de control. El uso de las EDPC se presenta como una alternativa muy prometedora para el control de dichas enfermedades.



TUSAL®, fungicida biológico a base de *Trichoderma harzianum*, en fase de desarrollo y registro por Newbiotechnic, S.A.

Más información:

Understanding *Trichoderma*: between biotechnology and microbial ecology
Dr. Enrique Monte (Asesor Científico, Newbiotechnic, S.A.)

Descargar artículo completo (pdf) → Understanding_Trichoderma.pdf (45 KB)

© Springer-Verlag and SEM 2001

Editorial

Understanding *Trichoderma*: between biotechnology and microbial ecology

Enrique Monte
1,

The author is Associate Professor of Microbiology at the Department of Microbiology and Genetics, and the Spanish-Lusitanian Institute of Agronomical Research of the University of Salamanca. He is also lecturer on Biocontrol at the Faculty of Agronomy of the University of Buenos Aires (Argentina). His project "IPM in Strawberries" was awarded the 1999 Severo Ochoa Prize. Since 1999, he has coordinated research at the biotechnology company NBT (Seville, Spain) and at the Andalusian Foundation of Research and Development.

Plant diseases, caused primarily by fungal and bacterial pathogens, causesevere losses of agricultural and horticultural crops every year. These losses can result in reduced food supplies, poorer-quality agricultural products, economic hardship for growers and processors, and, ultimately, higher prices. For many diseases, traditional chemical control methods are not always economical nor are they effective, and fumigation as well as other chemical control methods may have unwanted health, safety, and environmental risks.

Biological control involves the use of beneficial microorganisms, such as specialized fungi and bacteria, to attack and control plant pathogens and the diseases they cause. Biological control offers an environmentally friendly approach to the management of plant disease and can be incorporated into cultural and physical controls and limited chemical usage for an effective integrated pest management (IPM) system. Biological control can be a major component in the development of more sustainable agriculture systems (Fig. 1). IPM is an approach to making pest and disease control decisions with increased information and involves the use of biological, physical, and chemical tactics to manage pest and pathogen populations in an economically efficient and ecologically sound way. However, within a given IPM strategy, the established view of biological control is that, even though it is safer than chemical control, it is less efficient and less reliable. To be realistic, we should not expect a very broad range of pest or disease control from biological agents, or that they control major pests or pest complexes in major crops in a wide range of environments. Biological control agents are, by their own nature, more limited than their chemical counterparts, and they need to be targeted carefully, starting with an appropriate characterization of the biocontrol agent and later selecting the antagonist for a given pathogen [7]. These types of considerations have encouraged microbiologists and plant pathologists to gain a better knowledge of biocontrol agents, to understand their mechanisms of control and to explore new biotechnological approaches.

Biocontrol agents can work several ways: (1) A biocontrol agent may grow faster or use its food source more efficiently than the pathogen, thereby crowding out the pathogen and taking over. The pathogens thus do not stand a chance! (2) A biocontrol agent may release a product that slows down or kills the pathogens in the vicinity of such a product; this process is called antibiosis. (3) A biocontrol agent may cause a plant to make a product that discourages or kills the pathogen; this

process is called induced resistance. The plant actually fights back. (4) A biocontrol agent may feed directly on or in a pathogen: this process is called parasitism. In this way the pathogen is destroyed.

Some biocontrol agents use only one of these strategies but the most successful biocontrol agents use several of them. This is the case of the fungus *Trichoderma*. Most *Trichoderma* strains have no sexual stage but instead produce only asexual spores. For a few strains, the sexual stage is known; however, these do not include strains that have usually been considered for biocontrol purposes. The sexual stage, when found, is within the Ascomycetes in the genus *Hypocrea*. Traditional taxonomy was based upon differences in morphology, primarily of the asexual sporulation apparatus, but molecular approaches are now being used [2]. Consequently, the taxa recently have gone from consisting of nine to at least 33 species. As an example, the best biocontrol species, *T. harzianum* Rifai, has been separated into an array of species: *T. harzianum* s.str., *T. inhamatum*, *T. longibrachiatum*, *T. atroviride* and *T. asperellum* [4]. The improved knowledge of *Trichoderma* has facilitated the use of these microorganisms for biocontrol as whole cells, protein formulations and gene sources for transgenic plants.

Trichoderma biocontrol strains

Trichoderma species have been investigated as biological control agents for over 70 years [5], but only relatively recently have strains become commercially available. This is largely a result of the change in public attitude towards the use of chemical pesticides, together with increased knowledge of their harmful side-effects. Biocontrol agents are widely regarded by the general public as "natural" and therefore non-threatening products. *Trichoderma* species act against target organisms in several ways [3]. Strains are actively parasitic on host fungi, through direct penetration of host hyphae and/or production of extracellular enzymes. In addition, species may produce antifungal antibiotics, and inhibition of pathogenicity-inducing hydrolytic enzymes has also been identified. Species may also be aggressive degraders of organic matter and act as competitors to fungal pathogens in their saprobic phases, especially when nutrients are a limiting factor. Some strains have been reported to promote the activities of saprobic bacteria and mycorrhizal fungi, while others act as plant-growth promoters, increasing plant size, foliar surface area and weight, and induce resistance towards plant pathogens. The dual roles of antagonistic activity against plant pathogens and promotion of soil fertility make *Trichoderma* strains appealing alternatives to hazardous fumigants and fungicides.

Many *Trichoderma* strains have been identified as having potential applications in biological control, and a partial list of plant pathogenic fungi affected by *Trichoderma* includes: *Armillaria*, *Botrytis*, *Chondrostereum*, *Colletotrichum*, *Dematophora*, *Diaporthe*, *Endothia*, *Fulvia*, *Fusarium*, *Fusicladium*, *Helminthosporium*, *Macrophomina*, *Monilia*, *Nectria*, *Phoma*, *Phytophthora*, *Plasmopara*, *Pseudoperonospora*, *Pythium*, *Rhizoctonia*, *Rhizopus*, *Sclerotinia*, *Sclerotium*, *Venturia*, *Verticillium*, and wood-rot fungi.

Once active strains have been identified using in vitro assays, a further selection must be done by studying other factors such as: (1) activity in vivo using experimentally induced diseases on plants, (2) tolerance of high temperatures (necessary to survive other IPM treatments), (3) suitability for formulation as foliar sprays and/or soil enhancements (e.g. high sporulation levels, rapid growth in bulk conditions), (4) specificity (strains should be inactive against beneficial organisms and plant crops), (5) long-term survival in field conditions, (6) interactions with other *Trichoderma* strains already present in the crop systems, (7) compatibility with agrochemicals used on the crop.

Commercial products currently on the open market or under registration include:

- Bio-Fungus (Belgium) against *Sclerotinia*, *Phytophthora*, *Rhizoctonia solani*, *Pythium* spp., *Fusarium*, *Verticillium*
- Trichodex (Israel) against *Botrytis* of vegetables and grapevines
- Binab-T (Sweden) for control of wound decay and wood rot
- Root Pro (Israel) against *R. solani*, *Pythium* spp., *Fusarium* spp., and *Sclerotium rolfsii*
- RootShield (also sold as Bio-Trek T-22G) (USA) against *Pythium* spp., *R. solani*, *Fusarium* spp.
- SoilGard (formerly GlioGard) (USA) for damping-off diseases caused by *Pythium* and *Rhizoctonia* spp.
- Supresivit (Denmark) against various fungi
- Trichoject, Trichopel, Trichodowels and Trichoseal (New Zealand) for control of *Armillaria*, *Botryosphaeria*, *Chondrosternum*, *Fusarium*, *Nectria*, *Phytophthora*, *Pythium*, *Rhizoctonia*
- TUSAL (Spain) for damping-off diseases caused by *Pythium*, *Phoma* and *Rhizoctonia* species, rhizomania disease of sugar beet and drop of lettuce
- Trichoderma 2000 (Israel) against *R. solani*, *S. rolfsii*, *Pythium* spp., *Fusarium* spp.
- Trieco (India) against *Rhizoctonia* spp., *Pythium* spp., *Fusarium* spp., root rot, seedling rot, collar rot, red rot, damping-off, *Fusarium* wilt

---

Trichoderma protein formulations

Trichoderma strains have developed highly effective antagonistic mechanisms to survive and colonize the competitive environment of the rhizosphere, phyllosphere and spermosphere. One of its main mechanisms, mycoparasitism, relies on the recognition, binding and enzymatic disruption of the host-fungus cell wall. A major part of the *Trichoderma* antifungal system consists of a number of genes encoding for an astonishing variety of secreted lytic enzymes, including endochitinases, *N*-acetyl--glucosaminidases, chitin 1,4--chitobiosidases, proteases, endo- and exoglucan -1,3-glucosidases, endoglucan -1,6-glucosidases, lipases, xylanases, mannanases, pectinases, pectin lyases, amylases, phospholipases, RNases, and DNases [5]. Particularly useful for biocontrol applications are chitinolytic and glucanolytic enzymes because of their ability to efficiently degrade the cell wall of plant pathogenic fungi by hydrolyzing biopolymers not present in plant tissues. Each of these two classes of enzymes contains a number of proteins with different enzyme activity, and some of the enzymes have been purified and characterized and their genes cloned. Most of the enzymes tested as purified proteins have shown very strong antifungal activity, especially when assayed in combinations, against a variety of fungi. A substantial amount of work performed mainly during the past 7 years has indicated that cell-wall-degrading enzymes (CWDEs) from *Trichoderma* strains have great potential in agriculture as active components in new fungicidal formulations [1]. This is because purified CWDEs from different strains of *T. harzianum* are highly effective in inhibiting spore germination and mycelial growth in a broad range of pathogens such as *Rhizoctonia*, *Fusarium*, *Alternaria*, *Ustilago*, *Venturia*, *Pythium*, *Phytophthora*, *Colletotrichum*, and especially *Botrytis*. In contrast to plant enzymes, chitinases and glucanases from *Trichoderma* can degrade not only the immature wall at hyphal apices but also the strong chitin-glucan complexes of mature cell walls, as well as survival structures such as sclerotia and chlamydospores, which reduces not only disease symptoms but also pathogen spread. In particular, enzymes absent from plants such as -1,6-glucanases can degrade important fungal cell-wall structures such as -1,6-

glucans by linking chitin or -1,3-glucans to cell-wall proteins. *Trichoderma* enzymes have diverse structural and kinetic properties, which increase the probability of avoiding inhibitory mechanisms.

The antifungal activity of *Trichoderma* CWDEs can be enhanced synergistically by combining enzymes with different lytic activities (such as exo- and endochitinases and/or glucanases). For instance, a combination of an endochitinase, an exochitinase and a -1,3-glucanase purified from *T. harzianum* has an effective dose (ED50) on *Botrytis* of about 1 ppm, which is comparable to the effective dose of most chemical fungicides. The inhibitory activity of chemical fungicides on *Botrytis* and other plant pathogens can be greatly enhanced by the addition of minute quantities (10-20 ppm) of *Trichoderma* CWDEs. For example, the fungicidal effect of azole compounds was enhanced up to 100-fold when used in conjunction with an endochitinase from *T. harzianum*, and a much greater improvement was obtained by adding small doses of two or more enzymes. Fungicides synergistic with the *Trichoderma* CWDEs include several compounds used for chemical control of plant diseases, such as azoles, benzimidazoles and pyrimidines. CWDEs from *Trichoderma* are synergistic with some plant pathogenesis-related proteins such as thaumatin-like proteins, which suggests that it is possible to use these enzymes as foliar sprays, enhancing natural plant defense mechanisms. Work on the antifungal activities of *Trichoderma* chitinolytic and glucanolytic enzymes has been performed primarily on *Botrytis*. Tests show that *Trichoderma* chitinases and glucanases have no effect on the plant even when relatively large quantities are injected into plant tissues.

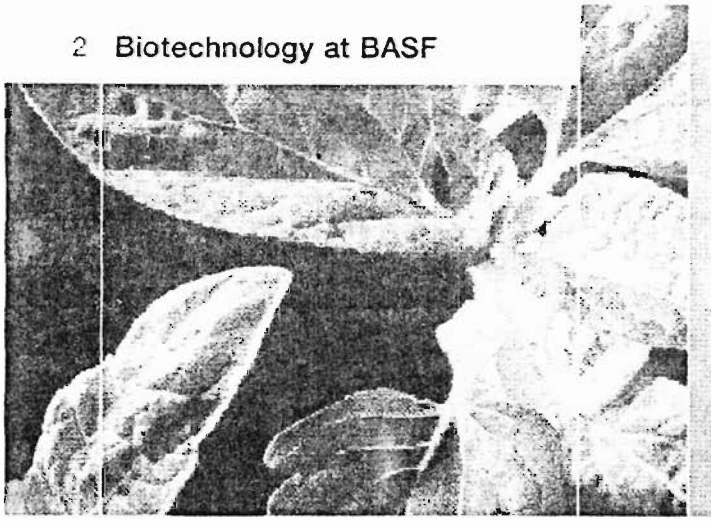
CWDEs are not harmful to humans or animals, as indicated by EPA tests for registration of strains of *Trichoderma* for use as biocontrol agents in the United States, and they degrade into environmentally friendly residues. CWDEs can be effectively combined with whole-organism *Trichoderma* control, with considerable opportunities for synergism. CWDEs are particularly suited to post-harvest control. Low-temperature controlled storage conditions will favor these applications as the level of enzyme activities will be more easily predicted than in the greenhouse or the field. Purified CWDEs or mixtures of CWDEs with high antifungal activity obtained from *Trichoderma* culture filtrates can be included in commercial formulations since they are easily characterized, stable, resistant to drying, freezing, temperatures up to 60°C, and have broad pH and temperature optima. As a dry powder, they can be stored at room temperature for years without a major reduction in activity.

Trichoderma source of genes

Trichoderma spp. have evolved numerous mechanisms for attacking other fungi and for enhancing plant and root growth. Several new general methods for biocontrol and for enhancement of plant growth have recently been demonstrated, and it is now clear that there must be hundreds of separate genes and gene products involved in the processes of mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, solubilization and sequestration of inorganic nutrients, induced resistance and inactivation of the pathogen's enzymes. Biocontrol microbes, almost by definition, contain many genes that encode products that permit biocontrol to occur. Several genes have been cloned from *Trichoderma* spp. that offer great promise as transgenes to produce crops resistant to plant diseases [6]. Transgenic expression of high levels of chitinolytic and glucanolytic *Trichoderma* enzymes do not affect plant morphology, development or yield, or infection by arbuscular mycorrhizal fungi. Most of these genes have been patented and are commercially available, but a number are in development to be used in agricultural biotechnology. These genes, which are contained in *Trichoderma* spp. and in many other beneficial genomes, are the basis for future "natural" organic crop protection and production. To facilitate a healthier and cleaner agriculture is our challenge and goal, and only the best biotechnology tools will be the ones to reach farmers' hands.

Biotechnology at BASF

BASF



Key technology of the 21st century

Together with microelectronics and information technologies, biotechnology is one of the most promising technologies of the 21st century. In order to take advantage of the large economic potential of biotechnology, BASF is strongly committed to develop and utilize the most competitive new technologies that this dynamic area has to offer.

Biotechnology provides BASF with opportunities in areas that include nutrition, agriculture and fine chemicals. In addition to the potential of these areas, biotechnology can help BASF save raw materials and energy during production. In both plant biotechnology and biocatalysis, the goal of BASF is to be among the world's leading companies within a few years. To achieve this goal, we have greatly expanded our entrepreneurial and scientific competence in biotechnology. Our knowledge of the world of genes and proteins is increasing day by day.

Genes – universal building plans for all living creatures

As different as they may be, all living creatures are made up of cells. These cells mainly consist of water and obtain their shape primarily through proteins. Proteins not only provide the cell with its structure, but also play a key role in many different metabolic processes. This is why every organism consists of a large number of different proteins. To produce a specific protein, the body requires a kind of building instruction. The information for this is stored in the corresponding genes. There are genes in every cell. This means that every cell contains the building plans required to create all necessary proteins.

Genes are the carriers of the genetic code and determine certain characteristics, such as the eye colour of living creatures. With about 30,000 genes, a human being has 20 times as many genes as a bacterium. The construction of genes

Page 4
Biocatalysis

BASF has successfully used biotechnological processes for years to produce vitamins, enzymes, amino acids and intermediates. The substances are produced in a particularly efficient and resource-saving way known as biocatalysis.

Page 6
Plant biotechnology

In 1998, BASF founded BASF Plant Science (BPS) at seven sites in four countries for our activities in agricultural biotechnology. Supported by functional genomic research, BPS develops crop plants that have been optimized by means of biotechnology techniques.

and the "language" in which the information is contained follows the same principles in all living creatures. This is why a bacterium can also "read" a gene for a protein from a cell of the human body.

What do biotechnology and genetic engineering involve?

In principle, biotechnological processes have been used for thousands of years. For as long as we can remember, we have been using yeasts to initiate fermentation processes, to produce, for example, beer, sour dough or yeast dough. Modern biotechnology is the targeted utilization of microorganisms, plants, cell cultures or isolated enzymes to produce chemical, agricultural and pharmaceutical products. Genetic engineering is a branch of biotechnology. It is concerned with the specific transformation of the genetic code of simple life forms, such as fungi, yeasts and bacteria, through to higher organisms, such as plants and animals.

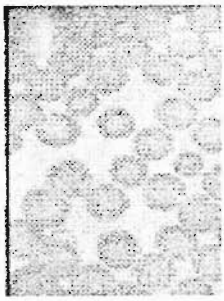
In biotechnology, the main focus of BASF is biocatalysis and plant biotechnology. BASF utilizes biocatalytic processes in the fields of fine chemicals and intermediates to produce, for example, amino acids, enzymes and vitamins. In plant biotechnology, BASF focuses on the development of crops that enable healthier nutrition of people by providing better ingredients, and on the development of plants with improved cultivation properties that are more resistant to cold, for example.

Opportunities and benefits of biotechnology

Biotechnology provides great potential in the area of plant breeding. Man has optimized today's crops over thousands of years by means of traditional breeding methods. As a result, these crops are only remotely similar to their "natural" predecessors. However, plant improvement achieved through traditional plant breeding, requires a great deal of time. For example, it took almost 100 years to improve the sugar content of sugar beets from three percent to almost 20 percent using this technique.

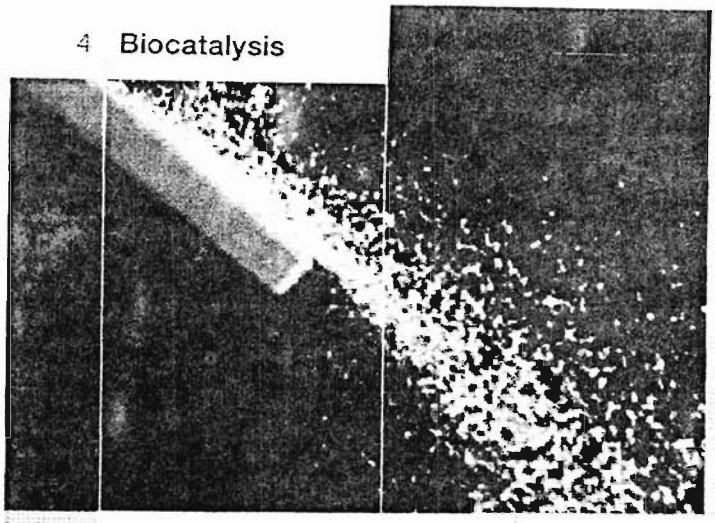
Plant biotechnology speeds up the process and optimizes traditional methods. Specific characteristics can be bred by introducing or removing, well-characterized genes in existing crops. In addition, plant biotechnology allows us to combine positive qualities from the genetic code of various species. These combinations enable development of completely new opportunities for BASF, including research into high-performance and resource-saving crop plants.

However, potential for opportunities arising from the use of biotechnology involves much more than plant science. Biocatalysis is another important area for BASF. This technology allows BASF to manufacture products in a single step by means of living cells or enzymes. This means that BASF can eliminate multistage syntheses and save valuable resources.



Page 9
The BASF Research Verbund

The results of biotechnological research will have a decisive influence on our century. Through increasing understanding of the genetic code, scientists will be able to develop more useful products for various areas of our lives.



Nature is the best laboratory

In conventional processes, vitamins and amino acids are produced exclusively by a series of complex chemical synthesis steps. This requires a great deal of energy and raw materials. New methods mean that these substances can be produced in a more efficient and resource-saving way using living cells or isolated enzymes. As a result, the multi-level processes of classical chemistry can be replaced by a biological process. At BASF, we call this process biocatalysis.

Because of its particular efficiency in manufacturing high-quality products, biocatalysis is an extremely interesting business area from an economic point of view. Products produced through biocatalysis are used in human and animal nutrition and in the production of agricultural and pharmaceutical products.

Vitamin B_2

The body requires vitamins for various vital functions. The human body is unable to produce sufficient amounts of vitamins. They need to be taken in via food. Vitamin B_2 belongs to the group of water-soluble vitamins. It is used as a supplement in food and beverages. In animal feed, vitamin B_2 guarantees the health and performance of animals. A lack of vitamin B_2 manifests itself in delayed growth and poor utilisation of food.

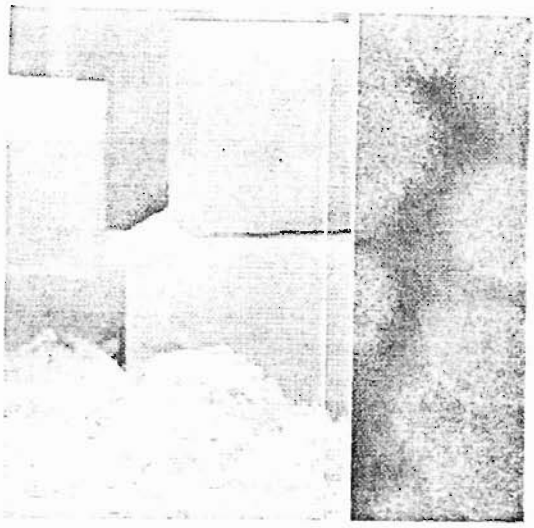
BASF is the pioneer of biocatalytical vitamin B_2 production. Chemical synthesis of vitamin B_2 was replaced at BASF by a biocatalytical process as early as 1990. Since then, vitamin B_2 has been produced by means of fermentation (transformation of substances through microorganisms)

Enzymes

Enzymes are proteins that enable or accelerate biological processes without themselves being changed. The transformation of substances would be many times slower without enzymes. The name of many enzymes ends in "ase," such as phytase.

Biocatalysis allows innovative and resource-saving production processes. It complements our expertise in classical chemistry.

Dr. Christian Dudeck, President of the Fine Chemicals division



with the help of the *Ashbya gossypii* fungus. This fungus feeds itself from plant oils, in other words from renewable raw materials. *Ashbya gossypii* naturally possesses enzymes that it uses to produce vitamin B_2 through its metabolism. In this process, the amount of vitamin B_2 produced depends on the quantity of enzymes and the growth conditions of the fungus.

In cooperation with scientists at the University of Salamanca in Spain, it was possible to identify the genes responsible for the production of the enzymes. The results of this research mean that production has been increased by a further 20 percent.

Chiral products

Many chemical substances for pharmaceuticals or crop protection products occur in two forms that are mirror images of each other, as the right hand is of the left. Traditional chemical syntheses lead to both forms, of which only one, however, is biologically effective. In many cases, the other form has no effect at all and may sometimes even have undesired side effects. For example, researchers at BASF have discovered a way of producing only the active form of the amine phenylethylamine – an important building block and auxiliary reagent used in the production of active ingredients for pharmaceuticals and agricultural products – with the help of the enzyme lipase.

Phytase and lysine

The enzyme phytase and the essential amino acid lysine provide further examples of the potential of biotechnology. They are both used as animal feed additives.

The use of phytase, for example, leads to 30 percent less phosphate in liquid manure and thus significantly reduces the burden on the environment. Phytase can only be produced economically using genetic engineering processes.

Animal feeds must contain sufficient lysine to prevent signs of deficiency. BASF uses *Corynebacterium glutamicum* to produce lysine by means of biotechnology. Biotechnologically produced lysine is an alternative to other sources of protein, such as soybeans and meat and bone meal.

Outlook

It is presumed that, in ten to fifteen years' time, all vitamins can be produced using fermentation, biotechnology or plant biotechnology. In addition to this, almost all amino acids will be produced by means of biotechnology. Biotechnological processes will replace conventional ones in the production of a wealth of specialty chemicals.

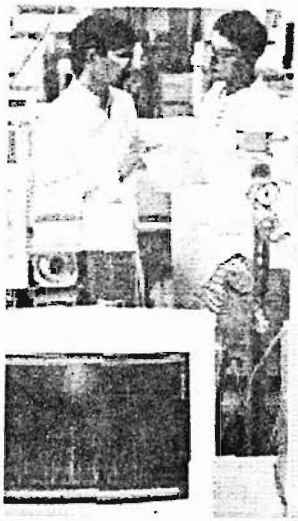
Fermentation

For centuries foods such as beer, wine, dairy products and sour dough bread have been produced through fermentation. Fermentation is the transformation of substances by means of microorganisms. In the production of yogurt, for example, lactic acid bacteria break down lactose to form lactic acid. Lactic acid gives yogurt its flavour and makes it more stable.

How does a fungus produce vitamin B_2?

BASF uses special sterile stirrer vessels, so-called fermenters, for the production of vitamin B_2. Growth conditions can be influenced through the availability of nutrients, temperature and air composition (e.g., addition of oxygen). An *Ashbya gossypii* culture is added to the fermenter together with various nutrients, such as vegetable oil.

The ideal growth temperature for *Ashbya gossypii* is set. The microorganisms multiply and produce vitamin B_2 in the form of yellow crystals, which are separated from the excess liquid (fermentation broth). In this way, BASF produces more than 1,000 metric tons of vitamin B_2 a year.



Understanding and improving plants

There is almost no other research area where new knowledge is gained as rapidly as it is in plant biotechnology. Researchers at BASF Plant Science analyze the genetic code of plants gene by gene to find out which genetic information causes which characteristic in the plant. This knowledge serves as a key for modifying plants to survive extreme conditions, such as salty soil or drought, to increase the content of vitamins or to enable complex chemical substances to develop in them. Through the specific modification of genes, we equip plants precisely with the desired characteristics.

With the technologies we have developed and the knowledge that we are currently gaining about plant genomes, BASF Plant Science aims to be one of the world's leading plant biotechnology companies in a few years' time. The first biotechnologically optimized plants from BASF should be ready for the market in 2005. We generally expect a development period of eight to 10 years.

As a result of progress in plant biotechnology, new plants with the desired characteristics can now be developed in a completely targeted manner. With traditional breeding methods, however, all the genes of the two related crossbreeding partners are mixed. It is thus left to chance which characteristics are dominant in the new generation and which may be lost. The goal of plant biotechnology is to recognize connections between genetic information and biological characteristics and to use them consciously.

Signposts in the genetic maze

There are about 25.000 genes in the

Plant biotechnology at BASF

BASF works to provide crop plants for three areas:

1. more resistant species that can better survive drought or cold and thus help to ensure harvest yields;
2. plants that, through optimized composition of their ingredients such as oil, starch or protein, contribute to healthier nutrition;
3. plants that, as "green chemical factories," produce substances that can currently only be produced by means of expensive chemical processes. This will save resources and costs, as well as benefiting the environment.

BASF plans to be one of the world's leading companies in plant biotechnology.

Dr. Hans Kast, Managing Director of BASF Plant Science

genetic code of each plant. The number and structure of genes is very similar in all plants – despite there being such diverse forms of life in the plant world, such as mosses, meadow flowers, cacti and redwood trees. This is because only some of the genes in plant cells are actually used.

By decoding the information that is stored in a gene, scientists have only taken the first step. In order to understand the connection between the genetic code and characteristics of the plant, they have to find out the exact function of every gene in the plant. metanomics, which is part of BASF Plant Science, wants to close the gap between genetic information and genetic function. By 2004, the researchers plan to have understood the function and characteristics of each of the 25,000 plant genes.

metanomics: Using new methods to gain new knowledge

metanomics was founded in May 1998 as a joint venture of BASF Plant Science and two leading staff members of the Max Planck Institute for Molecular Plant Physiology. The company's name (composed of metabolic and genomics) refers to a revolutionary method and has, in the meantime, come to represent a new branch of research: Plant metabolism is investigated for changes that occur when a single gene is modified in the plant's genetic code. The information from this modified gene must then be linked with its function within the plant.

In this process, the researchers block a single gene in a large number of plants or stimulate its activity. All the other genes in the plant, i.e., out 25,000, remain unchanged. The experimental plants thus created are investigated in detail with regard to changes in their metabolism. The researchers are able to use the data to understand, step by step, the connection between genotype and metabolic processes. Overall, metanomics is planning about 500,000 individual analyses using this method and about 100,000 experiments can be carried out a year.

The research method that has been developed by metanomics delivers results that can be applied rapidly, such as the identification of so-called key genes that lead to the production of certain ingredients in the plant. Patent applications have already been filed for the first key genes.

SunGene: How genes can be controlled

The development of technologies that make genetic modifications possible in plants – also called enabling technologies – is one of the special research areas of SunGene, a joint venture of BASF Plant Science and the Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, which was founded in 1998. metanomics and SunGene collaborate closely. While metanomics identifies key genes, SunGene's special competence is in optimizing and testing genes and controlling their activity.

BASF Plant Science

BASF Plant Science is BASF's plant biotechnology company. This joint venture with the Swedish seed breeding company Svalöf Weibull was founded in 1998 and is now represented at seven sites in four countries. The international technology network efficiently brings internal and external know-how together to achieve rapid success in plant biotechnology. Individual companies, such as metanomics in Berlin or SunGene in Gatersleben, play an important role because they can make an ideal contribution to this research and development platform through their flexibility and special competence. BASF's partnership with a leading seed breeding company guarantees good access to the market for newly developed products. Over the next 10 years, we are planning to invest EUR 700 million in the expansion of plant biotechnology.

If a new characteristic is to be developed in just one part of the plant, genetic activity needs to be regulated through certain control elements in the genetic code known as promoters. SunGene is doing research on promoters that are active in, for example, leaves, roots or fruits, to allow the new characteristics to become effective only in that part of the plant where they will result in the desired benefit.

Making plants more resistant

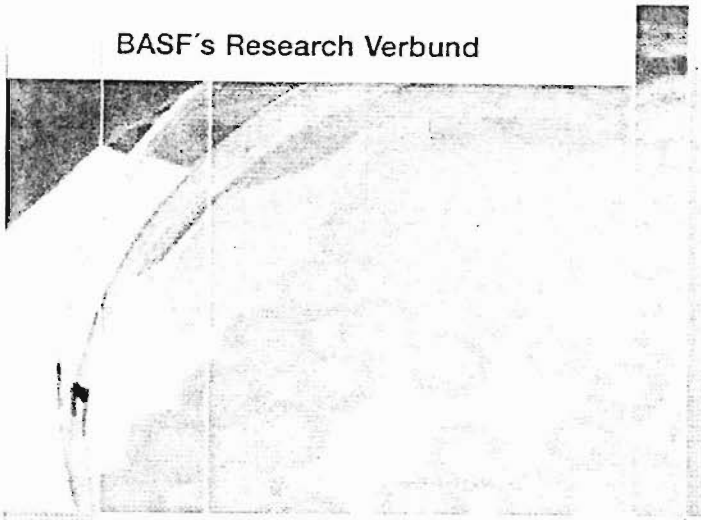
There is a special reason for research efforts concerning the genetic activity in leaves and roots: Roots are the decisive organ for water absorption, while leaves are the point at which the plant loses water. There is a close connection between the structure of these parts of a plant and the climate in which a plant grows. While cacti thrive in dry and hot areas, most moss species are adapted to a cool and damp climate. The researchers' aim is to understand in detail the adaptation mechanisms to these special growth conditions in order to transfer them to high-performance crop plants. If it becomes possible to reduce the loss of water via leaves, crop plants could be cultivated in significantly more arid areas than is possible today.

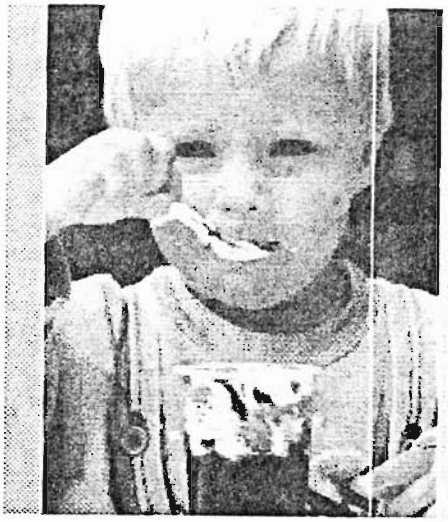
Drought is not the only problem for many farmers: In numerous areas – both in developing countries and in industrialized states – the soil is too salty for crop plants to grow in. Nature again provides a solution here: The moss *Physcomitrella patens* has developed a protective function that continuously pumps salt out of the cells. The researchers have managed to discover the gene responsible for this and to insert it into a test plant that cannot normally exist in a salty environment. The experiment has shown that the genetically modified plant can now grow in salty soil, too.

Enhancing plants may require several steps: Genetic engineering methods have made it possible to develop extremely valuable fatty acids in oil plants. However, the desired substance is broken down immediately in the plant's metabolism. The CONFAB (controlling novel fatty acid breakdown) research project, which is promoted by the European Union, combines the specialist knowledge of six universities from five countries and BASF Plant Science as an industrial partner with the aim of solving the problem. After just half of the three-year project, there are test plants in which the causes of the breakdown of fatty acid have been controlled by means of genetic engineering. The mechanisms that have been discovered within the context of the project can probably be transferred to other cultivated plants.

Building on existing knowledge

Plant biotechnology follows on from our competence in the area of cropt protection and plant nutrition. Over decades, BASF has acquired know-how in fundamental and applied biology. Building on our broad agricultural expertise as a producer of fertilizers and crop protection products as well as of animal nutrition products, we are developing plants with improved cultivation and quality characteristics.





We work towards products for the future

Our business success is based on innovative products and applications. This is why BASF, as a research-based company, is always looking for trend-setting ideas and technologies.

Competence through research
New knowledge is developed throughout BASF 's worldwide Research Verbund: This network consists of BASF's central laboratories in Ludwigshafen, technology platforms such as BASF Plant Science, participating interests in start-up companies and several hundred cooperative ventures with universities, research institutes and industrial partners. The BASF Research Verbund is made up of 8,000 people around the world. We invest more than EUR 1 billion in new knowledge and new abilities every year. And we do so successfully, because this results, among other things, in a wealth of patent applications. Every year, BASF protects its newly acquired knowledge with over 800 first applications. We currently hold about 94,000 patents and applications.

Requirements of future markets
BASF's research activities do not stop at patent applications because technology is not an end in itself. It must be economically viable and create products that result in added value for BASF and our customers. Biotechnology has this potential. It will change fundamentally the business areas of nutrition, agriculture and fine chemicals. Based on our competence in these areas, we are actively shaping the market.

The appeal of a technology is derived from its potential to create a benefit for society and to meet the requirements of our customers. Biotechnology offers this opportunity.

Dr. Stefan Marcinowski, Member of the Board of Executive Directors and Research Executive Director

Market leader in a wealth of areas
As one of the world's leading companies in the chemical industry, BASF has customers in over 170 countries. More than 90,000 employees contribute to the development, production and distribution of a wide and successful range of products including high value chemicals, plastics, colorants and pigments, automotive and industrial coatings, agricultural products, fine chemicals, oil and gas. We are international market leader in many areas with our products and processes.

We have already laid the foundation for this. It is based on intensive knowledge of our customers and markets. Our biotechnological know-how is significantly complemented by our experiences and abilities in the development and marketing of successful products.

The value of technology

Contemporary technology cannot exist only in economic terms, but its application must also be oriented toward the values of our times. BASF is committed to the principle of Sustainable Development.

Sustainable means meeting the needs of the economic, ecological and social requirements of today's society without compromising the development opportunities of future generations. Because biotechnology allows the resource-saving production of food products and valuable ingredients, it contributes to a sustainable economy.

We want to utilize the manifold possibilities of biotechnology. In this process, we carefully weigh up opportunities and risks and make safety our top priority. We also consciously set ethical limits, for example, by categorically rejecting any manipulation of the human genome. We bear great responsibility here – but as a chemical company, this responsibility is nothing new to us. In its 130-year existence, BASF has gained the trust of politicians and society through its responsible use of new technologies. We see ourselves as part of society and take concerns that have been expressed as well as possible risks extremely seriously. This is why we also participate actively in public debate.

BASF's dialogue with the general public

Social acceptance of biotechnology and genetic engineering is an important precondition for BASF. This is why it goes without saying that BASF implements an active information policy, conducts constant dialogue with the general public and provides consumer information, in the form of product labeling. For example we use our chemistry and biotechnology laboratory, Xplore!, to introduce this future technology to school children.

Glossary

Ashbya gossypii
The *Ashbya gossypii* is a microorganism that produces vitamin B2 in its metabolism. This method of producing the vitamin is more efficient than chemical synthesis. Genetic engineering has further increased the performance of *Ashbya gossypii*.

Biocatalysis
The aim of biocatalysis is to produce efficiently in a single step the most varied chemical substances by means of microorganism or enzymes. Traditional chemical processes are usually more expensive because they require several synthesis steps.

Breeding
In classical plant breeding methods, suitable crossbreeding partners are selected in an attempt to provide plants with the desired characteristics. The results, however, depends on chance. Modern biotechnology, however, is able to produce organisms with extremely specific characteristics.

Genetic modification
We speak of genetic modification when a new gene is added to a cell's genetic information (gene transfer) or if an existing gene is removed, blocked or influenced in its activity. This can affect the metabolic processes of the cell.

Genome research
The aim of genome research is to understand the structure and function of the genetic code of living creatures. This process not only includes the decoding of the genetic code, but also the investigation of the precise influence that a gene has on a cell or on an organism.

Metabolism
Metabolism describes all the biochemical processes in an organism, such as the transformation of substances contained in food into the body's own proteins or blood glucose. Metabolic processes occur in all living creatures.

Plant biotechnology
Plant biotechnology optimizes classical plant breeding methods. In this way, plants can be developed that provide ingredients for healthier nutrition, grow in unfavorable conditions or produce substances that are otherwise produced by means of a complex chemical process.

Research and Technology Verbund
At BASF, new insights in biotechnological research are developed in an international Research and Technology Verbund. In this network, BASF is developing new processes and applications together with subsidiaries and partner companies as well as in cooperation with universities.

Stress tolerance
Drought, cold and salty soil prevent the optimal growth of crops. These unfavorable conditions are also referred to as "stress." Genetic engineering can help make plants more resistant to this "stress."

Publisher:
BASF Aktiengesellschaft
Corporate Department Communications
67056 Ludwigshafen
Germany
Internet: www.basf.com/biotechnology
E-Mail: info.service@basf-ag.de

ZDA031135D

BASF

Consejo Superior de Investigaciones Científicas
Spanish Council for Scientific Research

Environmental applications

MINISTERIO DE CIENCIA Y TECNOLOGÍA
CSIC
Consejo Superior de Investigaciones Científicas

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA

CSIC
Consejo Superior de Investigaciones Científicas

Mass production of ß-carotene by Dunaliella salina in the southern Spanish coast

Abstract

A spanish research instute has study the operating conditions of an experimental instalation for the mass culture of D. salina, a ß-carotene rich strain, which makes feasible industrial production of this pigment. The advantages are: generation of a product by microalgae as alternative to chemical synthesis and development of agroalimentary sector in regions with saline habitats of negligible economic exploitation. Collaboration sought: financial resources and/or development support.

Description

Work carried out has been about the optimization of Dunaliella salina growth outdoors in open ponds, both in batch and semicontinuous regime, aa well as in a closed tubular photobioreactor. Production of ß-carotene in both systems has also been compared. Obtained results show that, in open systems, biomass and carotenoids productivities increase with solar irradiance, being semicontinuous regime more effective. In these systems, a carotenoids productivity of about 320 mg m-2 day-1, constituting 8.2% of the biomass dry weight, has been obtained. In the closed tubular photobioreactor carotenoids content of the obtained biomass is higher, about 12%, increasing, thus, its commercial value. Moreover, carotenoids profile of the biomass obtained shows the presence of other pigments in less amount (astaxanthin and lutein), being able to increase in the same way this biomass value.

Innovative aspects & Competitive advantages

The purpose of this work is to formulate the fundaments of biotechnological production of ß-carotene by Dunaliella saline and the establishment of the basic structure to a possible process for industrial production.

The final application will be to install, in early future, a pre-industrial plant for microalgae production, which makes feasible the development of an agroalimentary industrial sector, that involves social and economic advantages in Andalusia.

Generation of products with commercial interest by microalgae represents an issue of great scientific relevance related with the fields of biotechnology, aquaculture and marines sciences.

For its privileged climatic conditions, high irradiance and elevate number of sunlight hours, Andalusia is a suitable zone for the establishment of microalgae mass culture. The productive process combines characteristic to be directed as a soft biotechnology and can be an alternative to the exploitation of saline and other hiperhaline habitats, extensive natural resources with scarce or negligible economic exploitation in this region.

Current stage of development

Development phase.

Intellectual Property Rights

Patent applied for but not yet granted.

Collaboration sought

Finance / Further research and/or development support.

OTT Contact person
Juan Martínez Armesto
OTT - CSIC
Phone +34 954500976
Fax +34 954224331
E-mail armesto@cica.es

Researcher
Dr. Miguel Garcá Guerrero

Valorisation of wastes from vegetable handling: Obtention of bioactive compounds and dietary fibre

Abstract
A Spanish Research Group has developed a method for the extraction of bioactive compounds present in the subproducts of the vegetable processing industries. The method is simple and not expensive. Those compounds are responsible for the healthy promoting properties of the Mediterranean diet. By this method extracts enriched in bioactive compounds could be obtained, especially antioxidant and anticarcinogenic substances, which could be added to juices and other foods in order to functionalise them.

Description
The subproducts from the vegetable processing industries as welll as the wastes from the industrialisation of these products represent tones of wastes produced per day with the generation of management and environmental problems. These subproducts and wastes are generally very rich in bioactive substances which are considered to be responsible for the health promoting properties of the dietary habits in which fruit and vegetables are relevant ingredients such as the Mediterraneam diet. A research group from a Spanish Research Organisation has developed a technology which involves several simple steps allowing the obtention of food extracts enriched in these bioactive compounds, mainly antioxidant and anticarcinogenic substances. Furthermore, using this technology it is possible to obatin dietary fibre of a much higher nutritional quality than the fibre available in the market at present. The food extracts and ingredients obtained by this technology could be added to fruit juices and other foods like soups, creams etc, giving them the character of functional foods. Tomato juices eriched with antioxidant extracts obtained from subproducts of the artichoke processing industry have already been developped by the research group.

Innovative aspects & Competitive advantages
Valorisation of subproducts and wastes which become coproducts with added value. Significant reduction of the management and environmental problems due to the reutilisation of the subproducts and wastes.
The tecnology is relatively easy to implement, allowing the valorisation of subproducts and wastes as welll as reducing the management and enviromental problems associated with this type of industries.

Current stage of development
Development phase.

Intellectual Property Rights
Know-how.

Collaboration sought
Finance.

OTT Contact person
Yolanda Hernando Sáiz
OTT - CSIC
Phone +34 968396276
Fax +34 968396213
E-mail yhernando@cebas.csic.es

Researcher
Dr. Francisco A. Tomás Barberán

Genetically Modified Plant Resistant To Media Containing Heavy Metals

Abstract
A Snish research centre has developed a genetically modified plant to solve heavy metal contamination of soils and waters. This plant shows an increase of its capacity of of heavy metal binding up to 300%, this plant is tolerant to toxic concentrations of heavy metals and able to accumulate them in the aerial parts. The centre is looking for environmental companies for license agreements and research collaboration.

Description
The heavy metal contamination of the biosphere has drastically increased and one aspect of the phytoremediation technique is the use of plants tolerant to one or more heavy metals and able to accumulate them in the aerial parts. The plants have developed mechanisms to reduce the toxicity of heavy metals and metaloids by complex formation with organic ligands. Thus, cadmium, mercury, lead and arsenite are very reactive with thiol groups (-SH), and cysteine- rich peptides able to quelate these metals have been identified in plants. On the one hand, metalotioneins (MTs), that are proteins of low molecular weight and high cysteine content. On the other, phytochelatins (PCs), genetically uncoded peptides that are synthesized from glutathione (delta-glu-cys-beta-gly, GSH). GSH is an essential molecule in the cell, and its biosynthesis pathway is fine regulated in plants. The heavy metal induction of the quelating agents MTs and PCs triggers the biosynthesis of their precursor molecules, being cysteine the most important because it contains the -SH group.
We have developed a genetically modified plant that is resistant to media containing heavy metals and able to accumulate them to higher quantities than wild type plants are able. By the overexpression of the gene coding for O-acetylserine(thiol)lyase enzyme, which catalyzes the synthesis of cysteine, this plant has increased its cysteine-rich molecule synthesis capacity up to 300%. Tolerance analysis has shown that the genetically modified plant is able to germinate and grow in media containing toxic concentrations of cadmium, arsenite, arsenate, mercury, zinc, cupper and nickel, where the wild type plants are not viable. We have also analyzed the metal accumulation in the aerial parts of the genetically modified plant, and an important accumulation capability is observed.

Innovative aspects & Competitive advantages
The obtaining of a genetically modified plant that shows an increase of its capacity of synthesis of heavy metal and metaloid sequestring peptides up to 300%.
This plant is able to germinate and grow in media containing toxic concentrations of one or more of the metals As, Cd, Cu, Hg, Ni and Zn.
This plant accumulates these metals in its aerial parts, stems and leaves.
The genetically modified plant is able to grow in media contaminated with one or more of the most important contaminant metals.
The plant derives from the wild type strain of Arabidopsis thaliana with a short generation time and therefore the necessary time for recovery of a contaminated soil is reduced.
The plant accumulates the metals in its aerial parts which facilitates their elimination by harvesting stems and leaves.

Current stage of development
Available for demonstration.

Intellectual Property Rights
Patent applied for but not yet granted.

Collaboration sought
Finance / Further research and/or development support / Licence agreement.

OTT Contact person
Juan Martí nez Armesto
OTT - CSIC
Phone +34 954500976
Fax +34 954224331
E-mail armesto@cica.es

Researcher
Dr. Luis C. Romero

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA
CSIC
Consejo Superior de Investigaciones Científicas

Biological containment system for genetically engineered microorganisms

Abstract
A Spanish public research centre has studied the use of cytotoxic phosphotransferases (zeta proteins) to effectively kill i) the released genetically engineered microorganisms (GEMs) or to limit the function of the released GEMs in a controlled manner, ii) the GEMs that underwent an undesirable development, and iii) the GEMs that have lost their extrachromosomal element. A biotechnology company is sought for license agreement.

Description
This invention relates to a method of conditionally controlling the survivability of a recombinant microbial cell population, providing in the cells genes coding for two related zeta cytotoxic polypeptides (phosphotransferases), and their corresponding antitoxins (epsilon or epsilon-like proteins) that by binding to the cytotoxic proteins inhibit the toxic effect hereof. The citotoxic genes are operably linked to a gene whose product binds to a regulatable DNA sequence, and a regulatable regulatory DNA sequence is conditionally controlling the expression of the antitoxin polypeptides. In the present context, the expression "conditionally controlling" refers to a construction of the microbial cell which permits that the genes coding for the cytotoxic polypetides can be expressed under certain pre-determined environmental conditions whereas under other conditions, the genes are not expressed. Hence, the survivability of the microbial cells can be made dependent on certain pre-selected conditions. A regulatable regulatory DNA sequence is operably fused to the 5´region of the genes coding for the cytotoxic polypeptides. In accordance with the invention, such a regulatory sequence can be one with which one of the genes coding for the cytotoxic polypetides is naturally associated (sequence of omega binding site) or it can be a sequence with which the genes are not naturally associated. As defined herein the negatively functioning regulatory DNA sequences may indicate a DNA sequence which directly regulates the expression of the genes coding for the cytotoxic polypeptides at the level of transcription.
Factors regulating the activity of the promoter as defined above may be selected from a variety of factors. In accordance with the invention, advantageous promoter regulating factors, thus would control the expression of the genes encoding the cytotoxic polypeptides and may be determined by the physiological state, environment of the cells and/or by an inducible event promoted by an extrachromosomal element. In the present context, the term "physiological state of the cells" denotes the presence or absence of a certain chemical substance in the environment such as the fermentation medium in which the cell is propagated, that is not present in a second environment to which the cell is released, or when a factor required for the growth or survival of the cell is no longer present, or the factor is one which, when it is depleted or exhausted from an environment of the cell, has the desired effect, viz. that the gene is expressed.

Innovative aspects & Competitive advantages
The phosphotrasferases (zeta family of polypeptides) in the absence of the anti-phosphotrasferases (epsilon family of polypeptides) from Gram-positive bacteria induce the "viable but non-culturable state" of the cell, namely a long-lived phosphotrasferase toxic protein (275 to 295 amino acids long polypeptide) that interacts with a labile small anti-phosphotrasferase (85 to 95 amino acids long polypeptide) that antagonizes the toxic effect of the first (antitoxin). The labile antitoxin is degraded by a specific bacterial protease. In this programmed cell death systems, a plasmid-borne phosphotrasferase stabilizes the inheritance of both theta and sigma type replicons of all Gram-positive bacteria in which the genes are expressed. The phosphotrasferase cell killing functions defined herein may also be referred to as cytotoxic polypeptide, which phosphorilates a protein essential for bacterial duplication, and the anti-phosphotrasferase as antitoxin.
The present invention relates to the use of related zeta cytotoxins that show a loss of bacterial growth of 6 orders of magnitud each system (bacteriolytic effect) in the absence of their respective antitoxins. As defined herein they provide an active biological containment.

Current stage of development
Available for demonstration.

Intellectual Property Rights
Patent applied for but not yet granted.

Consejo Superior de Investigaciones Científicas
Spanish Council for Scientific Research

Medical
&
Pharmaceutical applications

MINISTERIO DE CIENCIA Y TECNOLOGÍA
CSIC
Consejo Superior de Investigaciones Científicas

Gene therapy approaches using transcriptional repressor DREAM

Abstract
A spanish research centre has developed transgenic mice useful to useful to define new strategies for the therapeutic control of pain and pathologic processes in oncology, neurodegenerative diseases, etc. These transgenic mice overexpress mutant forms of protein DREAM involved in this processes. Collaboration type: license agreement and financial support for research.

Description
Expression of the human prodynorphin gene is regulated through derepresion of its downstream regulatory element (DRE) sequence which acts as a location-dependent gene silencer. Prodynorphin gene encodes opiate peptides palliating the sensation of pain. DREAM protein (downstream regulatory element antagonist modulator) acts a transcription represor factor binding DRE sequence. Binding to DRE site blocks prodynorphin expression and other genes involved in cell proliferation, differentiation and/or cellular death. Therefore regulation by DREAM and/or its mutated forms, of the expresion of those genes implicates DREAM in the control of such important aspects as cell proliferation, differentiation and death in tumoural, degenerative, autoimmune and learning disorders, and also in pain.

DREAM and/or its mutated forms are useful in gene therapy for these pathologic processes. We have developed transgenic mice to study pain and gene therapy. These transgenic mice overexpress mutated forms of DREAM represor.

Innovative aspects & Competitive advantages
DREAM is a DNA binding transcriptional regulator and it is a novel tool in gene therapy for proliferative disorders. It is also involved in pain processes.
DREAM is a novel target protein to identify novel antitumoral compounds. The strategy of blocking the derepression of genes involved in pain, cell proliferation processes, etc and regulated through DRE by the use of mutated forms of DREAM is of therapeutic interest.

Current stage of development
Development phase.

Intellectual Property Rights
Patent applied for but not yet granted.

Collaboration sought
Further research and/or development support / Licence agreement.

OTT Contact person
Irene Herrera Insúa
OTT - CSIC
Phone +34 915616800 (ext. 3232)
Fax +34 915855196
E-mail I.Herrera@orgc.csic.es

Researcher
Dr. José Ramón Naranjo

MINISTERIO DE CIENCIA Y TECNOLOGÍA

CSIC

Consejo Superior de Investigaciones Científicas

Inhibitors of the tissue-type plasminogen activator (t-PA) with antitumor activity

Abstract

A Spanish research institution has developed inhibitors of the tissue-type plasminogen activator (t-PA) with antitumor activity. t-PA activity is required for the tumoral process in pancreatic cancer. We offer different procedures for the inhibition of the expression or the activity of t-PA, as well as two cellular systems genetically manipulated to detect potentially active substances able to inhibit t-PA activity. Collaboration type: license agreement and/or financial support for development.

Description

The overexpression of tissue plasminogen activator (t-PA) in exocrine pancreatic tumors might be a determinant for the aggressive biological behavior of these tumors. We have suppressed endogenous t-PA production by antisense oligonucleotides or transcripts in CAPAN-1 and RWP-1 cell lines. Reciprocally, the t-PA non-expressing BxPC-3 and PANC-1 cells, were stably transfected to overexpress t-PA. Recombinant t-PA (rt-PA) and chemical inhibitors were also used on these cells. Clones were assayed for invasion and growth in vitro and in vivo. In vitro, specific inhibition of t-PA expression or activity significantly inhibited the proliferation of t-PA-producing RWP-1, CAPAN-1 and SK-PC-1 cells. Antisense constructs were used to generate RWP-1 clones stably suppressed for t-PA expression (AS clones). These clones had a significantly reduced invasion and proliferation on plastic and in soft agar. The addition of rt-PA rescued the growth of the AS clones to parental levels, and was mitogenic for other independent pancreas cell lines. This effect did not require plasmin activity. In athymic mice, RWP-1 AS clones produced tumors five fold smaller than control clones. The AS tumors contained a significatively reduced number of Ki67-positive nuclei, fewer mitotic cells, and a remarkably reduced angiogenic network. Finally, the generation of tetracycline-repressed t-PA transfectants in PANC-1 cells, confirm the activity of t-PA in invasion and proliferation in vitro and in vivo. Conclusions. t-PA, in addition to its known role in invasion, plays other critical roles in pancreas tumor progression, stimulating cancer cell proliferation and tumor-associated angiogenesis.

Innovative aspects & Competitive advantages

1. We have demonstrated that pancreas tumors requires t-PA for their growth, invasion and angiogenesis.
2. We have generated inhibitory molecules (oligonucleotids and RNA) that specifically inhibit t-PA expression. Also, we have used specific chemical inhibitors that block t-PA activity. These inhibitors can block the growth, invasion and angiogenesis.
3. We have developed two cellular systems for the conditional expression of t-PA. These systems can be used to detect substances with inhibitory activity for t-PA.

We have demonstrated that t-PA activates the proliferation of epithelial tumor cells and the tumor angiogenesis. The advantage of using inhibitors of this protease in anticancer therapy is that of simultaneously targeting different mechanisms of tumor growth and progression: the growth and invasion of cancer cells and the process of angiogenesis.

Current stage of development

Available for demonstration.

Intellectual Property Rights

Patent applied for but not yet granted.

Collaboration sought

Finance / Further research and/or development support / Licence agreement.

OTT Contact person
Irene Herrera Insúa
OTT - CSIC
Phone +34 915616800 (ext. 3232)
Fax +34 915855196
E-mail I.Herrera@orgc.csic.es

Researcher
Dra. Rosanna Paciucci

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA

CSIC

Consejo Superior de Investigaciones Científicas

Calpain inhibitors for the treatment of degenerative diseases

Abstract

A Spanish research centre has developed a series of biologically active compounds that can be interesting for the pharmaceutical industry. These compounds can be useful for the treatment of degenerative diseases, especially neurodegenerative diseases, such as multiple sclerosis, Alzheimer and cerebral ischemia. This group is searching for pharmaceutical companies which would be interested in financing and help with further research.

Description

We have synthesized a variety of novel compounds that have been tested biologically, finding that they are highly powerful and selective inhibitors of calpain.

Calpain is an enzyme belonging to the group of the cysteine proteases, which has an active metabolic role. Calpain requires Ca (II) ion for activation. When the intracellular concentration of Ca (II) is at the physiological level ("normal" concentration), calpain works normally. However, when the intracellular concentration of Ca (II) increase (it happens, for instance, after a stroke), calpain is overactivated causing the hydrolysis (degradation) of many important proteins inside the cell; and, as a consequence, the degeneration of the cell occurs.

Thus, the overactivation of calpain is involved in many patho-physiological conditions that are related to cellular degeneration. This fact is especially striking on the neuron, and the overactivation of calpain is involved in the ethiology and development of several neurodegenerative diseases, such as multiple sclerosis, Alzheimer, cerebral ischemia, and so on.

Therefore, the use of selective inhibitors of calpain can be a therapeutically useful strategy for the treatment of such diseases. We have obtained compounds that are the most powerful and selective calpain inhibitors reported up to now, with IC50 values in the picomolar scale.

On the other hand, the syntheses of the calpain inhibitors are easy, and some of their characteristics are:

1) Short: less than three synthetic steps, although usually one step is enough.
2) Easy to perform at large scale.
3) High yield and selectivity.
4) Compounds are easily purified.

Innovative aspects & Competitive advantages

Therapeutical applications of calpain inhibitors have not been developed by the pharmaceutical industry.

However, since calpain is involved in several important physiological processes, this enzyme can be an important therapeutic target for the treatment and prevention of different diseases, especially neurodegenerative diseases.

An advantage of selective inhibitors of calpain in neurodegeneration versus other therapeutic strategies (for instance, the antagonism of neurotransmitters, such as glutamate or NMDA) is that it is expected to cause less side effect.

As indicated above, it is a novel strategy that has not been fully developed by the pharmaceutical industry, with a potential big market.

Current stage of development

Development phase.

Intellectual Property Rights

Patent applied for but not yet granted.

Collaboration sought

Finance / Further research and/or development support.

OTT Contact person
Katyssulla Costa
OTT - CSIC
Phone +34 915855376
Fax +34 915855196
E-mail kcosta@orgc.csic.es

Researcher
Dr. Bernardo Herradón García

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA
CSIC
Consejo Superior de Investigaciones Científicas

Homogeneous assay for high throughput screening of paclitaxel biomimetics

Abstract
A Spanish research institution has developed a new method to detect substances that may have antitumoral activity and mimic paclitaxel, a compound that targets cellular microtubules. This method is the first homogenous assay to detect substances that may replace paclitaxel at the microtubule-binding site and constitute potential anticancer agents. The simplicity of this new method compares favourably with other screenings. Sought: license agreement/ technical co-operation/ financial resources.

Description
The ubiquitous Taxol R (paclitaxel) binding site of microtubules also binds newly discovered ligands. A Spanish research institution has designed a homogeneous assay for high-throughput detection of Taxol biomimetics, based on the displacement of 7-O-[N-(2,7-difluoro-4'-fluoresceincarbonyl)-L-alanyl]Taxol from its binding site in diluted solutions of preserved microtubules. The state of this reference ligand is measured by fluorescence anisotropy in a micro plate reader, with varying concentrations of non-fluorescent competitors. The binding equilibrium constant of Taxol has a value Kb=3.7x107M-1.
In a first application of the method, they have found that baccatin III, an analogue of Taxol without the C-13 side chain, binds with Kb=1.5x105 M-1, whereas the side chain methyl ester is inactive. This was unexpected from the structure-activity relationship of taxoids, but compatible with models of Taxol docked at the microtubule site. Baccatin III binding has been confirmed by displacement of 3H-Taxol and by direct HPLC measurements of its co-sedimentation with microtubules, among other methods. Consequently, baccatin III induces microtubule bundles and multi-polar spindles in Ptk2 and U937 cells, and mitotic arrest and apoptotic death of the U937 cells, at concentrations 200-500 fold larger than Taxol. The simplest analysis of these results strongly suggests that the interaction of the C-2 C-4 substituted taxane ring system with the microtubule binding site provides most (ca. 75%) of the free energy change of Taxol binding and is sufficient to activate microtubule stabilisation and transmit the antitumoral effects of Taxol, whereas the C-13 side chain provides a weak specific anchor.

Innovative aspects & Competitive advantages
The fluorescent method of detection of ligands binding to the Taxol site of microtubules developed in this work constitutes a first homogeneous assay for any other substances acting on this important antitumoral target. Since multiple samples can easily be analysed with the fluorescence polarisation method, this assay should be a useful tool for the evaluation of the binding affinity of newly designed compounds of the Taxol, epothilone, eleutherobin and discodermolide families. It should also be applicable to the measurement of active Taxol-like contents of natural sources, and to high-throughput screening for new Taxol biomimetics, in a complementary fashion to cellular screens for mitotic inhibitors, such as that employed in the discovery of monastrol. An interesting property of the fluorescence anisotropy assay is its sensitivity for the detection of mid-affinity ligands. This is made possible by the combination of a highly fluorescent taxoid with frozen stabilised microtubules, permitting the large dilution necessary for effective displacement of the probe by weaker binders, which would otherwise pass undetected. This has been exemplified by the detection of the binding of baccatin III, providing new insight into the molecular recognition of Taxol by microtubules.
The simplicity of this new method compares favourably with established microtubule stabilisation screens and with the competitive assays employing radio-labelled Taxol. On the other hand, taxane-specific monoclonal antibodies offer possibly unsurpassed sensitivity for the determination of contents of drug and closely related compounds, however, they may fail to recognize chemically unrelated ligands of the microtubule Taxol binding site.

Current stage of development
Development phase.

Intellectual Property Rights
Patent applied for but not yet granted.

Collaboration sought
Finance / Further research and/or development support / Licence agreement.

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA

CSIC
Consejo Superior de Investigaciones Científicas

PTOV1, a new protein overexpressed in prostate cancer

Abstract

A new protein and its gene, PTOV1, have been identified as a new tumor marker in prostate cancer. Polyclonal monospecific antibodies have been generated for the detection of PTOV1 by Western blot, immunocytochemistry and immunohistochemistry. Using these antibodies, it has been shown that over 75% of samples with prostate carcinoma overexpress PTOV1. Also, it has been shown that PTOV1 is overexpressed in the preneoplastic lesions (PIN), or prostate intraepithelial neoplasia.

Description

We have identified a prieviously unknown protein, which we have designated PTOV1. By semiquantitative RT-PCR and immunohistochemical analyses we have shown that PTOV1 is overexpressed in 75% of human prostate adenocarcinomas. We have characterized the human gene for PTOV1, and assigned it to chromosome 19q13.2. We have determined that PTOV1 consists of a tandem duplication of a ptov domain, of which one copy is present on a second protein, that we have designated PTOV2.PTOV1 is a new protein that consists almost entirely in a tandem duplication of a new class of protein sequence repeats. These repeats are present in a second protein, which has been designated PTOV2.

We have identified a amino acid sequence as a good immunogen for the generation of anti-PTOV1 antibodies. We can use polyclonal monospecific anti-PTOV1 antibodies to detect, together with morphological analysis, prostate adenocarcinomas with great confidence. Also, these antibodies allow the detection of the preneoplastic lesions called PIN (prostate intraepithelial neoplasia), since the majority of these lesions overexpress PTOV1.

Innovative aspects & Competitive advantages

1. PTOV1 is a previously unknown protein.
2. Anti-PTOV1 antibodies are useful complementary tools in studies of prostate tumor pathology and biology.

Few molecular markers are found that are consistently altered in prostate cancer. PTOV1 is overexpressed in epithelial cells in at least 75% of prostate adenocarcinomas, which makes it one of the most prevalent molecular alterations in this class of tumors. The use in immunohistochemical analysis of anti-PTOV1 antibodies, together with conventional morphological analysis, sould be a useful tool for the confirmation of a diagnosis for malignancy in prostate pathology.

Current stage of development

Available for demonstration.

Intellectual Property Rights

Patent applied for but not yet granted.

Collaboration sought

Finance / Licence agreement / Manufacturing agreement.

OTT Contact person
Irene Herrera Insúa
OTT - CSIC
Phone +34 915616800 (ext. 3232)
Fax +34 915855196
E-mail I.Herrera@orgc.csic.es

Researcher
Dr. Timothy Thomson

MINISTERIO DE CIENCIA Y TECNOLOGÍA

CSIC

Consejo Superior de Investigaciones Científicas

In vitro diagnostic method for hepatitis G virus based on synthetic peptides

Abstract

This invention consists of the combination in the same molecule of peptide sequences belonging to structural and non structural proteins of hepatitis G virus and fatty acids of different hydrocarbonate chain length through an amide bond. These molecules are used to develop diagnostic systems of hepatitis G based on synthetic peptides.

Description

Hepatitis G virus (VHG) is present in asymptomatic blood donors with normal levels of ALT and is transmitted by blood. Its prevalence in the general population is in the range of 1-2%, although it is higher in individuals exposed to blood products. The infection is detected in serum through amplification of its genome by RT-PCR, however the development of enzyme-immunoanalysis techniques (indirect or competitive) would represent an important advantage in terms of simplicity, time and cost.

With this aim, in the present invention peptide molecules have been designed and synthesized. These molecules are formed by immunoreactive sequences of hepatitis G virus: structural proteins (E1, E2) and non-structural proteins (NS3) linked by means of an amide bond to fatty acids with hydrocarbonate chains of different length.

Synthetic peptide sequences would eliminate aspects such as non-specific reactions, difficult reproducibility and variability in sensitivity of the enzyme-immunoanalysis.

Small size peptide sequences present in general a low adsorption to solid surfaces. In order to avoid this problem fatty acids have been used as carriers of lineal peptides of relatively small molecular size. This approach increases the antigenicity of sequences by improving its adsorption capacity to the plates used in enzyme-immunoassays.

The obtention procedure is made by following several steps:

a) peptide solid-phase synthesis
b) fatty acid incorporation
c) lipopeptide cleavage from the resin and elimination of amino acids side protection groups
d) final product purification.

This technique is useful as a diagnostic tool for the acute phase (specific IgM) or as marker past infection (specific IgG).

Innovative aspects & Competitive advantages

This is a diagnostic method based on synthetic peptides from conserved regions of hepatitis G virus.

Peptides are linked to fatty acids in order to increase the specific recognition of antibodies in an enzyme-immunoassay.

This method allows the detection of antibodies in people who do not have an active infection (negative RNA-VHG).

This method allows to investigate the prevalence of infection in population studies.

At the present there is not any comercial system for the detection of anti-VHG antibodies based on peptides.

This invention presents advantages such as simplicity, time and cost.

Current stage of development

Development phase.

Intellectual Property Rights

Patent applied for but not yet granted.

Collaboration sought

Finance / Further research and/or development support / Licence agreement.

OTT Contact person
Irene Herrera Insúa
OTT - CSIC
Phone +34 915616800 (ext. 3232)
Fax +34 915855196
E-mail I.Herrera@orgc.csic.es

Researcher
Dra. Isabel Haro Villar

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA
CSIC
Consejo Superior de Investigaciones Científicas

First inhibitor of dihydroceramide desaturase. Method to decrease intracellular levels of ceramide

Abstract
This technology is related to synthesis of a cyclopropenyl analog of ceramide as a potent inhibitor of dihydroceramide desaturase, which is the last enzyme in the biosynthesis "de novo" of ceramide. Ceramide is a lipid which plays an important role on the regulation of several biological processes of the cell. Some diseases are related to ceramide accumulation and subsequent apoptosis induction, e.g. diabetes II. Therefore, this compund might be useful for future treatments of these pathologies.

Description
The inventors of this technology offer have synthesized a potent inhibitor of dihydroceramide desaturase. Dihydroceramide desaturase is the last enzyme of the biosynthesis "de novo" of ceramide. This inhibitor is an analog of cyclopropenylceramide. Inhibition assays performed in rat liver microsomes have demonstrated that this compounds efficiently inhibits desaturation of dyhydroceramide.
Ceramide is a lipid which plays an important role on the regulation of several biological processes of the cell and is a key molecule in the biosynthesis of sphingolipids and glycosphingolipids. Intracellular ceramide mediates the response to external stimuli in several important processes such as cellular differentiation, apoptosis, cellular growth supression, etc. There are extracellular and stress agents, such as tumor necrosis factor a, interleukine-1 b, 1-a-25 dihydroxivitamin D3, neurotrophins, Fas ligand, desametaxone, chemotherapeutic agents, ionized radiations, etc. which produce an endogenous increase of ceramide levels. There are several disease which are related to ceramide accumulation and subsequent apoptosis induction.
Therefore, development of molecules with ability to block enzymes involved in ceramide biosynthesis and metabolism brings up the discovery of new drugs. Taking into consideration that dihydroceramide lacks the biological effects of ceramide, analogs of cyclopropenylceramide could lead to opportunities for therapeutical intervention in different diseases related to ceramide accumulation due to increased de novo biosynthesis of ceramide, by interfering with the last step of this pathway, namely the desaturation reaction.
Moreover, despite the general acceptance of ceramide as a key second messenger, some aspects of ceramide-mediated signal transduction are controversial. Selective inhibition of the different enzymes involved in ceramide biosynthesis and metabolism can help to solve such discrepancies and to better understand the precise role of ceramide in cell biology. Therefore, this compound is a valuable tool for the elucidation of the role of dihydroceramide desaturase in ceramide-mediated biological processes.

Innovative aspects & Competitive advantages
Cyclopropenylceramide is an useful tool in research of the role of dihydroceramide desaturase in different cell biology processes. This is the first efficient inhibitor described for this enzyme.
Interfering with the de novo ceramide biosynthetic pathway with this dihydroceramide desaturase inhibitor we can envisage novel approaches to therapeutic interventions in different diseases related to ceramide accumulation due to increased de novo biosynthesis. This molecule is the lead compound for a novel family of compounds with improved chemical, biochemical and biological properties.
This is the first and only inhibitor of dihydroceramide desaturase.

Current stage of development
Available for demonstration.

Intellectual Property Rights
Patent applied for but not yet granted.

Collaboration sought
Finance / Further research and/or development support / Licence agreement.

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA
CSIC
Consejo Superior de Investigaciones Científicas

Method for the determination of the rate of mitochondrial respiration and its application to the search for regulators of the uncoupling proteins (UCPs)

Abstract

Energy production in the cell (respiration) is adjusted to its consumption rate. However, there are cases where dissipation is required like when heat is produced to maintain body temperature. The activity of the uncoupling proteins (UCPs) is central. A procedure has been designed that allows the monitorization of respiration. This method can be used, i.e., to identify compounds that regulate the activity of the UCPs. These compounds would be potential drugs to treat obesity or as antipyretic.

Description

Mitochondria are the cellular organelles where ATP is produced from the energy released during the oxidation of sugars and lipids. This process, termed oxidative phosphorylation, can be divided in two parts: respiration (substrate oxidation) and ATP synthesis. Both are coupled in such way that rate of substrate oxidation is adjusted to cellular demand for ATP synthesis. This coupling has important implications for cellular economy since reserves are not wasted. However, certain physiological situations require energy dissipation. Maintenance of body temperature is one example: in mammals non-shivering thermogenesis is achieved by uncoupling oxidative phosphorylation. This mechanism is also used to eliminate excess calories ingested with the diet. More recently it has been demonstrated that energy dissipation is important to lower the rate of free radical production, these reactive species cause cell damage that can eventually lead to cell death. The proteins that catalyze this energy dissipation are termed uncoupling proteins (UCPs). The present invention describes a procedure to determine the rate of mitochondrial respiration. The inventors have used this procedure as basis for a new screening method to evaluate the ability of compounds to modulate the activity of the UCPs. This compounds could become potential drugs for treating diseases due to changes in thermogenic activity of tissues where these proteins are present. A decrease in such activity could lead to obesity and related diseases (diabetes, hypertension, etc.), while an increase could result in fever or an excess of weight lost like in cachexia.UCPs belong to a superfamily of related proteins. UCP1 is only present in the brown adipose tissue of mammals and has an important role in keeping body temperature. When UCP1 is activated, it allows the re-entry into the mitochondria of the protons pumped by the respiratory chain without ATP synthesis. UCP2 and UCP3 play an important role in energy dissipation in humans. The thermogenic capacity of UCP2 and UCP3, and the fact that their activation could allow the elimination of excess fat, has made them targets for the treatment of obesity and related disorders. The present invention describes a procedure to determine the activity of the UCPs taking advantage of the fact that changes in UCP activity will necessarily lead to changes in the rate of mitochondrial respiration.This procedure can also be used to evaluate the activity of chemical agents on other elements involved in the mitochondrial oxidative phosphorylation. It would allow, for example, the evaluation of the capacity of compounds to inhibit respiration through an effect on the respiratory chain complexes. In the same context, it could help to assess their effects on the mitochondrial inner membrane that could decrease the efficiency of oxidative phosphorylation.

Innovative aspects & Competitive advantages

The present invention is a high throughput screening procedure that presents important advantages compared to existing protocols. Identification of new therapeutic compounds (obesity, diabetes, development of new antipyretics, etc.) with this method is easy, cheap, highly reproducible and useful to process high number of samples. This invention is highly suitable for companies that need to screen a large number of compounds. This procedure based in the measurement of fluorescence in standard micro-titer plates, it is available and ready to be tested.

Current stage of development

Available for demonstration.

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA

CSIC
Consejo Superior de Investigaciones Científicas

New compounds with enhanced antiangiogenic activity

Abstract

A spanish public research centre has developed a family of inhibitors of angiogenesis and cellular proliferation. These compounds are useful in cancer treatment, non tumoral angiodependent diseases, rheumatoid diseases, endometriosis, obesity, atherosclerosis or restenosis. It is sought a pharmaceutical company for license agreement.

Description

Inhibition of angiogenesis-promoting factors such as fibroblast growth factors is considered as a potential procedure for inhibiting solid tumour growth and other pathophysiological situations in which inhibition of endothelial cell proliferation would be a desirable therapeutic goal. Although several peptide-based angiogenesis inhibitors are currently under study, the development of compounds of small molecular size is, nevertheless, a pharmacological goal of considerable interest. We have already shown that certain naphthalene sulfonates constitute minimal functional substitutes of the antiangiogenic compounds of the suramin and suradista family. Using those data as a lead we have carried out a rational search for new angiogenesis inhibitors that could provide new pharmacological insights for the development of antiangiogenic treatments. The results of the study strongly underline the relevance of the stereochemistry for an efficient inhibition of acidic fibroblast growth factor mitogenic activity by the naphthalene sulfonate family, and allow us to formulate rules to aid in searching for new inhibitors and pharmaceutical developments. The compounds resulting of our studies are the object of an already filed patent.

Innovative aspects & Competitive advantages

A family of inhibitory compounds of cellular proliferation (in vitro tested) and angiogenesis processes (in vitro and in vivo tested) is offered. Toxic effects have not been detected. Low molecular weight, efficacy at low concentrations, low toxicity at inhibitory concentrations are innovative aspects of this technology offer.
This compounds have demonstrated an excellent activity as inhibitors of angiogenesis and low toxicity in animal models and in vitro assays. Their synthesis is very cheap. They are twice more active than suramines and suradists.

Current stage of development

Development phase.

Intellectual Property Rights

Patent applied for but not yet granted.

Collaboration sought

Licence agreement.

OTT Contact person
Irene Herrera Insúa
OTT - CSIC
Phone +34 915616800 (ext. 3232)
Fax +34 915855196
E-mail I.Herrera@orgc.csic.es

Researcher
Dr. Serafi n Valverde López

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA

CSIC
Consejo Superior de Investigaciones Científicas

NOR-1, new target for diagnosis and therapy of cardiovascular diseases

Abstract

A spanish research centre has described NOR-1 as a new target for the diagnostic and therapy of human cardiovascular diseases. NOR-1 is useful for the identification of new therapeutic compounds for these diseases and the design of new protocols for gene therapy. The institution is seeking a biomedical/biotechnology company for a license and/or R&D agreement to develop this technology.

Description

The finding of this technology offer is the identification of the transcription factor NOR-1 as an early-induction gene in vascular smooth muscle cells (VSMS), endothelial cells and monocytes/macrophages, exposed to mitogenic stimuli. In addition, we have shown the induction of NOR-1 in human atherosclerotic lesions and in the porcine model of balloon angioplasty (a procedure that activates VSMC to migrate and proliferate in the intima). Finally, we have shown the inhibition of VSMC migration and proliferation in an in vitro model of lesion induction, by antisense oligonucleotides against NOR-1. Based in this knowledge and in the methods developed in the study, this invention provides:

a) A procedure for the diagnosis and follow up of cardiovascular diseases in which NOR-1(*) is induced, or for the monitorization, follow up or evaluation of the efficacy of pharmacological treatments of these diseases.

b) A procedure for the identification of potential compounds (or pharmacological formulations) to modulate the activity of NOR-1, and thus, potential therapeutic compounds of cardiovascular diseases in which NOR-1(*) is induced.

c) A procedure for gene therapy for the treatment of cardiovascular diseases in which NOR-1 is activated, in particular restenosis post-PTCA.

Note: NOR-1(*) indicates NOR-1 and the other members of the NGFI-B family (Nur77 and Nurr1), because we have shown that these genes are concomitantly induced in conditions in which NOR-1 is induced.

Innovative aspects & Competitive advantages

In this finding a nuclear orphan receptor (NOR-1) is identified as a new target useful in the cardiovascular area. NOR1, as a transcription factor inducible by mitogens and potentially regulated by other mechanisms, including binding of ligands (physiological or pharmacological) and by post-translational (phosphorylation), can be an ideal target in therapeutic approaches of cardiovascular diseases as well as in the diagnosis and follow up (natural or after pharmacological treatment) of these pathologies.

Since NOR-1 is a transcription factor that regulates the expression of other genes involved in the activation of the main cells involved in the atherosclerotic process (VSMC, endothelial cells and monocytes/macrophages), if it is used as a marker in the molecular diagnosis and follow up of this pathology the number of genes required to carried out this purpose will be reduced.

In addition, targeting NOR-1 increases the chance of success in gene therapy approaches in which only one gene is blocked. Indeed, we have shown that blocking NOR-1 in VSMC cultures the migration and proliferation of VSMC can be significantly reduced.

Finally, since NOR-1 is a nuclear orphan receptor (with unknown ligand) himself is a tool for the identification of molecules able to modulate its activity, and thus, susceptible of be used as drugs in atherosclerosis treatment.

Current stage of development

Development phase.

Intellectual Property Rights

Patent applied for but not yet granted.

Collaboration sought

Licence agreement.

OTT Contact person
Irene Herrera Insúa
OTT - CSIC
Phone +34 915616800 (ext. 3232)
Fax +34 915855196
E-mail I.Herrera@orgc.csic.es

Researcher
Dra. Lina Badimon

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA

CSIC
Consejo Superior de Investigaciones Científicas

New screening system for immunomodulatory compounds of T lymphocytes activation.

Abstract

A spanish research institution offers a screening system based on the Nck-CD3epsilon interaction to search for agents that, by blocking or increasing the interaction, modulate T cell activation. This therapeutic target offers the advantage of complete cell specificity. We seek a collaboration with a pharmaceutical company interested in the development of new immunomodulatory agents in the form of a research grant, joint venture or a license agreement.

Description

We have recently found that the adapter protein Nck is directly recruited to the T cell antigen receptor (TCR). This recruitment is very fast, after a few seconds of TCR engagement and is mediated by the direct interaction of the N-terminal SH3 domain of Nck (SH3.1) with a proline-rich sequence (PRS) in CD3epsilon. Nck recruitment to the TCR is independent of tyrosine quinase activation or other tyrosine kinase activities and evidences the existence of a conformational change in the TCR that results in exposure of the PRS. Nck plays an important role in the reorganization of the actin cytoskeleton that in on the other hand fundamental for T cell activation. To demonstrate if Nck recruitment to the TCR plays an important role in T cell activation we used to different systems to inhibit the interaction in vivo. First, we overexpressed the SH3.1 domain in T cells by transfection. Second, we transduced T cells with a monoclonal antibody that specifically binds the PRS. Both procedures resulted in blockade of recruitment of endogenous Nck to the PRS and T cell activation, measured through cytokine release and cell proliferation. We have investigated the mechanism of action and demonstrated that the inhibition of Nck-CD3epsilon interaction blocks the formation of T cell : antigen presenting cell conjugates, inhibits the polymerization of the actin cytoskeleton and the maturation of the immune synapse. From all these results we conclude that the inhibition of Nck-CD3epsilon interaction has very important consequences for cell activation and therefore this interaction could constitute a good molecular target for the search and design of new immunosupressive drugs.

Innovative aspects & Competitive advantages

The discovery of the Nck-CD3epsilon interaction is innovative because it is the first time that a signal transduction pathway that emanates directly from the TCR in a tyrosine kinase-independent fashion is uncover. Other procedures to screen for new T cell activation inhibitors are based on the inhibition of enzymatic activities placed far downtream the TCR in the signal transduction pathway. However, the inhibition of the Nck-CD3epsilon interaction is placed at the initiation of the cascade.

Although Nck is ubiquitous, CD3epsilon expression and function are T cell restricted. Therefore, the modulation of Nck-CD3epsilon interaction would have effects exclusively on activation of T cells and not of other cell types. On the other hand, the recruitment of Nck to the TCR is a tyrosine kinase-independent process; the inhibition of the interaction could have a differential effect on different cell activation events. This may result in a selective effect on some aspects of the immune response that could lead to a reduction of detrimental effects of the immune response without causing a generalized immunosuppression.

Current stage of development

Development phase.

Intellectual Property Rights

Patent applied for but not yet granted.

Collaboration sought

Finance / Licence agreement / Joint Venture agreement.

OTT Contact person
Irene Herrera Insúa
OTT - CSIC
Phone +34 915616800 (ext. 3232)
Fax +34 915855196
E-mail I.Herrera@orgc.csic.es

Researcher
Dr. Balbino Alarcón Sánchez

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA

CSIC
Consejo Superior de Investigaciones Científicas

Assay system for Cot kinase protein activity modulators

Abstract

Spanish research centre performs research about different aspects of a Cot kinase, an enzyme involved in tumoral processes. Cot kinase could be a very good target for drugs designed to block tumour proliferation and angiogenesis. We have developed assay systems of Cot kinase activity in intact cells as well as in vitro. We offer to bio-companies the possibility to test the regulation of Cot kinase actvity by anti-tumurogenic drugs. Type of collaboraction sought: technical cooperation.

Description

Human Cot kinase gene as well as its murine homologue (tpl-2), were originally identified by independent groups as oncogenes. A deregulation of Cot kinase has proposed to play a role in human cancer breast as well as in Hogkin´s lymphomas. Cot kinase activity has been implicated in cell proliferation by regulating G0/G1 as well as G1/S cell cycle transition (1,2,3). Cot kinase has also a role in angiogenesis since is capable to up-regulate the expression of COX-2 gene (4). The Cot/tpl-2 gene encodes a MAP kinase kinase kinase that is capable of switching on several MAP kinase cascades including the Erk-1/Erk-2, Erk-5 , p38g and Jnk kinase pathways. These signal transduction pathways connect Cot kinase activity with the up-regulation of several transcription factors, such as AP-1, NFAT, NFkB, and E2F (1,4,5). All these data indicate that Cot kinase could be a very good target for drugs designed to block tumour proliferation and angiogenesis.

This research group routinely assays Cot kinase activity by co-expressing in different cell systems the COX-2 or the interleukin-2 promoters linked to the luciferase gene (3,4) or by co-transfecting the response elements of the above described transcription factors (1,4,5), together with different plasmids encoding the proton-oncogenic and oncogenic version of Cot kinase. Luciferase activity is measured 24 h after transfection. They also test Cot kinase activity "in vitro" (6), by transfecting the different Cot kinase plasmids with the HA tagging in HEK293 cells and 24 h after transfection Cot kinase is immunoprecipitated. Then a two step kinase assay similar as the developed previously for RAF kinase is performed.

Innovative aspects & Competitive advantages

We are one of the few groups in the world to be in a disposition of offer this type Cot kinase assays.
We have all the infrastructure necessary to perform the above mentioned assays.

Current stage of development

Available for demonstration.

Intellectual Property Rights

Others.

Collaboration sought

Finance / Technical co-operation / Manufacturing agreement.

OTT Contact person
Irene Herrera Insúa
OTT - CSIC
Phone +34 915616800 (ext. 3232)
Fax +34 915855196
E-mail I.Herrera@orgc.csic.es

Researcher
Dra. Susana Alemany

New method to hyperproduce penicillin and cephalosporin antibiotics

Abstract
A Spanish public research institution has developed a procedure to hyperproduce beta-lactamic antibiotics, by manipulation of a regulating gene, present in the biosynthetic route of the compound. The research group is looking for pharmaceutical companies and enterprises in the area of biotechnology which are interested in license agreements and financing.

Description
The object of this invention is the design by genetic engineering of a genetically altered merodiploid strain of Penicillium chrysogenum, in which the activity of a regulating gene that controls penicillin synthesis has been altered. All these strains modified genetically, are notable overproducers of penicillin in relation to the original strain and show high transcription levels of at least two genes of the biosynthesis of the antibiotic.
The present invention describes a new procedure which allows the increase of the penicillin biosynthesis, through the genetically modification of the regulating gene PacC. For the first time, a mutant form of this gene is presented in P. chrysogenum, called pacC33, which encodes a protein with a gain in the PacC function. The mutated gene codifies a truncated PacC protein, which provides a PacC gain of function, independently of the presence or absence of a transduced signal by the route of the pal genes. The strain P. Chrysogenum NRRL1951 are transformed with the construction, selected and purified. The resulting strains from the process of transformation are overproducers of penicillin G, when comparing with the strain NRRL1951.

The information obtained with this patent allows us to develop other complete or partial mutant forms, both by synthesis of new DNA sequences and by the isolation and identification of natural forms, of homologue genes in other ascomycetes, which encode new proteins with a function gain in relation to the wild-type protein PacC.

Innovative aspects & Competitive advantages
This invention represents the first case where the biosynthesis of penicillin and cephalosporin has been improved by the manipulation of a regulating gene, and in this way, presents an outstanding development over the previous existing technologies.
The manipulation by genetic engineering of a regulating gene to simultaneously increase the function of several structural genes under its control has the advantage of a general application to other secondary metabolites. Another advantage is the fact that it does not require the prior identification of the structural genes, whose expression is modified in the receiving organism.

Current stage of development
Available for demonstration.

Intellectual Property Rights
Patent applied for but not yet granted.

Collaboration sought
Finance / Licence agreement.

OTT Contact person
Katyssulla Costa
OTT - CSIC
Phone +34 915855376
Fax +34 915855196
E-mail kcosta@orgc.csic.es

Researcher
Dr. Miguel Angel Peñalva

Cold-curing acrylic formulations with low toxicity activators for use in bone cement precursors compositions

Abstract
A Spanisresearch institute has developed compositions for use in bone cement precusor compositions and to novel polymeric bone cement formed therefrom. They have been obtained by application of activators derived from diaminodiphenylcarbinol that present toxic effects inferior to those of the traditional activators. They are looking for companies of medical sector specially in surgery, traumatology and odontology interested in license agreement and finacial resources.

Description
The aim of the present invention is the development of cold-curing acrylic formulations with low toxicity activators derived from diaminodiphenylcarbinol. The proposed activators are similar to 4,4'-bis-dimethylaminobenzydrol (BZN), which is the most representative compound of the series. Lethal dose 50 in mice of BZN is 3 times superior to that of DMT. This activator provides exotherms of decreasing peak temperature with values 10°C lower than those obtained for the commercial formulations. Mechanical porperties of the cured cements are comparable to those of commercial cements with higher values of tensile strength. The tissue response to the implantation of a cement formulated with BZN in the femur of rabbits reveales an early and more abundant osseous neoformation, starting in a period of 1-2 days and following progressively at different periods of time, with high cellular activity.

Innovative aspects & Competitive advantages
Use of activators with antiseptic properties for the curing of acrylic bone cements.

- The proposed activators possess increased hydrophobicity in comparison with DMT and therefore, a decrease in necrosis resulting from leaching of these compounds from the bone cement into the surrounding tissues and systemic circulation is expected.
- The proposed activators present values of lethal doses 50 superior to that of DMT.
- The proposed activators are less cytotoxic than DMT on polymorphonuclear leucocytes.
- The proposed activators present higher activity againts different microorganisms than DMT .
- Bone cements formulated with the proposed activators cure at lower temperatures than compositions known in the art.
- The cements formulated with the proposed activators present beneficial effects on the regeneration process of the bone when they are implanted in the dough stage in the femur of rabbits and cure "in situ". The surrounding bone at the site of implantation showed an early and more intense osseous neoformation in comparison to that observed in presence of commercial formulations.

Current stage of development
Available for demonstration.

Intellectual Property Rights
Patent applied for but not yet granted.

Collaboration sought
Finance / Licence agreement.

OTT Contact person
Almudena Fernández Arizpe
OTT - CSIC
Phone +34 915616800 (ext. 3231)
Fax +34 915855196
E-mail A.Fernandez@orgc.csic.es

Researcher
Dr. Julio San Román del Barrio

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA
CSIC
Consejo Superior de Investigaciones Científicas

Cold-curing injectable bioactive acrylic formulations to be applied in minimally invasive surgery

Abstract

A Spanish research group has developed self-curing injectable formulations based on composites of poly(methyl methacrylate)/bioactive components (bioglass or silica nanoparticles) and an anti-inflammatory drug bearing phosphate groups as "fosfosal" to be applied in minimally invasive percutaneous vertebroplasty (PVP) and as biomedical stabilisation agents in osteoporotic process with loss of bone mass. The group is looking for companies of medical sector interested in license agreements.

Description

The aim of the present invention is the development of cold-curing acrylic formulations based on composites of poy(methyl methacrylate/bioactive components and an anti-inflammatory drug besring phosphate groups as "fosfosal". These formulations present values of setting time ranging between 20 and 25 min and values of maximum temperature around 60° C. When these systems are immersed in the physiological fluid, the bioactive components and the drug are dissolved in this medium and the precipitation of calcium phosphate salts (mainly calcium hydroxyapatite) takes place. This phenomenon reveals the capability of these systems to stimulate the calcification process in the bone. In addition, it has been observed that the presence of "fosfosal" accelerates notably the formation of hydroxiapatite even when the systems are implanted intramuscularly. On the other hand, it has been confirmed through "in vivo" experiments that the incorporation of this drug provides a reduction in the local inflammatory response at the site of implantation. The cured composites guarantee the stabilisation of this product until its dissolution in the medium with the hydrolysis of the molecule and the release of the salicylic acid.

Innovative aspects & Competitive advantages

Development of injectable formulations bearing bioactive components capable of stimulate the osseous regeneration, and an anti-inflammatory drug to be used in minimally invasive surgery.

- The proposed formulations present values of setting time between 20 and 25 min, offering the possibility of their use as injectable systems in minimally invasive surgery.
- The proposed formulations present values of maximum temperature around 60° C providing a decrease in necrosis of the surrounding tissues.
- The dissolution of the bioactive components and the drug in the physiological medium give rise to the precipitation of a calcium phosphate layer, mainly hydroxiapatite, providing a route of stimulation of the osseous regeneration process as well as the formation of a chemical bond with the adjacent bone.
- The presence of the drug bearing phosphate groups accelerates the precipitation of the hydroxyapatite layer mentioned above.
- The cured composites can be considered as "fosfosal" controlled release systems.
- The incorporation of this drug to these formulations could provide a positive effect of the response to the implantation of these materials clinically as has been observed in "in vivo" experimental models, in which a reduction of the local inflammatory response has been detected.

Current stage of development

Available for demonstration.

Intellectual Property Rights

Patent applied for but not yet granted.

Collaboration sought

Licence agreement.

New therapeutic compound used for the treatment of the Leishmania infection

Abstract
A Spanish research group from a public institution has developed a compound that inhibits the infection with the Leishmania parasite. This compound blocks the DC-SIGN receptor, which is present in the process of binding and uptake of the parasite by human cells. A screening system based in these results has been developed for the identification of molecules that can block and avoid the binding. The group searches for pharmaceutical companies interested in license agreements and financing.

Description
Leishmaniasis is a parasitic disease transmitted by a mosquito, which presents a broad spectrum of clinical symptoms, from local skin wounds to visceral forms that are associated with death risks. The development of different clinical symptoms can be explained by infection with different species of Leishmania and to the immunological state of the host.
Leishmania infects cells that are a key factor in the host immune response.
The technology presented here is based in the fact that the Leishmania parasites bind specifically and with high affinity to the receptor DC-SIGN. This receptor can be found in the surface of dendritic cells and is involved in the process of cell-to-cell adhesion. Blocking the DC-SIGN receptor could modulate, and more specifically reduce, the adhesion of the parasite to the eukariotic cells, and in this way avoid the infection caused by this parasite.
This technology offers an antibody that is able to block the binding of the Leishmania parasite to the DC-SIGN receptor, preventing the development of the disease.
This technology also offers a method for the identification and evaluation of compounds, which can block or avoid the binding between the Leishmania parasite and the DC-SIGN receptor. This procedure is based in the use of the DC-SIGN receptor as a target, for the design of therapeutic compounds.
The therapeutic compounds identified by this method will have the capacity to block or avoid the binding of the parasite to the DC-SIGN receptor, which can be found in the surface of the phagocytes in mammals. In this way, it is inhibited the internalisation of the parasite by this cells and the subsequent infection.

Innovative aspects & Competitive advantages
Development of an antibody able to prevent the infections of the Leishmania parasite and a method for the identification of therapeutic compounds used in the prevention of these infections. The innovative aspect of this method is the use of the molecules implied directly in the entry of the parasite in the cells, as targets in the identification of new compounds.
Nowadays, the only effective treatment against leishmaniasis is based exclusively in chemotherapy, as there is no vaccine efficient for humans and the programs for the control of mosquitoes and animal reservoirs are only effective in few special circumstances. On the other hand, medicines used against leishmaniasis frequently present toxic effects and develop resistances.
The use of the antibody developed by this research group allows for the first time the prevention of the infection by the parasite.
The new system developed for the screening of compounds allows the identification of new molecules, which inhibit the entry of the parasite in the host cell. In this way, these medicines would reduce the number of parasites that infect an individual. This favours the immune mechanisms of destruction of the parasite, increasing its efficacy.

Current stage of development
Available for demonstration.

Intellectual Property Rights
Patent applied for but not yet granted.

Collaboration sought
Finance / Licence agreement.

OTT Contact person
Katyssulla Costa
OTT - CSIC
Phone +34 915855376
Fax +34 915855196
E-mail kcosta@orgc.csic.es

Researcher
Dr. Angel L. Corbi

MINISTERIO DE CIENCIA Y TECNOLOGÍA

CSIC

Consejo Superior de Investigaciones Científicas

Rapid Method For Detection Of Infective Prion Proteins (PrPSc) In Blood

Abstract

A spanish public research institution has developed a rapid method to detect infective prion proteins (PrPSc) in ovine blood affected by a Transmissible Spongiform Encephalotaphy (TSE) by laser-Raman or infrared spectroscopy. This method can be also applied to, among others, bovines, goats, humans, birds, etc. The type of collaboration sought is license agreement and/or financial support with Biotech/Food and Livestock Industries.

Description

This invention provides a new procedure to detect infective prion proteins (PrPSc) in blood from animals (ovines, bovines, goats, humans, birds, etc.). The results obtained through this rapid procedure correlate at 100% with the biodiagnostic post-mortem tests (validated by the International Organization of Epizoothies, OIE) of brain samples obtained from the corresponding positive and negative animals.

This invention consists of the following steps:

a) isolation of a lysed fraction of cellular elements stemming from animal blood, enriched in PrPSc, and

b) identification, by laser-Raman or infrared spectroscopies, of PrPSc proteins in that lysed fraction of cellular elements.

The spectroscopic analysis of the final pellets includes the following steps:

1. Measurement of the Raman or infrared spectrum of samples in the amide I region (1600-1700 cm-1), where the characteristic bands of PrPSc protein beta-structures appear.
2. Determination of the percentages of beta-structure using established methods.

Innovative aspects & Competitive advantages

This innovation solves the technical problem of providing a reliable in-vivo, rapid test for the biodiagnostic of these pathologies. Currently, the diagnostic of transmissible spongiform encephalopathies (TSE) is founded on the clinical suspect of the disease in question and the confirmation through the diagnostic methods validated and authorized by the European Union (Regulations 999/2001) for the scrapie diagnostic. All of these methods are applied on samples obtained post-mortem using preferably assays of imunohistochemistry, immunoblotting, etc. However, this kind of assays are time-consuming, expensive and complicated, and, what is more important, they are not sufficiently reliable (S. B. Prusiner, 2002). By contrast, laser-Raman and infrared spectroscopies show the innovative aspect that they can detect infective prion proteins in blood samples through a rapid, reliable and non-invasive method without the need of using antibodies or other more laborious techniques.

The advantages provided by this invention, related to the state of the art in the current diagnostic techniques, lyes on the fact that one can rapidly and uninvasively act in-vivo on a biological fluid such as blood. Furthermore, this fluid is easily extracted without previous sacrifice of the animal in question. This implies obviously great economic advantages. By contrast, as described above, the current techniques use post-mortem diagnostic methods, which involve the inherent economic cost of sacrificing the analyzed animals and results of unsuficient reliability. Moreover, this spectroscopic method is itself less expensive and more rapid than the other methos of the techniques used so far.

Current stage of development

Available for demonstration.

Intellectual Property Rights

Patent applied for but not yet granted.

Collaboration sought

Finance / Licence agreement.

OTT Contact person
Irene Herrera Insúa
OTT - CSIC
Phone +34 915616800 (ext. 3232)
Fax +34 915855196
E-mail I.Herrera@orgc.csic.es

Researcher
Dr. Pedro Carmona Hernández

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA
CSIC
Consejo Superior de Investigaciones Científicas

New Na+/H+ exchanger inhibitor peptide (PINHE) and its applications

Abstract

Three spanish research public institutions have developed a peptide (PINHE) that acts as a physiological inhibitor of eukaryotic Na+/H+ exchanger. It can be used for several pathologies as in cardiovascular diseases, kidney, brain, metabolical disorders, tumoral cell proliferation, HCL hyperproduction, ischaemia, also as a contraceptive in male mammals, antibiotic, inmunomodulator agent, and salt-stress modulator in plants. It is sought license agreement and financial support for development.

Description

The invention show here describes a peptide (PINHE) derived from H7 halocin, with a low molecular weight and defined by its aminoacidic sequence or other analogue, and that acts as a natural-origin physiological inhibitor of Na+/H+ exchanger (NHE) in eukaryots. PINHE is obtained and purified after fractioning (including cold acetone precipitation) of the supernatant from a culture of the haloarchaea Haloferax gibonsii alicante SPH7. This haloarchaea produces PINHE in a natural way. PINHE can also be artificially sintetisize or modify, cloned or expressed using genetic engineering techniques based in its aminoacidic sequence. PINHE is estable and active in a wide range of saline concentration, temperature, and pH. It is also trypsin resistant and pronase sensitive. It can be lyophillized and reconstituted without activity loss. The use of this NHE inhibitor peptide or its pharmacological accepted derived salts, in the development of a pharmacological composition, is another additional subject of this invention. Several uses and applications can be derived from its reversible action as a eukaryal Na+/H+ exchanger inhibitor and its derived salts. These uses are: (1) Treatment or profilasis of pathologies in mammals, human beings preferently, caused by the hyperactivity or an abnormal activity of NHE as for example: cardiovascular diseases (high blood presure, myocardium infract, arrhythmias, heart-attack, etc.), brain diseases (brain infarct, stroke, brain edema, etc.) renal diseases (renal faillure), metabolic disorders and diseases (diabetes mellitus, hyperlipidemias etc.), diseases caused by hyperproduction of chloridic acid in gastric mucosa, diseases caused by epithelial transport alterations, diseases caused by alteration of cellular volume, pathological cell -proliferative processes (cancer, tumoral growth, fibrosis, etc.), alteration caused by an excesive activity of the inmune system (inflamation, hypersensibility), (2) other pathologies or diseases that develop an ischemic process and post-ischaemia perfusion injury due to hyperactivity of NHE, among others are: ischaemia by primary or secondary vascular alterations in different tissues or organs, by post-infarct-reperfusion, by organ or tissue transplantation, by cardiatic or vascular surgery, by ischaemia in physiological situation produced by physical exercise, pregnancy, childbirth, menstruation, etc., by traumatic injuries or any other pathology that implies tissular hypoperfusion (hemorragic shock, termal shock, neurogenic shock, etc.) and in any other surgery procedure in general, (3) also as a contraceptive in male mammals, antibiotic or inmunomodulator agent and in any field as modulator in plants saline-stress.

Innovative aspects & Competitive advantages

At the moment PINHE is the only described peptide from Archeae Domain that it has inhibitory effect on Sodium-Hydrogen-Exchanger of mammal cells including human. This opens a new field for the search of molecules with therapeutically interest inside the Archaea Domain.

The most remakables advantages of PINHE (shared with halocin H7) in comparison with other NHE inhibitor substance are:

- PINHE is a peptide and shows reversibility in its therapeutical action and an easy way to metabolize as protein substance.
- PINHE is a natural substance of antibiotic kind, its microbicidal capacity is based in its inhibitory action of Sodium-Proton exchanger.
- PINHE is a specific inhibitor, active at low concentrations with low probability to cause secondary effects.
- PINHE can operate in a wide range of salt concentrations, temperature and pH, that make it in the choosed substance in many process.
- PINHE is resistant to trypsin allowing to use it in the intestinal tract.
- PINHE is pronase-sensitive so it is possible to inactivate it at will.

Moreover to above, PINHE has additional advantages if we compared with Halocin H7 that it allows to assure that PINHE is a substance with a bigger potential from the applied point of view.

The main advantages of PINHE comparing to Halocin H7 are:

Consejo Superior de Investigaciones Científicas
Spanish Council for Scientific Research

Agricultural & Nutritional applications

MINISTERIO DE CIENCIA Y TECNOLOGÍA

CSIC
Consejo Superior de Investigaciones Científicas

Innovation

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA

CSIC
Consejo Superior de Investigaciones Científicas

Sequence of maize nucleotides codifying a protein with transglutaminase activity and their use as a food additive

Abstract

A Spanish Public Research Institution (IBMB-CSIC) licenses the first plant trasglutaminas patent as a biotechnological technology useful to agrofood industry. This enzyme catalyses covalent links formation between proteins to make networks, allowing food texture, firmness, elasticity, or fat and salt content modifications in the foodstuffs industry: processed fish, meat and sausage, dairy products, gelatines, etc. We look for patent licence agreements and additional developments collaborations.

Description

Our group from a Spanish Public Research Centre sited in Barcelona (IBMB-CSIC), has identified two sequences of nucleotides codifying two proteins with transglutaminase activity from maize cDNA. These were the first plant transglutaminase cloned. Protein activity has been tested in experiments starting from extracts of the codifying proteins. Moreover, these proteins show the specific characteristics assigned to the enzyme. This enzyme catalyses the formation of covalent links between proteins to make networks of high molecular weight.

The specific function of transglutaminase allows their biotechnological application in the foodstuffs industry: fish products (surimi), processed meat and sausages, chesses and yoghurt, ice creams, gelatines, chocolate etc. because food texture, firmness, elasticity, or fat and salt content can be modified.

Moreover, starting from these DNA molecules and by means of appropiate vectors, new transformed cells can be obtained, (bacteria or yeast GRAS). These transformed cells can over-produce the recombinant protein with transglutaminase activity and consequently their availability to the industry.

Innovative aspects & Competitive advantages

The use of transglutaminase in bio alimentary processes, allows the obtention of new products in the base of a technology relatively easy to use. Moreover, it is enable to extend the range of products, without modifying technological aspects of the machinery or instruments of a well-established industrial process. On the other hand, in some manufactures of fish, meat etc. allows a best raw material utilization improving products texture and humidity. Moreover, especially in lactic derivatives it can also be useful to prepare hypocaloric products.

This patent represent the first transglutaminase cloned in plants. Consequently it allows the preparation of a trendy technology to obtain a product with well-known foodstuffs market advantage because of actual use in the american market as a food additive of a patented product from bacteria with similar characteristics.

Current stage of development

Development phase.

Intellectual Property Rights

Patent applied for but not yet granted.

Collaboration sought

Licence agreement.

OTT Contact person
Luis Guasch Pereira
OTT - CSIC
Phone +34 915616800 (ext. 3210)
Fax +34 915855196
E-mail l.guasch@orgc.csic.es

Researcher
Dr. José Mª Torné

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA
CSIC
Consejo Superior de Investigaciones Científicas

Overproduction Of Diacetyl Using Inmobilised Cells Of Lactobacillus Casei In A Continous By Means Of "Food Grade" Genetic Tools

Abstract

Spanish Public Research Institution licences a technology. An integrative vector allows stable insertion of Lactococcus lactis ilvBN genes into Lactobacillus casei chromosomal lactose operon, yielding highly stable food-grade mutants, following lactose genes expression pattern. No antibiotic resistance genes or foreign DNA traces. Expertise offered in handling immobilised cell bioreactors of L. casei to a dairy or biotechnological industry. Application: diacetyl overproduction from milk whey.

Description

The main characteristic of lactic bacteria is the lactic acid production during the carbohydrate fermentation by glycolisis. L. casei is very convenient for industrial lactic acid production because of the almost exclusive production of L-lactic acid by some strains, the only one that mammals assimilate. Besides, L. casei can use different carbon sources, resists non-favourable environmental conditions and mild salt concentration, and grows between 10º a 42º C.

To optimise the fermentation process, continuous production can be used by means of stationary-state fermentation tanks and cell-immobilised bioreactors that allow size reduction. In this way there are immobilised cells of L. casei, L. rhamnosus o L. delbrueckii subsp. Bulgaricus over different inert holders like plastic polyurethane or porous glass balls, embedded in agriculture wastes. This accuration system was set in the lab accurately, yielding 100% sugar conversion for semi-determinate media and close to 99% for milk whey treated with proteases.

Depending on growth conditions of L.casei, other technologically very important products can be obtained like diacetyl, acetic acid or acetaldehyde. These are responsible for the specific aroma and flavour of dairy fermented products, and therefore are added to butter, fresh cheeses and salad sauces.

By means of Metabolic Engineering and Molecular Biology, "Food Grade" Lactobacillus Casei strains have been obtained in their lab, that cause Overproduction Of Diacetyl in culture media using immobilised cells in a continuous patented process. These strains have been obtained by inactivation of the L-Lactate dehydrogenase gene and over-expression of a-hydroxyacetic acid sintase enzyme.

Milk whey elimination is an important technological and economic problem. Its components can be reused because of their excellent commercialisation possibilities.

Ultra filtration and crystallisation to recuperate lactose and the different methods for selective separation of b-lactoalbumine and a-lactoalbumine (mainly ultra filtration) demand high investment on equipments and maintenance expenses (cleaning and membrane recycling) that can be only paid off over daily 150/200000 litres of whey. Because of its low hygroscopic properties, lactose is used as a very convenient excipient in pharmaceutical and dietetic products. Proteic fraction recovered from milk whey, depending on purity, has various applications as an emulsifier in nutrition, baby food, dietetic and cosmetic, and also as a food additive for soups and meat products.

Cheese production in Spain is extremely scattered into low-sized SMEs. That means that a large amount of the total milk whey production (total estimation 4.222 m3/day) could be directed to animal feeding or dehydration, but is considered mainly as waste, causing an environmental and legal problems. These SMEs produce large enough amounts (500 to 5.000 litres a day) to overpass the capacity of local purifying plants. However, it is not enough to pay off recovering plants.

Innovative aspects & Competitive advantages

This new biotechnological process with a lower cost can purify and recover whey by means of microbial fermentation that yields L-lactic acid and diacetyl. Its economic feasibility depends on development plans.

The recovering of milk whey to produce industrial-grade citric acid, ethanol, and methane requires 500.000 litres minimum to be profitable.

Enzymatic synthesis of the antioxidant hydroxytyrosol

Abstract

A Spanish research group has developed a method to synthetise the antioxidant hydroxytyrosol. The method is cheap, easy and hydroxytyrosol is obtained by enzymatic synthesis, avoiding pollutant and toxic compounds. The synthetised hydroxytyrosol can be use as food ingredient to enhance health-promoting properties and stability of the functionalized food. It can be used for tomato juice, butter, vegetable oils, etc. We look for agrofood, farmaceutical or chemical companies for license agreement.

Description

There are consistent evidences regarding the health-beneficial properties (protection against cardiovascular diseases and cancer) of the virgin olive oil. The responsible for such beneficial properties are both an adequate fatty acid profile and the presence of antioxidants such as the phenolic compounds. The most important phenolic compound is the antioxidant hydroxytyrosol which has been widely studied demonstrating its health-beneficial properties as well as its good bioavailability. However, the hydroxytyrosol is not commercially available. Therefore, researchers must either isolate or synthesized it. The chemical synthesis implies the use of toxic and/or pollutant reagents. The protocols are slow and require high qualified staff to be carried out. In addition, the precursors used in the chemical synthesis (oleuropein, 3,4-dihydroxyphenyl acetic acid) are expensive, appart from the additional cost of the rest of reagents (solvents, acids, etc.). Moreover, the use of HPLC equipment is essential. There are also several protocols based on the extraction of hydroxytyrosol from the olive oil, leaves and olive mill waste waters. Usually, the protocol involves 4 steps: extraction with ethyl acetate, sodium sulfate, 2 low performance chromatographic steps and one more step with thin layer chromatography. The main disadvantage, appart from the use of pollutant and toxic reagents is the low yield (around 1.5%).

The present invention allows to synthesize the antioxidant hydroxytyrosol from a commecially available precursor (tyrosol 18.7 €/g, Aldrich) using the enzyme tyrosinase (commercial enzyme from mushroom), in the presence of a reductor such as vitamin C. The advantages are the following:

- The reaction medium is at neutral pH, at room temperature and in aqueous medium.
- Organic solvents, acids, alkalis, or other pollutant/toxic reagents are not used.
- The yield can be 100%. The overall process can be 1 or 2 steps depending on the final use of the molecule. The protocol is fully adjustable, i.e the final amount of molecule can be chosen whenever decided.
- The protocol does not require expert staff to be carried out.
- The enzyme can be re-used. The antioxidant can be used alone or in combination with vitamin C.
- The process can be adapted to a bio-reactor although it is not essential.
- The present invention could be used by food industries to obtain "functional foods" (hydroxytyrosol can be combined with many foodstuffs) or also it could be used by chemical (or pharmaceutical) companies to obtain this molecule in order to make it commercially available either for food companies or researchers.

Innovative aspects & Competitive advantages

The present invention allows for the obtantion of the antioxidant hydroxytyrosol by enzymatic synthesis. The molecule can be further used as food ingredient. The use of pollutant and/or toxic reagents is avoided. The synthesized hydroxytyrosol is free of pollutant compounds since all the reagents used in the invention are suitalble for human consumption. The hydroxytyrosol can be used to "functionalize" foodstuffs (in this case the functional food would have the health-beneficial properties of the virgin olive oil). This has been demonstrated (laboratory scale) in tomato juice using antioxidant assays and a panel of trained people to asses changes in the sensory properties.

- To obtain a "natural" molecule (the hydroxytyrosol) using a "natural" process (enzymatic synthesis) without pollutant and/or toxic reagents. Besides, the procedure is cheap, easy to perform and little time consuming.
- To use this molecule as food additive to get new "functional foods" in accordance with the new market tendency. In this case, the functional foods (Spanish gazpacho, tomato juice, sauces, butter, vegetable oils, etc.) would have the health-beneficial properties of the virgin olive oil and also the stability of these foodstuffs could be enhanced by preventing rancidity processess.

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA
CSIC
Consejo Superior de Investigaciones Científicas

Production of chemicals of industrial application by microalgae and cyanobacteria in outdoor closed tubular bioreactors

Abstract

Indoor and outdoor cultures (outdoor closed tubular reactors) of microalgae and cyanobacteria (blue-green algae) for the production of chemicals of industrial application: Carotenoids (astaxanthin and lutein), phycobiliproteins, fatty acids, exopolysaccharides, and bioactive compounds. Isolation and selection of strains, improvement of the strains by random mutagenesis and flow cytometry, optimization of growth and productivity of the selected strains , production of the specific compounds in outdoor closed tubular reactors, effect of environmental and nutritional factors on the production of compounds in these reactors.

Description

For the selection of the most adequate strains we have made a search in the bibliography, as well as in the culture collections. Moreover, our group is expert in the isolation of microalgae and cyanobacteria from natural environments. Several interesting strains isolated by us are available in our group.

The selected strains are cultivated at laboratory scale to find out their optimal conditions for growth and production of the specific compounds.

55 l closed methyl polymetacrylate tubular photobioreactors are used, with an airlift system to recirculate the cell culture and an external horizontal loop, consisting of tubes (total surface 2,2 m2), which acts as solar receiver, submerged in a thermostatic pond of water. The airlift is made up of a degasser in which the pH, temperature and other probes are inserted and two tubes (the raiser and the downcomer). Compressed air is supplied into the raiser to move the cell suspension through the tubes and provide turbulence to the culture. The system is operated as a continuous culture during daylight period and as a batch during night, to avoid the culture washout.

The effect of different environmental and nutritional conditions are studied in the closed tubular reactors, such as, period of the year, cell density (dilution rate), turbulence (flow rate), temperature, pH, nutrients, etc.

Innovative aspects & Competitive advantages

Microalgae and cyanobacteria are rather new organisms to be used for industrial purposes. They have been used in the past only for biomass production. The use of microalgae for the production of chemicals of industrial application shows many innovative aspects, since these photosynthetic organisms can be grown combining the fermentative technology (closed reactors) used for bacteria and yeasts and the autotrophic conditions of outdoor cultures typical from plants. In addition, some of these compounds can only been found in microalgae and/or cyanobacteria; among these are phycobiliproteins and some carotenoids. They also show extremely high productiviites even in outdoor closed tubular reactors and wide tolerance to environmental and nutritional factors. Moreover, the nitrogen-fixing cyanobacteria do not require nitrogen in the medium, which makes their culture cheap and restricts the contamination by other microorganisms.

The photosynthetic metabolism of microalgae and cyanobacteria (blue-green algae) is the main competitive advantage with regard to other organisms, since solar energy can be used for the production of chemicals of industrial interest.

In addition, in the case of carotenoids, the contents in carotenoids of microalgae are much higher than those of yeasts (as Pfaffia) or bacteria, and the ester composition is similar to those found in animals. For those reasons microalgae are much better accepted for nutraceutical, pharmaceutical and cosmetic uses, as well as for aquaculture.

The use of closed tubular bioreactors outdoors for the cultivation of microalgae and cyanobacteria ensures axenity and therefore the high quality of the product. This technology shows, obviously, a high competitive advantage in comparison to outdoor open ponds, specially if the product is going to be used for human consumption, taking into account, in addition, that we are talking about products of high value in the market.

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA

CSIC
Consejo Superior de Investigaciones Científicas

Phenolic tetrahydro-beta-carbolines as antioxidants

Abstract

A Spanish Public Research Institution (IFI-CSIC) licenses the patent for obtention of a new class of antioxidant molecules based on tetrahydro-beta-carbolines containing phenolic substituents that are obtained from naturally occurring precursors such as amino acids or amines and phenolic aldehydes. These compounds can exhibit a potential use as antioxidants agents and against oxidative stress. We look for patent licence agreements and additional developments collaborations.

Description

Molecules of tetrahydro-beta-carboline class containing phenolic subtituents are obtained from naturally occurring amino acids or amines and phenolic aldehydes through a condensation reaction in aqueous or alcoholic solutions. These phenolic tetrahydro-beta-carbolines exhibit good antioxidant properties against radicals and also can inhibit lipid peroxidation in vitro. Therefore, the obtained tetrahydro-beta-carbolines or preparations containing them could exhibit further use as antioxidant agents and also against oxidative stress in pharmaceutical products, nutraceutical products, and functional foods.

Innovative aspects & Competitive advantages

Phenolic tetrahidro-beta-carbolines have two different sites of antioxidant action based on the indole moiety and the phenolic subtituents and therefore they can exhibit interesting properties as antioxidants and free radical scavengers.

Those molecules are antioxidants that differ from the classical phenolic compounds or vitamines and therefore they may represent a different and complementary pattern of antioxidant action and activity against free radicals. Also, they can be obtained from natural precursors such as amino acids and phenolic aldehydes.

Current stage of development

Available for demonstration.

Intellectual Property Rights

Patent applied for but not yet granted.

Collaboration sought

Finance / Further research and/or development support.

OTT Contact person
Luis Guasch Pereira
OTT - CSIC
Phone +34 915616800 (ext. 3210)
Fax +34 915855196
E-mail l.guasch@orgc.csic.es

Researcher
Dr. Tomas Herraiz

MINISTERIO DE CIENCIA Y TECNOLOGÍA

CSIC

Consejo Superior de Investigaciones Científicas

Serologic detection system of cucumber vein yellowing virus (CVYV)

Abstract

A public Spanish Research Centre has developed a system for serologic detection of Cucumber Vein Yellowing Virus (CVYV) in cucurbits. The system uses an antiserum with antibodies raised against a recombinant CP of CVYV. The system is easy to use as serologic methods such as ELISA are known and used by most parties involved in virus control. The system has a low cost and is simpler than other methods based in the detection of nucleic acids. Biotechnology companies for licence agreements are sought.

Description

Cucumber vein yellowing virus (CVYV) causes very severe epidemic events in cucumber and other cucurbits crops in eastern Mediterranean basin, provoking huge economical losses.

CVYV was first observed in Spain during autumn 2000, in protected cucumber crops of the Almeria province. Since then the virus has spread very quickly through this area, causing dramatic economical losses. To avoid this situation an effective strategy to control the virus is needed. Virus control by means of controlling its vector has been shown to be an ineffective control method for virus transmited by whiteflies. Among alternative control methods, there are some culture practices such as the use of non-infected propagation material and the use of cultivars genetically resistant to the virus. In both cases a specific, sensitive and fast detection method is needed. Among the most used plant viruses detection methods are those based in serologic techniques which imply the use of specific antibodies raised against a viral protein.

The present invention consist of a system for serologic detection of CVYV, using an antiserum containing antibodies against the capsid protein (CP) of CVYV. The antibodies had been generated using a recombinant CP.

The procedure for generating the antibodies involves the following steps: (i) obtention of a recombinant CP from CVYV, (ii) purification (optional) of the recombinant CP obtained and (iii) inmunisation of mamals or other animals or animal cells with the recombinant CP, followed by the obtention of the serum from those animals or the culture media from the animal cells.

The recombinat CP was obtained after generation of a genetic construction comprising the CP coding sequence fused to the coding sequence of a purificable protein, preceded by an inducible promoter. The recombinant CP is obtained after expression, in the appropriate host cells, of the fused protein followed by purification and proteolitic cleavage.

The antiserum obtained after inmunisation of animals with the recombinant CP have been used for CVYV detection in plants infected by the virus, showing their capability for its detection either by western-blot or by ELISA. The antiserum gave no reaction when the plants were not infected by CVYV showing that the serologic detection system generated by the present invention is specific for CVYV.

Innovative aspects & Competitive advantages

Antiserum with antibodies raised against a recombinat CVYV CP. Detection of CVYV by serological techniques.

Specificity, sensitivity, low cost, simplicity and speed of the CVYV detection method.

Current stage of development

Available for demonstration.

Intellectual Property Rights

Patent applied for but not yet granted.

Collaboration sought

Finance / Licence agreement.

OTT Contact person
Yolanda Hernando Sáiz
OTT - CSIC
Phone +34 968396276
Fax +34 968396213
E-mail yhernando@cebas.csic.es

Researcher
Dr. Juan José López-Moya

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA
CSIC
Consejo Superior de Investigaciones Científicas

Method For Detection Of Cucumber Vein Yellowing Virus (CVYV)

Abstract

A public Spanish Research Centre has developed a specific, sensitive and fast detection method for Cucumber vein yellowing virus (CVYV) in cucurbits (cucumber, melon and squash) by using a hibridization-based technology. This method has a reduced economic cost and an increased simplicity compared with other available methods for the CVYV detection having equivalent sensitivity and specificity levels. We look for a licence agreement with a biotechnology company.

Description

The cucumber vein-yellowing virus (CVYV) causes very severe epidemic events in cucumber and other cucurbitaceous crops in eastern Mediterranean basin, provoking huge economical losses.

CVYV was first observed in Spain during autumn 2000, in protected cucumber crops of the Almeria province (Spain). Since then the virus has spread very quickly through this zone, causing dramatic economical losses. To avoid this disaster an effective strategy to control the virus is needed. Virus control by means of controlling its vector has been revealed to be an ineffective control method for virus with the whitefly as a vector. Among alternative control methods, there are some culture practices such as the use of non-infected propagation material and the use of cultivars genetically resistant to the virus. In both cases a specific, sensitive and fast detection method is needed.

There are several detection methods of plant viruses; those based on serological techniques are the most widespread. However, to use these serological methods the knowledge of the specific virus antibodies is required. The antibodies for CVYV virus are not yet known, probably because of the difficulty of purification of viral particles. Hybridisation-based technology constitutes an alternative method to the serological one. Molecular hybridisation implies the preparation of a genetic construct that contains a nucleotide sequence homologous to the virus genome, which is used to synthesize the probes. The complete sequence of CVYV genome is still unknown.

Molecular hybridisation as virus detection method has been widely used, in particular detection by dot-blot hybridisation of plant nucleic acid extracts applied onto nylon membranes. Recently an easier and simpler hybridisation technique in tissue-prints has been developed. The goal of the present invention is the achievement of a detection method for CVYV by means of hybridisation-based technology. The method can be used with both plant nucleic acid extracts and tissue-prints.

Preparation of a genetic construct that contains a fragment of the CVYV-AILM genome was the first step. CVYV-AILM is the viral isolate infecting a melon plant from Almeria. The oligonucleotide primers MA162 and MA163 were used for the cloning of a DNA fragment complementary (cDNA) to the virus genome. As template for the cDNA synthesis, a total RNA extract of a CVYV-AILM infected plant was used. The plasmid containing this insert has been called pLMCVYV.

Complementary RNA (cRNA) probes were synthesised form the pLMCVYV plasmid, cRNA probes were used as they have an improved sensitivity and specificity compared with other type of probes. cRNA was prepared according to standard protocols for in vitro transcription.

The dot-blot hybridisation of plant nucleic acid extracts was performed applying one ml of total RNA plant extracts onto a nylon membrane positively charged, nucleic acids were fixed to the membrane using a ultraviolet crosslinker. Next, membrane was subjected to standard procedures for prehybridisation, hybridisation with the probe prepared as above described, and washes. Final detection of the probe was done by chemiluminiscence.

The tissue-prints for hybridisation came from the stems, petioles and major leaf veins that were sectioned with a razor blade. To avoid cross-contaminations, each section was prepared with a different and sterile blade. Sections so prepared were immediately applied onto a nylon membrane positively charged until the exudate was absorbed. Once prints were performed, membranes were maintained at room temperature until dried. The rest of the process is as described for hybridisation of plant nucleic acid extracts.

This detection method showed specificity for CVYV, being the minimum amount of virus RNA that can be detected using this method of 6 pg.

Innovative aspects & Competitive advantages

Obtention of a cDNA to the genomic CVYV RNA and its use in a hybridisation-based technique for the virus detection, in particular, for detection in tissue prints.

Specificity, sensitivity, low cost and speed of the CVYV detection method.

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA

CSIC
Consejo Superior de Investigaciones Científicas

Mustard oils with high oleic and/or low linolenic acid content

Abstract

Ethiopian mustard (Brassica carinata) lines with improved seed oil composition have been developed by conventional, non-transgenic procedures by the Institute for Sustainable Agriculture (CSIC). The lines produce oils with high oleic (>80%) and/or low linolenic acid content (<5%), of great value for food and non-food (bio-fuels, bio-lubricants, deep-frying, etc.) applications. Ethiopian mustard with particular oil profile can be produced in typical canola areas without isolation. Collaboration sought is financial resources for further research and licence contract with oil industries.

Description

Ethiopian mustard (Brassica carinata) is an annual oilseed crop closely related to rapeseed, but it exhibits a better adaptation to agro-ecological conditions of Southern Europe and other semiarid areas. Naturally occurring Ethiopian mustard forms produce a seed oil rich in erucic acid (>40%), which is toxic for human food and animal feed. The elimination of erucic acid by plant breeding resulted in a seed oil poor in monounsaturated oleic acid (<35%) and rich in polyunsaturated fatty acids (>35% linoleic, >20% linolenic acid). The high degree of polyunsaturation determines a very low oxidative stability, which represent a serious limitation for its commercial use. Through conventional breeding procedures, without producing transgenic plants, the Institute for Sustainable Agriculture has developed Ethiopian mustard lines that produce novel seed oil types. One of them is characterized by a high linoleic acid content (>45%) and a low linolenic acid content (<5%). This oil combines an optimal nutritional value with an improved oxidative stability. A second oil type possesses a very high concentration of monounsaturated oleic acid (>80%) and a low concentration of linolenic acid (<5%). The main characteristic of this oil is an exceptional oxidative stability coupled with an excellent nutritional value, having therefore a number of applications in both food and non-food applications, for example in the fields of bio-fuels and bio-lubricants. Since Ethiopian mustard is a crop very similar to rapeseed/canola, but their crossability is very low, it can be used for producing particular oil types in traditional areas of rapeseed/canola cultivation without need for isolation. Also, Ethiopian mustard is an optimal crop for producing specialty oils in semiarid regions of Southern Europe.

Innovative aspects & Competitive advantages

No other low linolenic or high oleico/low linolenic acid Ethiopian mustard lines have been developed to date.
Two main advantages in comparison with similar oil types in rapeseed/canola can be cited:
1) Ethiopian mustard is better adapted to semiarid conditions, as for example in Southern Europe, and
2) it is possible to produce particular oil types in traditional areas of rapeseed/canola cultivation, as the rate of crossability between both species is very low.

Current stage of development

Available for demonstration.

Intellectual Property Rights

Patent applied for but not yet granted.

Collaboration sought

Further research and/or development support / Licence agreement.

OTT Contact person
Consuelo Quilchano Gonzalo
OTT - CSIC
Phone +34 954500978
Fax +34 954224331
E-mail quilchano@cica.es

Researcher
Dr. Leonardo Velasco Varo

Lactose hydrolysis with immobilized thermoresistant lactase and its obtention method

Abstract

Spanish public research institution has designed a procedure for the production of the beta-galactosidase from Thermus sp. T2 fused with an IMAC domain consisting in a poly-His tagged in the N-terminal region of the protein, that make possible its purification and immobilization without loosing its thermoresistant hydrolytic power, potentially useful to milk industry simultaneously with thermic treatments. We look for dairy or biomedical enterprises for licence agreement or technical support.

Description

To avoid the presence of other enzymes as contaminants, such as proteases, that may have a severe impact on the stability of the enzyme leading to undesirable changes in dairy products during storage, and to produce it in a mesophile to reduce industrial productions cost, a purifying system was previously applied to overproduce the b-galactosidase of the thermophilic bacteria Thermus sp. strain T2 in a mesophile, which retains the biochemical properties of the native enzyme and can be purified in a single chromatographic step. To improve the potential biotechnological applications on agrofood industry of the b-galactosidase from Thermus sp. T2, and to apply other purification possibilities used in previous studies for other proteins we decided to create a new quimeric poly-His tagged b-galactosidase protein, which could facilitate the purification of the enzyme by metal-chelate chromatographic techniques. The use of immobilized metal-ion affinity chromatography (IMAC) to purify proteins fused with poly-His tags is becoming increasingly popular as a tool for the simple and inexpensive production of large quantities of pure industrial enzyme and pure enzymes for molecular biology studies. In most cases, the insertion at the terminal amino or carboxy positions of a small tag of six histidine residues hardly modifies the activity-stability properties of an enzyme. The usual protocol of purification for so tagged enzymes is based on their adsorption, along with many other host proteins, on commercial supports. Then, natural proteins (that usually adsorb less intensely than fused proteins) are adsorbed by using a gradient or step-wise elution system. Finally, poly-His tagged enzymes are eluted with high purification yields by using more drastic conditions, although in some cases there are some traces of contaminants. This stronger adsorption of the tagged proteins compared to that natural ones might be a consequence of probable differences in the exact mechanism of adsorption. This may permit the development of new purification protocols including a highly selective adsorption of the target protein on tailor-made chelating supports. Commercial chelating supports usually contain about 40 mmol of chelating groups per ml of support, attached to the support through medium or long spacer arms (7 or 12 carbon atoms). In this way, several weak specific (chelate) and non-specific (ionic hydrophobic) interactions between natural proteins and rhe supports may be occur. These supports were previously developed for IMAC of natural proteins. In that case, the requirements were different from the objective of selective adsorption: this first version of commercial chelating supports was designed to provide supports able to adsorb moss proteins. Now, when trying to perform selective adsorption of poly-his tagged proteins, such versions of commercial supports may be not the best choice. It may be expected that a poly-His tag could be able to promote a strong adsorption to tagged proteins by interacting with only one chelate moiety. Therefore, it may be assumed that chelating supports that were unable to promote multipoint interactions with proteins could be much more suitable for selectively adsorb the tagged proteins. It has been shown that similar adsorption between isolated histidine residues in natural proteins and single chelate moieties on the supports are very weak. The bgaA gene of the bacteria Thermus sp. Strain T2 encoding a b-galactosidase An active chimeric thermostable b-galactosidase was constructed by fusing the BgaA protein from Thermus sp. Strain T2 to a poly-His tagged in the N-terminal region of the protein, and expressed in its active form in a mesophile. Biochemical analyses showed that the tag did not alter maturation of the chimeric poly-His b-galactosidase (IMAC-Bga), that undergoes a complex pos-translational processing from an inactive monomeric precursor to the active heterodimeric enzyme. This enzyme has been used as a model to develop a novel and very simple procedure for one-step purification of poly-Histidine proteins via immobilised metal-ion affinity chromatography on tailor-made supports. It was intended to improve the selectivity

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA
CSIC
Consejo Superior de Investigaciones Científicas

New "HSF" transcription factors and their utilization in transgenic plants

Abstract

A Spanish Research Centre has identified and cloned a new HSF (Heat Stress Transcription Factor). Such HSF has unique characteristics that allow using it for producing transgenic plants, which specifically, and simultaneously, over express the HSF target genes (including embryonic sHSPs: Small Heat Stress Proteins). This would be useful for seed improvement biotechnology (of vigour, stress-tolerance, etc.) We look for biotechnology enterprises for license agreement.

Description

The new HSF (Heat Stress Transcription Factor), HaHSF9, presents unique characteristics such as protein expression limited to the embryonic stage of seeds and its capacity of transcriptional activation.

These two properties allow the use of this factor as an ideal tool for producing transgenic plants that specifically over express the genes regulated by this factor. Among other genes, the embryonic sHSP (small Heat Stress Proteins) genes are regulated by this factor. The presence of the sHSP genes are correlated to stress tolerance, repair of damages caused by stress in the seeds, and the seed vigour.

The ectopic over-expression of this factor HSF in transgenic plants, allows the simultaneous over-expression of different embryonic sHSP and other related genes, making possible a gain of function and seed improvement.

The HSFs are used to achieve gain of function in transgenic plants by means of their over-expression in seeds and/or ectopic (constitutive o inducible). The use of the HSFs comprises the following steps:

a) Construction of a chimeric gene adding the SHF sequences, especially HaHSFA9, to a primer and to other proper regulatory sequences, depending on the type of desired over-expression.

b) Introduction of the chimeric gene into the plant (using Agrobacterium)

c) Evaluation, in transgenic plants, of the expression of the HSF factor and the expression of the genes regulated by that HSF (especially the sHSP which are usually expressed in seeds).

The gain of function by means of these transcriptional factors HSF, could improve several seed properties such their germination capacity, their stress tolerance, etc., due to the over-expression of the activated genes by these transcriptional factors HSF.

In order to achieve a gain of function and/or to promote the expression of genes, it is also possible to use chimeric HSF factors that include partially sequences and characteristics of the HaHSFA9 factor (native or modified sequences). Likewise, recombinant HSF factors can be used for the same propose.

These HSF factors can come from any transgenic plant (mono or di-cotyledon plants).

Innovative aspects & Competitive advantages

The identification of a transcription factor that is specifically involved in the activation of embryonic sHSP genes, allows the SIMULTANEOUS over-expression, and gain of function, of such sHSPs in transgenic plants. We do not know of other factors that would produce similar effects.

The simultaneous over-expression of several embryonic sHSPs would improve the effect of gain of function (tolerance to water-stress) obtained until the moment using a single sHSP (). The improvement would be as much quantitative: greater effects produced when combining several sHSPs that are expressed at high levels by means of the transcriptional activation by a very specific HSF (HaHSFA9 or its functional equivalent); as qualitative: the different sHSPs activated by the HSF can have different functions, as it is deduced from correlations between the expression of the embryonic sHSPs and various phenotypes of interest in the improvement of seeds (tolerance to stress, vigour, etc). The activation-specificity of the used HSF would not produce negative effects to the development and growth of the transgenic plants. We have verified this with experiments performed since our patent application.

The activation of other probable target genes for the HSF (HSPs of other families: as HSP70, HSP90 etc) could complement the effects of the embryonic sHSPs.

The alternative possibilities to improve the gain of function of the embryonic sHSPs are very limited. The sexual crossing of different transgenic lines that over-express sHSPs (initially one in each line) is the only feasible possibility. However, this would be technically more complicated, and the possibilities of success are smaller, as they depend on the availability of cloned sHSPs, their appropriate election and combination, etc.

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA
CSIC
Consejo Superior de Investigaciones Científicas

Recombinant bovine pepsin and pepsinogen produced in prokaryotic and eukaryotic cells

Abstract
A Spanish Public Research Institution is offering licences recombinant bovine pepsin codified by a synthetic gene for bovine pepsinogen, expressible in various organisms. The resulting enzyme can be used for manufacturing cured cheese varieties, for the generation of bioactive peptides from various proteins or as a general purpose protease. Thus the use of animal tissues is avoided. A biotechnological company is required to licence or to collaborate to reach commercial production levels.

Description
A complete cDNA version of the bovine pepsinogen gene has been constructed, with the exception of the original signal peptide. Two different cDNA clones, coming from different bovine cDNA libraries, as well a synthetic sequences added to the 5' end of some oligonucleotides, have been used for this construction. This gene, cloned in appropriate vectors has been expressed in both Saccharomyces cerevisiæ and Escherichia coli, thus allowing the production of pepsinogen. This pepsinogen can be activated by acidification of the medium, thus rendering active pepsin. The enzyme can be purified from the growth medium, in the case of the expression in Saccharomyces cerevisiæ or by breaking the cells, for the enzyme produced in Escherichia coli. The possible applications of recombinant bovine pepsinogen and pepsin include their use for the manufacture of cured cheese varieties in combination with recombinant chymosin. This could help to the acceleration and improvement of cheese maturation as compared to those made by using recombinant chymosin exclusively. However, in contrast to the use of natural bovine pepsin coming from the abomasum of cows, the use of the recombinant enzyme is safer than the use of some animal tissues, specially in relation to the possible presence of prions.
On the other side it is known that the action of bovine pepsin on certain proteins, for example on caseins or casein macropeptide (resulting from the action of chymosin on casein), can result on the release of several peptides with biological activities potentially useful for the elaboration of functional foods. Similar to the above situation, the use of recombinant pepsin allows working with safer and pure enzymes
Recombiant bovine pepsine can also be used as a general purpose protease.

Innovative aspects & Competitive advantages
The main advantage of this invention is that it allows the production of bovine pepsinogen and pepsin in microorganisms by the use of recombinant DNA technology. In this way, any risk related to infectious diseases transmitted by animal tissues is avoided. The enzyme can be used for the manufacture of cured cheese varieties, in combination with bovine chymosin, as well as for the production of bioactive peptides or as a general purpose protease.
None of the cDNA clones available at the beginning of this work contained the complete bovine pepsinogen coding sequence. Site directed mutagenesis and synthetic sequences have been used in order to create a complete copy of the gene.
it would be possible to prepare mixtures of recombinant chymosin and pepsin; specially designed in order to accelerate maturation of some specific cured cheese varieties.
In this way the advantages of chymosin and pepsin would be simultaneously exploited by avoiding the problems associated to the use of some animal tissues for food production.
The facility of purification may constitute an advantae, not only for cheese-making, but also for the use of the enzyme for the production of bioactive peptides or as a general purpose protease.

Current stage of development
Development phase.

Intellectual Property Rights
Patent applied for but not yet granted.

Collaboration sought
Further research and/or development support / Licence agreement.

TECHNOLOGY OFFER

New Na+/H+ exchanger inhibitor peptide (PINHE) and its applications.

SUMMARY

Three spanish research public institutions have developed a peptide (PINHE) that acts as a physiological inhibitor of eukaryotic Na+/H+ exchanger. It can be used for several pathologies as in cardiovascular diseases, kidney, brain, metabolical disorders, tumoral cell proliferation, HCL hyperproduction, ischaemia, also as a contraceptive in male mammals, antibiotic, inmunomodulator agent, and salt-stress modulator in plants. It is sought license agreement and financial support for development.

TECHNOLOGY DESCRIPTION

The invention show here describes a peptide (PINHE) derived from H7 halocin, with a low molecular weight and defined by its aminoacidic sequence or other analogue, and that acts as a natural-origin physiological inhibitor of Na+/H+ exchanger (NHE) in eukaryots. PINHE is obtained and purified after fractioning (including cold acetone precipitation) of the supernatant from a culture of the haloarchaea Haloferax gibonsii alicante SPH7. This haloarchaea produces PINHE in a natural way. PINHE can also be artificially sintetisize or modify, cloned or expressed using genetic engineering techniques based in its aminoacidic sequence. PINHE is estable and active in a wide range of saline concentration, temperature, and pH. It is also trypsin resistant and pronase sensitive. It can be lyophillized and reconstituted without activity loss. The use of this NHE inhibitor peptide or its pharmacological accepted derived salts, in the development of a pharmacological composition, is another additional subject of this invention. Several uses and applications can be derived from its reversible action as a eukaryal Na+/H+ exchanger inhibitor and its derived salts. These uses are: (1) Treatment or profilasis of pathologies in mammals, human beings preferently, caused by the hyperactivity or an abnormal activity of NHE as for example: cardiovascular diseases (high blood presure, myocardium infract, arrhythmias, heart-attack, etc.), brain diseases (brain infarct, stroke, brain edema, etc.) renal diseases (renal faillure), metabolic disorders and diseases (diabetes mellitus, hyperlipidemias etc.), diseases caused by hyperproduction of chloridic acid in gastric mucosa, diseases caused by epithelial transport alterations, diseases caused by alteration of cellular volume, pathological cell –proliferative processes (cancer, tumoral growth, fibrosis, etc.), alteration caused by an excesive activity of the inmune system (inflamation, hypersensibility), (2) other pathologies or diseases that develop an ischemic process and post-ischaemia perfusion injury due to hyperactivity of NHE, among others are: ischaemia by primary or secondary vascular alterations in different tissues or organs, by post-infarct-reperfusion, by organ or tissue transplantation, by cardiatic or vascular surgery, by ischaemia in physiological situation produced by physical exercise, pregnancy, childbirth, menstruation, etc., by traumatic injuries or any other pathology that implies tissular hypoperfusion (hemorragic shock, termal shock, neurogenic shock, etc.) and in any other surgery procedure in general, (3) also as a contraceptive in male mammals, antibiotic or inmunomodulator agent and in any field as modulator in plants saline-stress.

MINISTERIO
DE CIENCIA
Y TECNOLOGÍA

CSIC
Consejo Superior de Investigaciones Científicas

Biological containment system for genetically engineered microorganisms

Abstract

A Spanish public research centre has studied the use of cytotoxic phosphotransferases (zeta proteins) to effectively kill i) the released genetically engineered microorganisms (GEMs) or to limit the function of the released GEMs in a controlled manner, ii) the GEMs that underwent an undesirable development, and iii) the GEMs that have lost their extrachromosomal element. A biotechnology company is sought for license agreement.

Description

This invention relates to a method of conditionally controlling the survivability of a recombinant microbial cell population, providing in the cells genes coding for two related zeta cytotoxic polypeptides (phosphotransferases), and their corresponding antitoxins (epsilon or epsilon-like proteins) that by binding to the cytotoxic proteins inhibit the toxic effect hereof. The citotoxic genes are operably linked to a gene whose product binds to a regulatable DNA sequence, and a regulatable regulatory DNA sequence is conditionally controlling the expression of the antitoxin polypeptides. In the present context, the expression "conditionally controlling" refers to a construction of the microbial cell which permits that the genes coding for the cytotoxic polypetides can be expressed under certain pre-determined environmental conditions whereas under other conditions, the genes are not expressed. Hence, the survivability of the microbial cells can be made dependent on certain pre-selected conditions. A regulatable regulatory DNA sequence is operably fused to the 5' region of the genes coding for the cytotoxic polypeptides. In accordance with the invention, such a regulatory sequence can be one with which one of the genes coding for the cytotoxic polypetides is naturally associated (sequence of omega binding site) or it can be a sequence with which the genes are not naturally associated. As defined herein the negatively functioning regulatory DNA sequences may indicate a DNA sequence which directly regulates the expression of the genes coding for the cytotoxic polypeptides at the level of transcription.

Factors regulating the activity of the promoter as defined above may be selected from a variety of factors. In accordance with the invention, advantageous promoter regulating factors, thus would control the expression of the genes encoding the cytotoxic polypeptides and may be determined by the physiological state, environment of the cells and/or by an inducible event promoted by an extrachromosomal element. In the present context, the term "physiological state of the cells" denotes the presence or absence of a certain chemical substance in the environment such as the fermentation medium in which the cell is propagated, that is not present in a second environment to which the cell is released, or when a factor required for the growth or survival of the cell is no longer present, or the factor is one which, when it is depleted or exhausted from an environment of the cell, has the desired effect, viz. that the gene is expressed.

Innovative aspects & Competitive advantages

The phosphotrasferases (zeta family of polypeptides) in the absence of the anti-phosphotrasferases (epsilon family of polypeptides) from Gram-positive bacteria induce the "viable but non-culturable state" of the cell, namely a long-lived phosphotrasferase toxic protein (275 to 295 amino acids long polypeptide) that interacts with a labile small anti-phosphotrasferase (85 to 95 amino acids long polypeptide) that antagonizes the toxic effect of the first (antitoxin). The labile antitoxin is degraded by a specific bacterial protease. In this programmed cell death systems, a plasmid-borne phosphotrasferase stabilizes the inheritance of both theta and sigma type replicons of all Gram-positive bacteria in which the genes are expressed. The phosphotrasferase cell killing functions defined herein may also be referred to as cytotoxic polypeptide, which phosphorilates a protein essential for bacterial duplication, and the anti-phosphotrasferase as antitoxin.

The present invention relates to the use of related zeta cytotoxins that show a loss of bacterial growth of 6 orders of magnitud each system (bacteriolytic effect) in the absence of their respective antitoxins. As defined herein they provide an active biological containment.

Current stage of development

Available for demonstration.

Intellectual Property Rights

Patent applied for but not yet granted.