VI International Conference on Polyphenols and Health

Buenos Aires, Argentina
October 16-19, 2013

VIInternational Conference on Polyphenols and Health
(ICPH-6)
Buenos Aires, Argentina
October 16-19, 2013

CONTENTS

CONGRESS VENUE
School of Law, University of Buenos Aires
Av. Figueroa Alcorta 2263
Buenos Aires, Argentina

Previous ICPHs

Vichy, France, 2003

Davis, USA, 2005

Kyoto, Japan, 2007

Harrogate, UK, 2009

Barcelona, Spain, 2011

Welcome

Welcome to Buenos Aires and to the VI International Conference on Polyphenols and Health (ICPH-6).

The series of biennial ICPHs started ten years ago in Vichy, France, and were always organized with the goal of maintaining the highest scientific level. In the path of this tradition, this year we present a program of exceptional quality, including speakers from all over the world, coming both from academia and also from the industry.

The resulted program was built considering two enriching situations. First, the sessions were selected from an open call which allowed any interested person or group to propose subjects and speakers. As a result, we have a democratically selected compendium of sessions, covering themes of the most current interest delivered by outstanding scientists. Second, the activities shared with the VIII Society for Free Radical Biology and Medicine-South American Group, which is taking place in the same venue from October 14th to 17th. This overlapping is an opportunity to foster interactions among scientists interested in the health properties of plant polyphenols.

As leaders of the organization we are very thankful to the financial contributions from several national and international public and private organizations, as well as from several companies that synergistically have made possible this ICPH-6.

Finally, we really hope that everybody can profit from the science of this conference, and additionally take advantage of the cultural possibilities that Buenos Aires offers, under the beauties of a sunny and blooming October.

Cesar G. Fraga
Chair

Patricia I. Oteiza
Co-chair

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Cesar G. Fraga, Argentina

SCIENTIFIC SECRETARY
Monica Galleano, Argentina

CO-CHAIR
Patricia I. Oteiza, USA

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Mariana Garces
Nicolas Lopez Goldenberg

ORAL AND POSTER PRESENTATIONS

ORAL SESSIONS

1 Metabolism of polyphenols in mammals
2 Metabolomics to assess dietary exposure and health effects of polyphenols
3 Molecular mechanisms of polyphenol actions
4 Polyphenols and cancer prevention
5 Polyphenols-gut interactions and health
6 Polyphenols and neurocognition
7 Polyphenols and cardiovascular health (I)
8 Polyphenols and long-term degenerative diseases
9 Polyphenols and cardiovascular health (II)
10 (Epi) genomic effects of polyphenols
11 Health effects of phenolic acids and resveratrol
12 ADME and health effects of isoflavones
13 ADME and health effects of anthocyanins
14 The road for evidence-based dietary recommendations for flavonoids: how do we get there?

POSTER TOPICS

1 Methodology for polyphenols determination
- 1.1. Plants and foods
- 1.2. Animal tissues
- 1.3. Human samples

2 ADME
- 2.1. Absorption, tissue distribution and excretion
- 2.2. Metabolism

3 Mechanisms of action
- 3.1. Antioxidants and redox regulation
- 3.2. Receptor mediated
- 3.3. Protein and membrane interactions

4 Dietary intervention
- 4.1. Cancer
- 4.2. Cardiovascular
- 4.3. Metabolic disorders
- 4.4. Intestine and Microbiota
- 4.5. Brain/Neurobiology

PROGRAM AT A GLANCE

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Session 4
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(Epi) genomic effects of polyphenols</td><td>ROOM B
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Health effects of phenolic acids and resveratrol</td></tr>
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VI International Conference on Polyphenols and Health
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WEDNESDAY 16

14:00-18:00 Registration

OPENING LECTURES

Opening Lectures in the Honor of Federico Leighton
at the School of Medicine, University of Buenos Aires,
Buenos Aires, Argentina
Sharing Activities with VIII SFRBM-SAG

18:30-18:45 Welcome address by *Cesar G Fraga, University of Buenos Aires, Buenos Aires, Argentina.*

PLENARY LECTURE 1

Chair: *Ricardo J Gelpi, University of Buenos Aires, Buenos Aires, Argentina.*

18:45-19:30
Myocardial stretch induces reactive oxygen species production: its mechanical counterpart.
Horacio Cingolani, University of La Plata, La Plata, Argentina.

PLENARY LECTURE 2

Chair:*Patricia Oteiza, University of California, Davis, USA.*

19:30-20:15
Redox biology and dietary polyphenols.
Helmut Sies, Heinrich-Heine University Dusseldorf, Dusseldorf, Germany.

20:15-21:00 WELCOME RECEPTION

THURSDAY 17

SESSION 1
Metabolism of polyphenols in mammals

Chairs: *Francene M Steinberg, University of California, Davis, USA; Peter CH Hollman, Wageningen University and RIKILT, Wageningen, the Netherlands.*

8:30-8:55
Absorption, metabolism and excretion of ^{14}C-labelled (-)-epicatechin in humans.
Alan Crozier, University of Glasgow, Glasgow, UK.

8:55-9:20
Studying the ADME of cocoa flavanols in humans: implications for future dietary intervention studies and investigations *in vitro*?
Hagen Schroeter, MARS Inc., McLean, USA.

9:20-9:45
Metabolism has a high impact on the complex puzzle of the health effects of polyphenols.
Peter CH Hollman, Wageningen University and RIKILT, Wageningen, the Netherlands.

9:45-10:10
Prenylation modulates the bioavailability of dietary flavonoids.
Junji Terao, University of Tokushima, Tokushima, Japan.

10:10-10:20
Effects of green tea catechins with or without caffeine on fat oxidation at rest and during exercise.
Silvina Lotito, Unilever R&D, Sharnbrook, UK.

10:20-10:30
Whole cell-dependent production of polyphenol conjugates using genetically engineered budding yeast
Shinichi Ikushiro, Toyama Prefectural University, Toyama, Japan.

10:30-11:00 Coffee break

SESSION 2
Metabolomics to assess dietary exposure and health effects of polyphenols

Chairs:*Claudine Manach, INRA, Clermont-Ferrand, France; Cristina Andres-Lacueva, University of Barcelona, Barcelona, Spain.*

11:00-11:25
When metabolomics helps to elucidate the contribution of polyphenols in the health effects of plant foods.
Claudine Manach, INRA, Clermont-Ferrand, France.

11:25-11:50
Non-targeted LC-MS metabolite profiling approach on human and animal studies to investigate the metabolic effect of whole grain rich diet.
Kati Hanhineva, University of Eastern Finland, Kuopio, Finland.

11:50-12:15
In vitro and *in vivo* metabolic fate of black tea polyphenols.
Doris Jacobs, Unilever R&D, Vlaardingen, the Netherlands.

12:15-12:40
Metabolomic strategies in clinical nutrition research: from polyphenol rich diet to revealing disease risk biomarkers.
Cristina Andres-Lacueva, University of Barcelona, Barcelona, Spain.

12:40-12:50
Phase II metabolites of cyanidin-3-glucoside reduce IL-6 and VCAM-1 production in CD40L-stimulated endothelial cells.
Hiren P Amin, University of East Anglia, Norwich, UK.

12:50-13:00
*nano*LC-MS/MS metabolomics of urinary biomarkers following intake of grape seed extract in a rodent model of menopause.
Helen Kim, University of Alabama at Birmingham, Birmingham, USA.

13:00-14:00 Lunch
POSTER VIEWING A
Topics 1 & 2

SESSION 3
Molecular mechanisms of polyphenol actions

Chairs, *Hernan Speisky, University of Chile, Santiago, Chile; Javier I Ottaviani, University of California, Davis, USA.*

14:00-14:25
Pleiotropic activity of the flavonoid quercetin in sensitizing B-cells isolated from chronic lymphocytic leukemia patients and leukemia cell lines to apoptosis.
Gian-Luigi Russo, National Research Council, Avellino, Italy.

14:25-14:50
Quercetin, among several flavonoids, selectively protects against non-steriodal anti-inflammatory drug-induced complex I inhibition and its mitochondrial and cellular consequences.
Hernan Speisky, University of Chile, Santiago, Chile.

14:50-15:15
Redox interactions of flavonoids at the membrane interface.
Brian Bandy, University of Saskatchewan, Saskatoon, Canada.

15:15-15:40
What have we learned about flavonoids-membrane interactions?
Sandra V Verstraeten, University of Buenos Aires, Buenos Aires, Argentina.

15:40-15:50
Polyphenols and atherosclerosis risk reduction: What is the central mechanism?
Joe A Vinson, University of Scranton, Scranton, USA.

15:50-16:00
The effects of quercetin-3-*O*-glucoside on *Caenorhabditis elegans* lifespan are related to deglycosylation and aglycone accumulation in the worm.
Celestino Santos-Buelga, University of Salamanca, Salamanca, Spain.

16:00-16:30 Coffee break

16:30-17:30 PLENARY LECTURE 3

Chair: *Junji Terao, University of Tokushima, Tokushima, Japan.*

The polyphenol metabolome and the exposome - Opportunities for nutritional epidemiology.
Augustin Scalbert, International Agency for Research on Cancer, Lyon, France.

17:30-19:00 POSTER PRESENTATION A
Topics 1 & 2

FRIDAY 18

ROOM A: SESSION 4
Polyphenols and cancer prevention

Chairs: *Bharat Aggarwal, University of Texas MD Anderson Cancer Center, Houston, USA; Norbert Latruffe, University of Burgundy, Dijon, France.*

8:30-8:55
Anti-inflammatory life style for prevention and treatment of cancer: facts and fiction.
Bharat Aggarwal, University of Texas MD Anderson Cancer Center, Houston, USA.

8:55-9:20
Polyphenols and colon cancer prevention.*Chinthalapally V Rao, PC Stephenson Oklahoma Cancer Center, Oklahoma City, USA.*

9:20-9:45
Dynamic modulation of Nrf2/Keap1 system by polyphenols.
De-Xing Hou, Kagoshima University, Kagoshima, Japan.

9:45-10:10
Silibinin and prostate cancer prevention.
Rajesh Agarwal, University of Colorado Skaggs Aurora, USA.

10:10-10:20
Anti-inflammatory, proapoptotic and anticarcinogenic properties of allicin.
Berthabetty Schwartz, The Hebrew University of Jerusalem, Rehovot, Israel.

10:20-10:30
Black soybean seed coat polyphenols prevent benzo(a)pyrene-induced DNA damage through modulating drug-metabolizing enzymes in hepatocytes.
Hitoshi Ashida, Kobe University, Kobe, Japan.

ROOM B: SESSION 5
Polyphenols-gut interactions and health

Sponsored by Herbalife

Chairs: *Francisco Tomas-Barberan, CEBAS-CSIC, Murcia, Spain; Daniele Del Rio, University of Parma, Parma, Italy.*

8:30-8:55
The nitrate: nitrate: nitric oxide and signaling pathways in the gut: the prominent role of polyhenols.
Joao Laranjinha, University of Coimbra, Coimbra, Portugal.

8:55-9:20
Identification of human gut bacteria capable of producing the anti-inflammatory and anticarcinogenic urolithins from ellagic acid and ellagitannins.
Francisco Tomas-Barberan, CEBAS-CSIC, Murcia, Spain.

9:20-9:45
New insights in the bioactivity of polyphenols: focus on colon-derived microbial metabolites.
Daniele Del Rio, University of Parma, Parma, Italy.

9:45-10:10
Does simulated gastrointestinal digestion and colonic fermentation alter the bioactivity of berry extracts?
Chris Gill, University of Ulster, Londonderry, Northern Ireland, UK.

10:10-10:20
Coffee phenolics degradation by human colonic microbiota.
Iziar Ludwig, University of Glasgow, Glasgow, UK.

10:20-10:30
The impact of consuming date fruits on the colon health and reducing cancer biomarkers.
Noura Eid, University of Reading, Reading, UK.

10:30-11:00 Coffee break

ROOM A: SESSION 6

Polyphenols and neurocognition

Sponsored by PepsiCo

Chairs:*Christine Morand, INRA, Saint Genes Champanelle, France; Jeremy P E Spencer, University of Reading, Reading, UK.*

11:00-11:25
Impact of dietary flavonoids on blood flow and vascular function - from clinical to nutrigenomic studies.
Christine Morand, INRA, Saint Genes Champanelle, France.

11:25-11:50
Flavonoids and neuro-cognitive improvements: the involvement of the vascular system as a mediator of benefits.
Jeremy PE Spencer, University of Reading, Reading, UK.

11:50-12:15
Polyphenols found in berry fruit improve cognitive function in inflammatory models of aging.
Barbara Shukitt-Hale, Tufts University, Boston, USA.

12:15-12:40
Natural product approaches to increasing stem cell function in the aged.
Paula C Bickford, University of South Florida, Tampa, USA.

12:40-12:50
Neuroprotection by quercetin: facts and pitfalls.
Federico Dajas, Institute of Biological Research Clemente Estable, Montevideo, Uruguay.

12:50-13:00
Consumption of a flavanone rich beverage is associated with acute benefits in objective and subjective measures of cognitive function.
Daniel J Lamport, University of Reading, Reading, UK.

ROOM B: SESSION 7

Polyphenols and cardiovascular health (I)

Sponsored by Mars Inc. & FLAVIOLA

Chairs:*Carl Keen, University of California, Davis, USA; Kevin Croft, University of Western Australia, Perth, Australia.*

11:00-11:25
Polyphenols, endothelial function and cardiovascular disease.
Thomas Luescher, University Hospital Zurich, Zurich, Switzerland.

11:25-11:50
The effect of dietary polyphenols on vascular function and blood pressure.
Kevin Croft, University of Western Australia, Perth, Australia.

11:50-12:15
Cardiovascular benefits of cocoa flavanols in the healthy general population: relevance to dietary recommendations.
Christian Heiss, University of Dusseldorf, Dusseldorf, Germany.

12:15-12:40
Flavanols and health: epidemiological considerations.
Gunter GC Kuhnle, University of Reading, Reading, UK.

12:40-12:50
Grape seed extract delivered in a beverage: effects on blood pressure and metabolic endpoints in individuals with pre-hypertension.
Britton Burton-Freeman, Illinois Institute of Technology, Chicago, USA.
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12:50-13:00
(-)-Epicatechin reduces blood pressure and endothelial dysfunction in genetically hypertensive rats by improvement of vascular nitric oxide bioavailability.
Iveta Bernatova, Slovak Academy of Sciences, Bratislava, Slovak Republic.

13:00-14:00 Lunch
POSTER VIEWING B
Topics 3 & 4

ROOM A: SESSION 8
Polyphenols and long-term degenerative diseases

Chairs: *Francisco Perez-Vizcaino, Complutense University of Madrid, Madrid, Spain; Fawaz G Haj, University of California, Davis, USA.*

14:00-14:25
New insights into metabolic regulation by protein-tyrosine phosphatase 1B.
Fawaz G Haj, University of California, Davis, USA.

14:25-14:50
New insights into the anti-inflammatory mechanisms of dietary flavonoids: involvement of mitochondrial dysfunction and autophagy.
Yoshichika Kawai, Nagoya University, Nagoya, Japan.

14:50-15:15
Quercetin reverses monocrotaline-induced pulmonary hypertension.
Francisco Perez-Vizcaino, Complutense University of Madrid, Madrid, Spain.

15:15-15:40
Potent and specific inhibition of VEGF signaling via slow tight-binding of polyphenols to VEGF: a novel paradigm for explaining the health benefits of dietary polyphenols.
Paul A Kroon, Institute of Food Research, Norwich, UK.

15:40-15:50
A grape extract containing resveratrol exerts a moderate immunomodulatory effect on peripheral blood mononuclear cells of patients with coronary artery disease.
Mar Larrosa, CEBAS-CSIC, Murcia, Spain.

15:50-16:00
Oleuropein and/or rutin consumption decreases the spontaneous development of osteoarthritis in Hartley guinea pig.
Maria-Noelle Horcajada, Nestle Research Center, Laussanne, Switzerland.

16:00-16:30 Coffee break

ROOM B: SESSION 9
Polyphenols and cardiovascular health (II)

Sponsored by Ocean Spray- PepsiCo-Unilever

Chairs: *Olga Pechanova, Slovak Academy of Sciences, Bratislava, Slovak Republic; Valerie Schini-Kerth, University of Strasbourg, Strasbourg; France.*

14:00-14:25
Natural product-derived polyphenols enhance the cardiovascular protective endothelial function in health and disease by targeting eNOS, oxidative stress and the angiotensin system.
Valerie Schini-Kerth, University of Strasbourg, Strasbourg, France.

14:25-14:50
Cranberries, flavonoids, and heart disease.
Joanna T Dwyer, Tufts University, Boston, USA.

14:50-15:15
The link between tea and tea flavonoids and cardiovascular health, with focus on vascular function.
Douglas Balentine, Unilever, USA.

15:15-15:40
Molecular basis of the effects of red wine polyphenols on cardiovascular diseases associated with alterations of angiogenesis.
Ramaroson Andriantsitohaina, INSERM, Angers, France.

15:40-15:50
Red wine polyphenols: beneficial effects in the cardiovascular and renal systems.
Olga Pechanova, Slovak Academy of Sciences, Bratislava, Slovak Republic.

15:50-16:00
Flavan3-ols bioactivities on metabolic syndrome–a new angle of observation.
Naomi Osakabe, Shibaura Institute of Technology, Tokyo, Japan.

16:00-16:30 Coffee break

PLENARY LECTURE 4

Chair: *Alan Crozier, University of Glasgow, Glasgow, UK.*

16:30-17:30
Wine polyphenols: define red wine quality and explain the French Paradox.
Andrew Waterhouse, University of California, Davis, USA.

17:30-19:00 **POSTER PRESENTATIONB**
Topics 3 & 4

SATURDAY 19

ROOM A: SESSION 10
(Epi) genomic effects of polyphenols

Chairs: *Dragan Milenkovic, INRA, Saint Genes Champanelle, France; Wim Vanden Berghe, University Antwerp, Wilrijk, Belgium.*

8:30-8:55
Polyphenols and cardiovascular health: gene expression relationship.
Dragan Milenkovic, INRA, Saint Genes Champanelle, France.

8:55-9:20
Cocoa flavanols modulate the transcription of genes involved in atherosclerosis pathways with complex epigenetic changes of their DNA methylation state.
Wim Vanden Berghe, University Antwerp, Wilrijk, Belgium.

9:20-9:45
Nutri-epigenetics and cancer prevention- an overview.
Clarissa Gerhauser, DKFZ, Heidelberg, Germany.

9:45-10:10
Resveratrol mimics the effect of calorie restriction on transcriptional targets of healthspan in white adipose tissue.
Jamie L Barger, LifeGen Technologies LLC, Madison, USA.

10:10-10:20
Opposite effects of didzein and genistein supplementation on whole genome gene expression profiles in adipose tissue of posmenopausal women.
Vera van der Velpen, Wageningen University, Wageningen, the Netherlands.

10:20-10:30
An integrated approach to understand the metabolic effects of a rosemary *(Rosmarinus officinalis L.)* extract rich in carnosic acid: critical differences between lean and obese phenotypes.
Maria Teresa García-Conesa, CEBAS-CSIC, Murcia, Spain.

ROOM B: SESSION 11

Sponsored by Nestlé

Health effects of phenolic acids and resveratrol

Chairs: *Silvia Berlanga Barros, University of Sao Paulo, Sao Paulo, Brazil; Lucas Actis-Goretta, Nestle Research Center, Lausanne, Switzerland.*

8:30-8:55
Standardized phenolic compounds attenuates Alzheimer's disease by preventing pathological β-amyloid misfolding and promotion of amyloid clearance in the brain.
Giulio Maria Pasinetti, Mount Sinai and JJ Peterson Kanazawa University, New York, USA.

8:55-9:20
Bioavailability and bioefficacy of chlorogenic acids in humans.
Lucas Actis-Goretta, Nestle Research Center, Lausanne, Switzerland.

9:20-9:45
Influence of resveratrol and of red wine polyphenols in the inhibition of digestive tract cancers *in vitro* and *in vivo*.*Norbert Latruffe, University of Burgundy, Dijon, France.*

9:45-10:10
Phenolic compounds as skin photoprotective agents.
Silvia Berlanga Barros, University of Sao Paulo, Sao Paulo, Brazil.

10:10-10:20
Resveratrol rescues insulin sensitivity in "obese" adipocytes.
Dario C Ramirez, University of San Luis, San Luis, Argentina.

10:20-10:30
Quantitation of novel glycine conjugates of chlorogenic acid from coffee after consumption by volunteers.
Nicolai U Kraut, University of Leeds, Leeds, UK.

10:30-11:00 Coffee break

ROOM A: SESSION 12
ADME and health effects of isoflavones

Chairs:*Adrian A Franke, University of Hawaii, Honolulu, USA; Stephen Barnes, University of Alabama at Birmingham, Birmingham, USA.*

11:00-11:25
Isoflavone and glyceollin modulation of gene expression in vivo.
Thomas C Register, Wake Forest School of Medicine, Winston-Salem, USA.

11:25-11:50
ADME of isoflavonoids after soy intake.
Adrian A Franke, University of Hawaii, Honolulu, USA.

11:50-12:15
Nanometabolomics using SWATH-MS on a 5600 TripleTOF mass spectrometer for the study of polyphenol uptake and metabolism in small animal models.
Stephen Barnes, University of Alabama at Birmingham, Birmingham, USA.

12:15-12:40
Urinary isoflavone variability in postmenopausal women during a three-year intervention study.
Mindy S Kurzer, University of Minnesota, St. Paul, USA.

12:40-12:50
Sociodemographic and lifestyle correlates of urine isoflavone concentration in the US population.
Michael E Rybak, National Center for Environmental Health, Atlanta, USA.

12:50-13:00
Daidzein metabolizing phenotypes influence metabolomic responses in individuals with cardiometabolic risk factors.
Francene M Steinberg, University of California, Davis, USA.

ROOM B: SESSION 13

ADME and health effects of anthocyanins

Sponsored by PepsiCo

Chairs: *Aedin Cassidy, University of East Anglia, Norwich, UK; Anne Marie Minihane,University of East Anglia, Norwich, UK.*

11:00-11:25
Cardiovascular effects of anthocyanins: evidence from intervention trials.
Aedin Cassidy, University of East Anglia, Norwich, UK.

11:25-11:50
Anthocyanins and disease – Strong evidence from epidemiological studies.
Eric B Rimm, Harvard School of Public Health and Harvard Medical School, Boston, USA.

11:50-12:15
Absorption and metabolism of anthocyanins: insights form ^{13}C labelling approaches.
Colin Kay, University of East Anglia, Norwich, UK.

12:15-12:40
The mechanisms of colour.
Sonia de Pascual-Teresa, CSIC, Madrid, Spain.

12:40-12:50
Strawberry-derived polyphenols and their metabolites in human plasma: Detection and identification by LC/MS Q-TOF.
Jack C Cappozzo, Illinois Institute of Technology, Chicago, USA.

12:50-13:00
Anthocyanins profile of new red-flesh apple varieties: a comparative study.
Flavio Ciesa, Research Centre for Agriculture and Forestry, Laimburg, Italy.

13:00-14:00 Lunch

SESSION 14
The road for evidence-based dietary recommendations for flavonoids: how do we get there?

Sponsored by ILSI North America

Chairs, *Douglas Balentine, Unilever, USA; John Erdman, University of Illinois, Urbana, USA.*

14:00 - 14:10
Handle with care: flavonoids are different than essential nutrients.
Douglas Balentine, Unilever, USA.

14:00 - 14:30
Reporting requirements for flavonoids research: a critical component in enhancing our understanding.
Mario Ferruzzi, Purdue University, West Lafayette, USA.

14:30 – 14:50
Flavonoids: from data to databases to adequate intakes.
Jeffrey Blumberg, Tufts University, Boston, USA.

14:50 - 15:10
Flavonoids and cardiovascular health - what progress has been made towards publich health recommendations for flavonoids?
Carl Keen, University of California, Davis, USA.

15:10 - 15:30
The evolving path towards dietary guidance for flavonoids: challenges, gaps and priorities moving forward.
John Erdman, University of Illinois, Urbana, USA.

15:30 - 16:00
Panel Discussion

16:00 - 16:30 – Awards and Closing Ceremony

- *Mars Research Awards*
  - *Outstanding Flavonoid Research Award*
  - *ICPH Flavonoid Research Award*
  - *Travel Awards*
  - *Young Investigators Awards*
- *ICPH Travel Awards*
- *OCC Young Investigators Awards*
- *Archives of Biochemistry and Biophysics-Elsevier Awards*
- *Food and Function-RSC Awards*

PLENARY LECTURES

Myocardial stretch induces reactive oxygen species production: Its mechanical counterpart.
Cingolani HE

Centro de Investigaciones Cardiovasculares. (CONICET-UNLP). Argentina.

Myocardial stretch elicits a biphasic contractile response: the Frank-Starling mechanism followed by the slow force response (SFR) or Anrep effect. Whereas the Frank-Starling mechanism is attributed to an increase in myofilament Ca^{+2} responsiveness, the SFR is due to a progressive increase in the calcium transient amplitude (CaT). However, the mechanism of this increase in CaT remains controversial.
The increase in myocardial muscle length from .96 to .98 % of the length at which the developed tension is maximal induces the SFR and an increase in ROS production (epifluorescence of H2 DCFDA). The SFR is cancelled by ROS scavenging with MPG or EUK. Interestingly, the SFR is also abolished by AT1 inhibition with Losartan and mimic by small concentrations of Angiotensin 2 (A2).It is also prevented by apocynin and by the mitochondrial K_{ATP} channel blockers 5HD and glybenclamide, suggesting a mitochondrial origin for the ROS produced. Myocardial stretch also increases the activity of the redox sensitive kinases $ERK_{1/2}$ and $p90^{RSK}$.These kinases are upstream of and phosphorylate the NHE-1, increasing the intracellular Na concentration and consequently the CaT through the Na/Ca exchanger.
In summary, myocardial stretch, through stimulating mitochondrial ROS production, triggers intracellular signals that lead to the Anrep effect.
Myocardial stretch activates de A2 AT1 receptor increasing NADPH oxidase (Nox)-dependent mitochondrial ROS production. As a consequence, the redox-sensitive kinase pathway of $ERK_{1/2}$-$p90^{RSK}$ is activated and the NHE-1 phosphorylated. NHE-1 stimulation increases intracellular Na^{+} concentration favoring Ca^{+2} entry to the cell and the SFR to stretch.

Redox biology and dietary polyphenols
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As the intricate molecular machinery of regulation by redox switches is being unravelled, the role of small molecules as messengers and as modulators becomes more clear. For example, nanomolar concentrations of H_2O_2 in cellular compartments serve in insulin signalling, and selenium as constituent of the active center of selenoproteins participates in regulation of carbohydrate metabolism. A network of sensitive thiol/disulfide switches exists in regulation of metabolism.
It is also becoming increasingly evident that polyphenols, the large class of phytochemicals, exert influence on redox regulation in direct and indirect ways. Focusing on flavonoids, and here on the subclass of dietary flavanols, recent research on their impact on redox signaling will be summarized. This pertains to the biochemistry of nitric oxide and nitrite/peroxynitrite, the role of phase II metabolism, cell-cell communication and the biological responses which have been attributed to health effects, notably in the endothelial lining of the cardiovascular system.
Thus, the major processes in health and disease, including development, proliferation, turnover, repair and aging, encompass subtle redox balances in space and time which are subject to modulation by micronutrients contained in our diet. Orchestrated research in nutrition, epigenetics and lifestyle is an exciting emerging field in redox biochemistry and physiology.

The polyphenol metabolome and the exposome – Opportunities for nutritional epidemiology
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The antioxidant hypothesis and the ability of polyphenols to scavenge free radicals has been the long-lasting paradigm substantiating much of the research conducted over the last decades. Along with this paradigm, it has been commonly assumed that most polyphenols would share similar biological properties and would have similar effects on human health. This has proved to be largely wrong. This over simplistic view contrasts with the considerable diversity of phenolic compounds in foods and of their metabolites formed in the body. They constitute together the polyphenol metabolome and a major fraction of the human exposome. Our limited capacity to deal with this complexity explains to a large extent our still limited understanding of their effects on human health at the level of populations. Modern technologies and particularly the different omics approaches contribute today to re-examine the health effects of polyphenols in a comprehensive way and in the context of the whole diet. Recent developments of the Phenol-Explorer database and of exposome-wide associations studies in large populations will be described to show how these powerful approaches can be used to measure individual exposures to the polyphenol metabolome and how they should contribute to identify its most meaningful components for human health.

Wine polyphenols: define red wine quality and explain the French Paradox
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Red wine grapes are very rich sources of multiple classes of polyphenolics, including stilbenoids, hydroxycinnamates, anthocyanins, flavonols and flavanols, both monomeric and polymeric. Each class contributes important factors to a wine's sensory qualities, and most have well described biological impactfor both the plant and the consumer. Condensed tannin and anthocyanins react to yield the key wine pigments that persist in long term aging. Their reaction with oxidation products leads to reductions in astringency and ultimately, precipitation from wine. Wine has a very long history of health proscriptions, starting with early Greek philosophers, through to protecting Australia-bound convicts on cramped sailboats from typhus. While the consumption of wine is complicated by the potential for physiological effects of excessive alcohol, individuals can generally give a good account of their wine consumption, so that it can be identified and quantified in epidemiological studies. Thus it is a very useful dietary component for analyzing the effects of phenolic intake, and early observations of wine's effects led to subsequent studies focused on dietary phenolics. Simple antioxidant properties were the initial focus for explaining Renaud's French Paradox in the modern era of wine and health. Current studies are demonstrating more complex pathways for humanhealth interactions. The central effects of these polyphenolics appears to be related to inflammatory pathways, affording benefits for cardiovascular health as well as potentially preventing some cancers. While the focus has been on flavonoids, it now appears that both simpler phenols and condensed tannin may also be important contributors to the French Paradox and finally to human health.

ORAL PRESENTATIONS

Absorption, metabolism and excretion of ^{14}C-labelled (–)-epicatechin in humans

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As data from dietary intervention studies are accumulating, interest in the nutritional- and pharmacological properties of flavanols is increasing, and so is the need for a comprehensive understanding of the absorption and metabolism of these nutrients in humans. Thus, we investigated the absorption and metabolism of (−)-epicatechin (EC) in a cohort of healthy men, following the oral intake of isotopically labelled (−)-[2-^{14}C]epicatechin. Samples of whole blood, plasma, urine and feces were collected over a period of 72h after intake of the labelled compound, and analysed by HPLC with MS^2 and on-line radioactivity detection, and NMR. Overall, total plasma radioactivity never exceeded 2% of intake, although 82% of the ingested radioactivity was recovered in urine. A further 12% of radioactivity was excreted in feces. Indicative of absorption in the small intestine, 12 glucuronidated-, sulphated- and methylated EC metabolites reached peak plasma concentrations (C_{max}) of 19-357 nM at 0.8-1.4h after ingestion (T_{max}). Plasma levels declined thereafter, with elimination half-life timesranging from 1.0-2.0h. We also identified microbiome-derived EC metabolites, in particular glucuronidated- and sulphated phenylvalerolactones and phenylvaleric acids, which reached a C_{max} of 39-272 nM 5.5-6.8h after intake, and which remained present in plasma for a prolonged period of time.

Studying the ADME of cocoa flavanols in humans: Implications for future dietary intervention studies and investigations *in vitro*?

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Diets rich in flavanols [F] and procyanidins [P] are causally related to health benefits in humans. Understanding the absorption, distribution, metabolism, and excretion [ADME] of F and P in humans is essential to meaningfully investigate the mechanisms-of-action [MOAs] that underlie the effects observed in vivo. F+P encompass 4 monomeric F stereoisomers, as well as numerous oligomeric P, F are absorbed following oral intake, and various F metabolites are systemically present in humans. However, with the exception of P dimers, P are not systemically present in humans. Moreover, studies have demonstrated that P do not break down in the human GI tract to give rise to monomeric F, thus these compounds do not directly contribute to the systemic pool of F metabolites. However, P may indirectly affect systemic biological functions. They may be viewed as components of the food matrix in which F are ingested, and as such they may affect factors, including digestibility, chemical stability, and nutrient-nutrient interactions, which modulate F absorption, and thus systemic pools of bioactive F metabolites. We also identified various phase II metabolites of 5-(3,4-dihydroxyphenyl)-γ-valerolactone, a gut microbiome-related catabolite of both, F+P, and P intake may, via γ-valerolactone metabolites, exert systemic effects in humans.

Metabolism has a high impact on the complex puzzle of the health effects of polyphenols

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Polyphenols exist as complex mixtures in plant foods. Epidemiology and human trials reduced this complexity, by studying specified foods; subclasses of polyphenols; individual polyphenols, or total antioxidant capacity (TAC). Implicitly, the following assumptions apply here: 1) a limited number of potent polyphenols exists; 2) well-defined natural potent mixtures of polyphenols exist; 3) polyphenols share a common biological activity (e.g. antioxidant activity). To find potent polyphenols (1st assumption), *in vitro* screening has been widely applied, but is of limited use because metabolism, which profoundly changes biological activity, has mostly not been considered. Anecdotal evidence for natural potent mixtures of polyphenols (2nd assumption) is widely distributed via the internet, but is very hard to verify. Additionally, ecological studies have revealed the potency of e.g. cocoa. However, new natural mixtures will probably not be discovered, as large differences in diets have disappeared (globalisation). Polyphenols share the antioxidant phenolic group which inspired researchers to measure their antioxidant activity, thus greatly reducing complexity (3rd assumption). Unfortunately, the elegant antioxidant hypothesis has to be rejected, because poor absorption and extensive metabolism annihilate any contribution to the endogenous body antioxidants. In conclusion, the above assumptions are hard to verify, and no quick answers are to be expected.

Prenylation modulates the bioavailability of dietary flavonoids

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Prenylflavonoids are widely distributed in plant foods and have attracted appreciable attention on their potential benefits for human health. We recently found that 8-prenylnaringenin (8-PN) exerted a greater preventive effect on muscle atrophy than nonprenylated naringenin (N) in a mouse model (1). The concentration of total 8-PN in the target muscle tissue was much higher than that of N, suggesting that prenylation increases the bioavailability of this flavonoid. Then, we aimed to estimate the effect of prenylation on the bioavailability of dietary quercetin (Q) (2). In Caco-2 cell, efflux of 8-prenyl quercetin (PQ) to the basolateral side was significantly lower than that of Q. After intragastric administration of PQ and Q to mice or rats, the area under the concentration-time curve for PQ in plasma and lymph was also lower than that Q. Interestingly, PQ and its *O*-methylated form accumulated much higher amount than Q and *O*-methylated Q in the liver and kidney after 18day of feeding. Despite the lower bioavailability of flavonoids in a single dose, prenylation may facilitate its accumulation in target issues in the case of continuous long-term dietary intake by modulating the fate of flavonoids from their absorption to excretion in the body. (1) Mukai et al. PLoS ONE 2012:7:e45048.(2) Mukai et al. J. Nutr. 2013:143:1-7.

Effects of green tea catechins with or without caffeine on fat oxidation at rest and during exercise

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Green tea consumption has been long claimed to increase fat oxidation (FatOx) and lead to fat loss in the long term. Green tea extract (GTE) is also popular among athletes, given the potential benefits of increased FatOx on exercise performance.

To test the hypothesis that GTE increases FatOx during exercise, we conducted two exploratory studies in healthy athletes, who consumed catechin-enriched GTE (2x600 mg catechins/day, +/- caffeine) daily for 7-28 days. On each experimental day, subjects completed a moderate intensity cycling exercise 2 h after GTE (600 mg catechins) or placebo. Whole body FatOx was measured during exercise (by indirect calorimetry), and blood samples were collected at different time points, at rest and throughout exercise, for metabolite analysis (metabolomics).

GTE did not significantly affect FatOx during exercise in either study. Instead, GTE increased FatOx markers at rest. Moreover, caffeinated (but not decaffeinated) GTE significantly increased markers of lipolysis (glycerol and free fatty acids). Taken together, our findings indicate that GTE increases FatOx and lipolysis at rest, through the combined effect of catechins and caffeine, but the value of GTE for exercise is not directly supported. Variation between reported effects may relate to the specific GTE compositions and subject characteristics.

Whole cell-dependent production of polyphenol conjugates using genetically engineered budding yeast

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Polyphenols are conjugated by UDP-glucuronosyltransferases (UGT) and sulfotransferase (SULT) isoforms and its biological effects depend, in partly, on formation of the conjugates. In order to synthesize the conjugates, we have developed several mammalian UGT or SULT expression systems in budding yeast. For the glucuronide production, mammalian UGT and UDP-glucose dehydrogenase (UGDH) were expressed in budding yeast using a multicopy plasmid vector and a genome integrated vector. For sulfate production, five human SULT isoforms were expressed in yeast. Using genetically engineered yeast containing human UGT1A1 and rat UGDH, glucuronide formation of quercetin was examined. Most glucuronide of quercetin was found in reaction medium with time-dependent production, suggesting the functional expression of both enzymes and the presence of endogenous transport system for glucuronide in yeast. Querectin with multiple glucuronidating sites was conjugated as isoform-dependent formation, suggesting that the regiospecific glucuronides of several drugs could be obtained using UGT isoforms. In the presence of glucose and ammonium sulfate, formation of sulfated quercetin was observed in whole-cell production system with SULT isoforms .These expression system of mammalian UGT or SULT in budding yeast would be a powerful tool for enzyme-assisted synthesis of various polyphenol metabolites including glucuronides and sulfates.

When metabolomics helps to elucidate the contribution of polyphenols in the health effects of plant foods

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In the field of polyphenols, research has mainly focused on the effects of a few isolated compounds or of particular subclasses such as flavanols or anthocyanins. However polyphenols are everyday consumed as complex mixtures present in plant foods, which also include phytochemicals from other families (terpenoids, alkaloids, glucosinolates…). The absorption and metabolism of polyphenols add another level of complexity, since ingested polyphenols undergo biotransformations which can show inter-individual variations. The last level of complexity is the pleiotropic effects described for polyphenols that go far beyond the direct antioxidant effect that initially fueled the interest for these compounds. Metabolomics can address these 3 levels of complexity. Examples will be given of metabolomic analyses to determine individual exposure to polyphenol and other phytochemical metabolites after consumption of target foods (coffee, blackberry) or dietary pattern (low and high fruit and vegetable diets). Variations induced in plasma and urine metabolomes by a one-month supplementation with isolated hesperidin or orange juice in a controlled intervention study will be compared.

Non-targeted LC-MS metabolite profiling approach on human and animal studies to investigate the metabolic effect of whole grain rich diet

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Growing epidemiological evidence shows that diets rich in whole grains can protect against various chronic diseases. The bran and germ are rich in micronutrients and bioactive phytochemicals which most likely contribute to the beneficial health effects, although the exact biological processes involved are not known completely. This is partly because the rich phytochemical composition of grains is not yet comprehensively characterized, and the effects of such diverse mixture on various biochemical processes are difficult to interpret with any presently used clinical biomarker analyses.

We have used the non-targeted LC-MS based metabolite profiling approach to characterize the composition of human plasma after consumption of polyphenol-rich bran to study the circulating metabolites modulated by intestinal microbiota and phase II metabolism. Additionally, this approach has been used to investigate how the endogenous metabolism is affected. The response is varied during several hours postprandially and at the fasting status. To complement the human studies, we have also done mouse trials using feeds enriched with rye bran to detect the appearance of phytochemical derivatives in various organs and whether they cause any changes on the endogenous metabolism on site. The data reveals interesting accumulation patterns in various organs, accompanied with endogenous metabolite changes, which will aid in interpreting the metabolic events related to whole grain or bran rich diets.

In vitro and in vivo metabolic fate of black tea polyphenols

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Health benefits of black tea are hypothesized to be mostly attributed to polyphenols, which are extensively metabolized by gut microbiota and phase II metabolism. A comprehensive view on the metabolites formed is still lacking.

Therefore, we aimed at identifying metabolites from black tea polyphenols using both *in vitro* and *in vivo* studies. Different state-of-the-art profiling techniques were applied to capture the vast diversity of polyphenol metabolites.

Initially, we explored the inter-individual variation in the gut microbial bioconversion of black tea extract from 10 healthy human subjects using *in vitro* fecal batch fermentations. We also designed a single- and continuous-dose experiment using a five-stage *in vitro* gastrointestinal model (TWINSHIME) to get more insight into the metabolism occurring in the different colon compartments.

Furthermore, we performed a randomized, open, placebo-controlled, cross-over study, in which twelve healthy men consumed a single bolus of black tea extract or a placebo. In total, 58 conjugated metabolites of black tea polyphenols were identified from plasma collected at several intervals over a period of 30 hours.

Taken together, these studies identified potential leads that should be further tested for their bioactivity in order to elucidate the mechanisms of actions underlying the health benefits of black tea.

Metabolomic strategies in clinical nutrition research: from polyphenols rich diet to revealing disease risk biomarkers.

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Background and objectives:An important challenge of modern nutrition is to correctly assess metabolic status of subjects after specific diets. Commonly used methods for estimating polyphenol dietary exposure, such as food frequency questionnaires (FFQs), 24-h recalls or diet diaries, are related to measurement error (both systematic and random error), causing the under- and over-reporting of dietary intake in different population groups. For this reason, it is necessary to find new nutritional biomarkers that represent food and nutrient exposure with more accuracy and precision. The Mediterranean diet shows a complex profile of bioactive-rich food sources including fruits, wine, nuts, olive oil. Methods:The PREDIMED study (www.predimed.org) is a parallel-group, single-blind, multicenter, randomized, controlled, 5 y feeding trial assessing the effects of the Mediterranean Diet supplemented with extra-virgin olive oil or nuts compared to a control Low Fat Diet .Spot urine samples and FFQ data from the participants were analyzed by LTQ(Thermo) and LCMSQtof followed by statistical analysis in a nutrimetabolomic study. Biomarker identifications were achieved combining computational- assisted identification. Results: The results reinforce the interest to combine dietary information with metabolic fingerprinting to obtain new food-related metabolome biomarkers. Conclusions: This study reveals the interest of metabolic phenotyping strategies in the identification of biomarkers related with clinical parameters. Acknowledgments:Funded by the Spanish Goverment: CICYT- AGL2009-13906-C02-01, Instituto de Salud Carlos III, CONSOLIDER INGENIO 2010 Programme, FUN-C-FOOD (CSD2007-063) and Merck Serono Foundation 2010.

Phase II metabolites of cyanidin-3-glucoside reduce IL-6 and VCAM-1 production in CD40L-stimulated endothelial cells

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Consumption of anthocyanins is associated with a reduced risk of cardiovascular disease; however, owing to their low bioavailability, their bioactivity *in vivo* is likely mediated by their phenolic metabolites. We examined the effect of cyanidin-3-glucoside (C3G) and seven of its established metabolites (at 0.1, 1 & 10μM) on the production of interleukin-6 (IL-6) and vascular cell adhesion molecule-1 (VCAM-1), by endothelial cells (HUVECs) following stimulation with CD40L-expressing Jurkat D1.1 cells. Protocatechuic acid (PCA), vanillic acid (VA), isovanillic acid (IVA), PCA-3-glucuronide, PCA-3-sulfate and PCA-4-sulfate significantly reduced CD40L-induced IL-6 production by 43% -to- 96%. In addition, PCA, VA, IVA & PCA-4-sulfate reduced CD40L-induced VCAM-1 production by 26% -to-64%.These data suggest that the metabolism of C3G increases its bioactivity in CD40L-stimulated cells and that sulphate & methyl conjugation of PCA exerted the greatest effect on its bioactivity. In conclusion, anthocyanin metabolism may modulate inflammation through reducing the production of proinflammatory mediators. Future investigations are required to establish the cellular mechanisms behind these reported activities.

NanoLC-MS/MS metabolomics of urinary biomarkers following intake of grape seed extract in a rodent model of menopause

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Differences in urinary biomolecules following ovariectomy, and the impact of dietary polyphenols (grapeseed extract [GSE]) in rodents were examined by nanoLC-tandem mass spectrometry, and the impact of dietary polyphenols (grapeseed extract [GSE]). Urines from sham-ovariectomy (OVX) and ovariectomized ((OVX) rats were analyzed, where both groups were given GSE, thus, any differences were due to the OVX. In positive mode LC-MS/MS, 5,589 peaks were resolved with unique retention times. Of these, 12% were significantly different in abundance ($p<0.05$), with 2% of this subgroup ($p<0.001$). Conclusive identification of significant peaks required both the METLIN software on the Scripps XCMS server (Tautenhahn et al., Anal. Chem, 2012) and the Human Metabolome Database (Wishart et al., Nucleic Acids Res, 2007). Experiments are ongoing to continue identification of peaks using MS/MS, as well as to populate the appropriate databases with conjugated forms of polyphenols to enable deeper analysis. In TARGETED metabolomics analysis, we determined that glucuronidated grapeseed polyphenols were increased in the urines of OVX rats, indicating that OVX increased the amount or activity of the relevant glucuronosyltransferases. This is the first metabolomics analysis of urinary biomolecules in a model of menopause, and of effects and fate of dietary polyphenols.

Pleiotropic activity of the flavonoid quercetin in sensitizing B-cells isolated from chronic lymphocytic leukemia patients and leukemia cell lines to apoptosis

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Quercetin is a flavonoid naturally present in food and beverages belonging to the large class of phytochemicals with potential anti-cancer properties. We reported that quercetin is able to sensitize several leukemia cell lines and B-cells isolated from patients affected by chronic lymphocytic leukemia (B-CLL) to rTRAIL and anti-CD95 dependent apoptosis. The molecule also potentiates the effect of fludarabine, a first-line chemotherapeutic drug against CLL. Response to therapy in CLL often depends upon the expression and activity of anti-apoptotic proteins belonging to the Bcl-2 family. Among these, Mcl-1 has been associated to apoptotic resistance in CLL. In B-CLL, quercetin lowers Mcl-1 protein level; similarly in U-937 cells, quercetin down-regulates Mcl-1 mRNA and protein levels acting on mRNA stability and protein degradation. Since CLL is characterized by overexpression of pro-survival Bcl-2 family members, treatments with their antagonists, such as ABT-737, represent a promising new therapeutic strategy. ABT-737 is a BH3 mimetic agent which binds Bcl-2, Bcl-XL and Bcl-w with high affinity, while weakly interacts with Mcl-1 and Bfl-1. The association between ABT-737 and quercetin synergistically induces apoptosis in B-cells and in five leukemic cell lines through the inhibition of PI_3K/Akt signalling pathway. Considering the low toxicity of quercetin towards normal peripheral blood cells, our experimental results are in favour of a potential use of the molecule in adjuvant chemotherapy in CLL or other types of cancer.

Quercetin, among several flavonoids, selectively protects against non-steroidal anti-inflammatory drug-induced complex I inhibition and its mitochondrial and cellular consequences

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The ability of five non-steroidal anti-inflammatory drugs (NSAIDs: indomethacin, diclofenac, piroxicam, ibuprofen and aspirin) to inhibit mitochondrial complex-I, and that of quercetin and a series of structurally-related flavonoids (kaempferol, isorhamnetin, apigenin, luteolin, galangin and epicatechin) to protect against such inhibition was addressed. All five NSAIDs (250 µM) were effective in early inhibiting complex-I activity in Caco-2 cells, and in incrementing subsequently the mitochondrial production of superoxide and in inducing a loss of ATP and cellular viability. A close relationship was found between NSAID's ability to inhibit complex-I and to increase superoxide (r^2=0.787). Quercetin (10 µM) protected against all NSAID-induced effects, showing a close relationship between its ability to prevent complex-I inhibition and superoxide increment (r^2=0.807). In contrast, none of the otherflavonoids was effective. Since all tested flavonoids share a similar superoxide-scavenging activity, the ability of quercetin to protect against the NSAID-induced mitochondrial and cellular damage is proposed to arise mainly from its selective complex-I protecting activity. Results suggest that intervening early at the level of complex-I inhibition could be effective in preventing NSAID-induced cytotoxicity.

Redox interactions of flavonoids at the membrane interface

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As amphiphiles, flavonoids will accumulate at the aqueous-lipid interface region of biomembranes, wherein they may participate in redox reactions. In *in vitro* studies using phospholipid liposomes and isolated mitochondria we compared the ability of different flavonoids to participate in biologically relevant redox reactions. Experiments with liposome-embedded cytochrome *c* showed the ability of certain flavonoids to mediate protection by ascorbate against peroxide-induced damage. Most effective were fully-conjugated flavonoid aglycones, such as quercetin and cyanidin. Other experiments with liposomes and isolated mitochondrial membranes showed the ability of quercetin to interact with both ascorbate and vitamin E, facilitating regeneration of vitamin E by ascorbate. Experiments with energized mitochondria and *in vivo* in rats injected *iv* with flavonoids, identified special mitochondriotropic and redox activities of anthocyanins, consistent with their unique charge and electronic properties among flavonoids. The bioaccumulation and redox activities of flavonoids at the interface of membranes provide one mechanism whereby relatively low physiological levels of flavonoids may exert biological effects and health benefits.

What have we learned about flavonoids-membrane interactions?

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One of the potential mechanisms involved in the positive health effects of flavonoids involves the capacity of certain polyphenols to interact with membrane lipids and proteins. Whereas some polyphenols can partially or totally penetrate lipid bilayers and reside in the hydrophobic portion of the membrane, others can only adsorb onto the membrane surface through interactions with the polar region of lipids. Among the latter are the procyanidins, large oligomers of (+)-catechin and (-)epicatechin. Procyanidins of more than two units are absorbed at the gastrointestinal tract and/or penetrate membranes. However, they can interact with membrane lipids and exert local actions both in artificial and biological membranes. In the last ten years our group has investigated whether the interaction of procyanidins with membrane lipids can protect cells from the oxidative and/or mechanical damage exerted by different physiological stresses. We have focused on the capacity of these compounds to maintain membrane physical properties and through this mechanism regulate cell physiology. In summary, the biological effects of select monomeric and polymeric flavonoids can be in part explained by their capacity to interact with membranes and modulate events initiated at the cell membrane.

Polyphenols and atherosclerosis risk reduction: What is the central mechanism?

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There are numerous epidemiological studies involving plant foods and beverages and a reduction of heart disease risk. Biochemical parameters that are affected include lipids, LDL oxidation, platelet aggregation, vascular reactivity, enzyme inhibition and gene expression. Our group has shown a benefit of diverse polyphenol sources such as chocolate, beer, tea, red wine, and citrus extract on an animal model of atherosclerosis. Polyphenols in plant foods may be changed in the GI tract and the absorbed polyphenols are metabolized to methylated, sulfated and glucuronidated forms in the intestinal wall and in the liver and other organs. Our group has recently shown that that binding of polyphenols/metabolites to proteins is a central mechanism meditating and linking the biochemical changes. We will illustrate this binding using lipoproteins and albumin as protein models and red blood cells as a cellular model.Ours and other published data indicate protein-bound polyphenols/metabolites function as plasma and vascular antioxidants. Our calculations of data from cell studies show that intracellular polyphenol/metabolites'concentration is in the µM region and thus these compounds may function as intracellular antioxidants in spite of the low plasma concentrations. We propose that binding to proteins can explain many healthful effects of polyphenols.

The effects of quercetin-3-*O*-glucoside on *Caenorhabditis elegans* lifespan are related to deglycosylation and aglycone accumulation in the worm

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Treatment with quercetin-3-*O*-glucoside (Q3Glc) or quercetin induced different effects in *C. elegans* lifespan apparently related to the accumulation of quercetin in the worm. Significant mean lifespan extension was observed in wild type worms exposed to low concentrations of Q3Glc (up to 25 µM), whereas greater concentrations (200 µM) caused a reduction in lifespan; in contrast, 200 µM of quercetin increased worm lifespan. It was found that Q3Glc was taken up by *C. elegans* in a concentration-dependent manner and deglycosylated to quercetin, which was accumulated in the worm, whilst quercetin aglycone was incorporated in lesser extent. Exposure of*klo-1* and *klo-2* mutant worms lacking β-glucosidase activity to 200 µM Q3Glc led to slight formation of quercetin and increased lifespan. These findings indicated thatthe effects of Q3Glc on worm lifespan were related to its deglycosylation and subsequent accumulation of the aglycone. They also indicated that Q3Glc was taken up by the nematode in greater extent than quercetin, suggesting that facilitated transport could be more important than passive diffusion for quercetin uptake. The obtained results support recent findings in human *ex vivo* models about the key role of tissue deglycosylation and aglycone release in the vascular effects of quercetin metabolites.

Anti-Inflammatory life style for prevention and treatment of cancer: facts and fiction

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Chronic infections, obesity, alcohol, tobacco, radiation, environmental pollutants, and high-calorie diet have been recognized as major risk factors for the most chronic diseases including cancer. All these risk factors are linked to chronic diseases through inflammation. While acute inflammation that persists for short-term mediates host defense against infections, chronic inflammation that lasts for long-term can predispose the host to various chronic illnesses, including cancer. Linkage between cancer and inflammation is indicated by numerous lines of evidence; first, transcription factors NF-κB and STAT3, two major pathways for inflammation, are activated by most cancer risk factors; second, an inflammatory condition precedes most cancers; third, NF-κB and STAT3 are constitutively active in most cancers; fourth, hypoxia and acidic conditions found in solid tumors activate NF-κB; fifth, chemotherapeutic agents and gamma-irradiation activate NF-κB and lead to chemoresistance and radioresistance; sixth, most gene products linked to inflammation, survival, proliferation, invasion, angiogenesis, and metastasis are regulated by NF-κB and STAT3; seventh, suppression of NF-κB and STAT3 inhibits the proliferation and invasion of tumors; and eighth, most chemopreventive agents mediate their effects through inhibition of NF-κB and STAT3 activation pathways. Thus suppression of these proinflammatory pathways may provide opportunities for both prevention and treatment of cancer. We will discuss the potential of various dietary agents, also called nutraceuticals derived from spices, lentils, nuts, fruits, and vegetables; and agents from traditional medicine in suppression of inflammatory pathways and their role in prevention and therapy of cancer

Polyphenols and colon cancer prevention

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The functional relationship between inflammation and cancer is not new. It is now clear that proliferation of cells alone does not cause cancer, sustained cell proliferation in an environment rich in inflammatory cells, growth factors, and activated stroma promotes neoplastic risk. These insights are further supported by regular use of antiinflammatory agents, both synthetic as well as naturally-occurring phytochemicals in preventing various cancers. As an example, colorectal tumor progression is significantly associated with inflammatory mediators. Protumorigenic inflammatory markers (NFκB, iNOS, COX-2, 5-LOX, etc.,) are well established targets for colorectal cancer prevention. While hundreds of naturally-occurring polyphenols tested both in vitro and in vivo models as effective inhibitors of inflammation and potential cancer preventive agents. However, very few polyphenols with antiinflammatory activity were proven to be effective inhibitors of colon tumorigenesis in well –established models. Our laboratory tested number of polyphenolic compounds for the prevention of colorectal cancer. These include turmeric derived curcumin, has shown to be very effective in preventing colon tumor progression by suppressing antiinflammatory pathways in a dose-depending manner. Equally, impressive observation that honey bee hives constituents such as caffeic acid phenethyl esters proven to be very effective in suppressing protumorigenic eicosanoid metabolism and inhibition of colon adenocarcinoma growth. Berries, oak bark and pomegranate polyphenolic compound such as ellagic acid tested in chemically-induced colon cancer shoed modest colon tumor inhibitory effects. However, isoflavones such as genistein had no significant colon tumor preventive effects in rat colon cancer model and shown to enhance the tumor promoting COX-2 metabolite formation. Recent evidence also suggests that strategies to improve the absorption of anticarcinogenic polyphenols such as curcumin with combination of other agents may significantly improve the cancer chemopreventive efficacies.

Dynamic modulation of Nrf2/Keap1 system bypolyphenols

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Nrf2 is a transcription factor that positively regulates the basal and inducible expression of a large battery of the genes encoding antioxidant enzymes, and Keap1is a major negative regulatorof Nrf2. Thus, Nrf2/Keap1 system is absolutely essential for the coordinate induction of these enzymes toprotect against oxidative stress.Under homeostatic conditions, Nrf2 is sequestered in cytosol and maintained at a low level through Keap-dependent ubiquitination and proteasomal degradation. Upon oxidative or electrophilic stress, a group ofKeap1 cysteines are oxidatively modified or Nrf2 is phosphorylated, resulting in the release of Nrf2 from Keap1 binding. As a consequence, Nrf2 enters into the nucleus where it heterodimerizes with small Maf or other uncertain proteins, binds to ARE element, and activates its downstream genes.The pathways based onhuge number of studies could be classified into two catalogues including Keap1-dependent and Keap1-independent pathways. Behind the antioxidant properties, polyphenols have been found to regulate the expressions of a large battery of antioxidant enzyme genes. Interestingly, these enzymes contain Nrf2/Keap1-mediated ARE in their gene promoters, suggesting that polyphenols may exert their antioxidant properties by modulating the Nrf2/Keap1 system. We chose some typical polyphenols such quercetin as well myricetin to investigate how polyphenols modulate Nrf2/Keap1 system to exert their antioxidant activities. Our data revealed that these polyphenols not only enhanced the steady-state level of Nrf2 at both transcriptional and posttranslational levels, but also reduced the steady-state level of Keap1 through 26S proteasome-independent degradation. Silencing of Nrf2 or Keap1 with their siRNA dramatically affected ARE activity induced by these polyphenols, suggesting that both Nrf2 up-regulation and Keap1 downregulation induced by polyphenols are essential for ARE-mediated activation. These findings provide an insight into the mechanisms underlying polyphenols in cytoprotection and cancer chemoprevention. Acknowledgements: This study was supported by the funds of Scholar Research from Kagoshima University, Japan, and from Hunan Agriculture University, China. References*:* 1. Tanigawa S. et al. (2007) Free Radic Biol Med, 42,1690. 2. Qin S. et al. (2012) J Ethnopharmacol, 140:131. 3. Qin S. et al. (2013) Mol Nutr Food Res, 57(3): 435. 4. Korenori Y.et al. (2013) Mol Nutr Food Res, 57: 854.

Silibinin and prostate cancer prevention

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Prostate cancer (PCA) is one of the most commonly diagnosed non-cutaneous malignancies in men. According to the American Cancer Society, in 2013, there will be an estimated 238,590 new cases and 29,720 deaths from PCA in the United States alone. In PCA patients diagnosed at an early stage of the disease with only localized growth of cancer, 5 year survival rate is near 100%. However, in PCA patients with metastatic disease, median survival is reduced to only 12-15 months. This underlies the importance of primary prevention and early intervention to halt disease progression to a metastatic stage, which is the main cause for high mortality in PCA patients. In developed countries, due to existing screening and diagnostic measures, PCA ismostly diagnosed at an early stage when the disease is localized in the prostate or surrounding tissues; however, at this stage, existing therapeutic measures, including radiotherapy, chemotherapy and anti-androgen therapy have significant side effects causing a great deal of harm along with the good. Therefore, patients diagnosed with localized indolent and asymptomatic disease are often not treated initially to avoid treatment side effects. We strongly believe that chemoprevention approaches could be extremely useful in early stage patients to prevent disease progression to an advanced metastatic stage while avoiding adverse effects. Furthermore, preventing or inhibiting the growth and the progression of PCA through non-toxic chemopreventive agents could be a useful strategyas prostate carcinogenesis involves multiple steps and usually requires more than a decade for its development into a clinically significant disease. In last 15 years, my research group has established the chemopreventive utility of a flavonoid named silibinin ($C_{25}H_{22}O_{10}$), which is isolated from the seeds of *Silybum marianum* (L.) Gaertn (Family Asteraceae). Now, there is ample literature demonstrating the cancer chemopreventive potential of silibinin against PCA in cell culture, animal models and human studies. Our detailed molecular studies have identified pleotropic mechanisms for silibinin's anti-PCA actionincluding the induction of cyclin dependent kinase inhibitors and E-cadherin as well as inhibition of epidermal growth factor receptor, integrins, and NF-κB pathways. In addition, we have identified that silibinin targets several tumor microenvironment components including endothelial cells, cancer-associated fibroblasts (CAFs), osteoclasts, to exert its cancer chemopreventive, angiopreventive and anti-metastatic efficacy against PCA. Lately, clinical trials are being conducted to examine the chemopreventive usefulness of silibinin in PCA patients. In my presentation, I will discuss mechanistic details of the chemopreventive efficacy of silibinin against PCA cells and tumor microenvironment components as well as its translational usefulness.

Anti-inflammatory, proapototic and anticarcinogenic properties of allicin

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Allicin is the biologically active component in the extract of freshly crushed garlic. The specific mechanism of allicin as anticancer agent remains unclear. We have developed a novel and simple method of isolation of an allicin isolate which was demonstrated to be stable in aqueous solution and amenable to be used *in vitro* and *in vivo*. This novel allicin isolate was demonstrated *in vitro* to induce translocation of NRF2 to the nucleus, and the activation of this transcription factor and to exert significant pro-apoptotic effects in four different human colon cancer cell lines. The anti-inflammatory effect of allicin *in vivo* was demonstrated in the dextran sulphate sodium treated mice. Two different regimes were used: a preventive or therapeutic. In both regimes (preventive and therapeutic) low doses of allicin exerted a remarkable anti-inflammatory effect. The markers tested were length of the colon, the severity of induced colitis, histopathology and TNF-α secretion from colonic tissue. All the mentioned markers were significantly downregulated following treatment with low doses of allicin both as prevention and therapeutic regimes. Our study demonstrates that the novel stable allicin extract can abolish colon cancer cells proliferation, induce apoptosis, activate cell defense mechanism by activating Nrf2 and suppress inflammation of the colon.

Black soybean seed coat polyphenols prevent B(a)P-induced DNA damage through modulating drug-metabolizing enzymes in hepatocytes

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Black soybean seed coat is a rich source of polyphenols that have been reported to have various physiological functions. The present study investigated the potential protective effects of black soybean seed coat extract (BE) on DNA damage in human hepatoma HepG2 cells and ICR mice. The results from micronucleus assay revealed that BE at concentrations up to 25 μg/mL was non-genotoxic in HepG2 cells. It is noteworthy that BE (at 4.85 μg/mL) and its main components, procyanidins (PCs) and cyanidin 3-glucoside (C3G), at 10 μM significantly reduced the genotoxicity induced by benzo[a]pyrene [B(a)P]. To obtain insights into the underlying mechanism, we investigated BE and its main components on drug-metabolizing enzyme expression in HepG2 cells and ICR mice. The results demonstrate that BE and its main components, PCs and C3G, down-regulated B(a)P-induced cytochrome P4501A1 (CYP1A1) expression by inhibiting the transformation of aryl hydrocarbon receptor. Moreover, they increased expression of detoxifying defense enzymes, glutathione S-transferases (GSTs) via increasing the binding of nuclear factor-erythroid-2-related factor 2 to antioxidant response elements. Collectively, we found that PCs and C3G, which are the main active compounds of BE, down-regulated CYP1A1 and up-regulated GST expression to protect B(a)P-induced DNA damage in hepatocytes effectively.

The nitrate: nitrite: nitric oxide and signaling pathways in the gut: the prominent role of polyphenols

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Following ingestion of nitrate and food or beverages-containing polyphenols (namely red wine), a rich chemistry occurs in the stomach in which nitric oxide ($^{\bullet}$NO), $^{\bullet}$NO-derived species and nitroso or nitrated derivatives may be formed. Most of these molecules may play an important role *in vivo*. In particular, polyphenol-catalyzed nitrite reduction to $^{\bullet}$NO may induce local vasodilation and ethanol (from wine) reacts with $^{\bullet}$NO-derived species yielding ethyl nitrite endowed with $^{\bullet}$NO-donating and vasorelaxant properties. Moreover, an operative nitrate:nitrite: $^{\bullet}$NO pathway in the stomach supports the occurrence of post-translational modifications that, by targeting specific proteins (namely pepsin) and modifying its functions may have implications for local physiology. Upon diet consumption, polyphenols can modify the extent of post-translational modifications, as observed in the case of stomach wall protein S- and N-nitrosation. On the other hand, and beyond the redox interactions with nitrite, polyphenols perturb signaling pathways in intestinal cells, preventing, for instance, local inflammatory processes, involving iNOS and COX-2. Data supporting these concepts will be presented and discussed, including the proof of concept for the ethyl nitrite and NO production from dietary nitrate in the presence of polyphenols in humans. NO was selectively measured by chemiluminescence and ethyl nitrite by mass spectrometry.

Thus, both, the redox participation of polyphenols in the nitrate:nitrite:NO pathway as well as its direct modulation of cellular inflammatory cascades reveal new pathways for the biological effects of dietary polyphenols with impact on human physiology and pathology, namely cardiovascular and gastrointestinal systems. Novel therapeutic strategies are therefore expected to follow the elucidation of the mechanisms supporting polyphenol biology in the gut.

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Identification of human gut bacteria capable of producing the anti-inflammatory and anticarcinogenic urolithins from ellagic acid and ellagitannins

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Urolithins are dibenzypyranone metabolites that exert anti-inflammatory activity *in vivo* and anticarcinogenic activity *in vitro* and are produced by the gut microbiota from the dietary polyphenols ellagic acid (EA) and ellagitannins. These urolithins have been suggested as the responsible for the health effects observed after ellagitannin-rich food intake (1-2). These foods include pomegranates, strawberries, raspberries, blackberries, camu-camu, walnuts and oak-aged wines among others. However, the identification of the gut bacteria involved in this process remains unknown. We have recently isolated a new bacterium, capable of metabolizing ellagic acid to urolithins, isolated from the faeces of a healthy urolithin-producing woman and characterized by determining phenotypic, biochemical and molecular features of the isolate. The strain was related to *Gordonibacter pamelaeae* DSM 19378T the type and only strain of *Gordonibacter* genus with about 97% 16S rRNA gene sequence similarity; they were obligatory anaerobic, non-spore-forming, Gram-positive, short-rod/coccobacilli and metabolized only a small number of carbon sources. L-fucose, D-fructose, D-turanose, D-galacturonic acid and α-ketobutyric acid were metabolized by the isolate while *G. pamelaeae* was negative for this metabolism. The new species *Gordonibacter urolithinfaciens*, sp. nov. was described, with strain CEBAS1/15PT as the type and only strain of *Gordonibacter urolithinfaciens*. Both *Gordonibacter* species were able to convert the polyphenol ellagic acid to various urolithins under anaerobic conditions. *G. urolithinfaciens* and *G. pamelaeae* grew similarly in presence and absence of ellagic acid at 30 µM concentration. Ellagic acid catabolism and urolithin formation occurred during the stationary phase of the growth of the bacterial. The HPLC-MS analyses showed that the sequential production of pentahydroxy-urolithin (urolithin M-5), tetrahydroxy-urolithin (urolithin M-6) and urolithin C. Other closely related species belonging to Coriobacteriaceae family including *Paraeggerthella hongkongensis* HKU10T, *Eggerthella sinensis* HKU14T and *Eggerthella lenta* DSM 2243T were not able to produce urolithins. This is the first report of two single bacteria capable of converting ellagic acid to urolithin metabolites. The identification of bacteria responsible for the urolithin production is a relevant objective due to the implication in health and in the potential development of functional foods. 1) Larrosa M et al. Mol Aspects Med. 2010, 31, 513-539.

New insights in the bioactivity of polyphenols: focus on colon-derived microbial metabolites

Del Rio D

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Over the last decade, considerable attention has been paid toward understanding the metabolic fate of dietary polyphenols within the human organism. Most phenolics, especially high molecular weight polymers, are poorly absorbed in the small intestine, and studies with ileostomists indicate that substantial amounts reach the colon where they are subjected to the wide array of enzymes produced by the anaerobic microbiota. This can result in the ring fission accompanied by reduction, decarboxylation, demethylation, and dehydroxylation reactions. These modifications generate several low molecular weight metabolites that appear to be efficiently absorbed into the circulatory system potentially undergoing subsequent hepatic phase II conjugations prior to excretion.

This lecture will describe recent research with innovative model systems and on-going experiments investigating the potential involvement of these colonic/hepatic metabolites in the preventative effects exerted *in vivo* by dietary polyphenols. These effects may be local, exerted at intestinal level, but also systemic, and the mechanisms through which these metabolites act may be different from what was previously envisaged. The effects and possible modes of action of specific phenolic metabolites in *in vitro* models of diabetic cardiomyopathy, Alzheimer's, Parkinson's, lateral amyotrophic sclerosis and atherosclerosis will be presented.

Does simulated gastrointestinal digestion and colonic fermentation alter the bioactivity of berry extracts

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Given the inverse correlation of fruit and vegetable consumption with CRC incidence, it is unsurprising that bioactive phytochemicals within berries are of interest with regard to their anticancer properties.Post-ingestion, berry phytochemicals undergo alterations in their structure and possibly their function as a result of events occurring in the human digestive tract. Substantial amounts of polyphenolic compounds in berries are not absorbed into circulation from the small intestine but pass into the large intestine where, it is reasonable to infer, they come into direct contact with the colonic epithelium.The microbiota within the colon plays a key role in the fate of phytochemicals that are not absorbed in the small intestine. Once in the colon, berry polyphenolics are subject to the fermentative action of the microbiota giving rise to a diversity of phenolic acids. These components may exert anti-cancer activity through direct interaction with the colonic epithelium. Thus, we simulated the impact of the colonic microbiota on berry phytochemicals by subjecting in vitro digested berry extracts to fermentation with human faecal inocula and consequently determined that the breakdown products retained their ability to modulate cellular processes associated with colon cancer, including DNA damage.

Coffee phenolics degradation by human colonic microbiota

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Coffee, one of the most consumed beverages, is a rich source of chlorogenic acids (CGA). The health impact of dietary (poly)phenols, such as CGA, depend on their bioavailability. As they pass along the gastrointestinal tract CGA are extensively metabolised and it is their metabolites rather than the parent compounds which predominate in the circulatory system. Previous studies have shown that ca.70% of the CGA ingested after coffee consumption reaches the colon in their intact form, where they will be subjected to microbial metabolism. Thus, the aim of this work was to study the colonic catabolism of CGA present in espresso coffee using an *in vitro* fermentation model with human faecal samples. HPLC-PDA-MS and GC-MS analyses were used to monitor CGA breakdown and identify and quantify the microbial catabolites. All CGAs were rapidly degraded, and up to 11 catabolites were detected during the 6h of faecal fermentation showing that the CGA were extensively catabolized by the microorganisms present in the human faecal samples. The rate and extent of the degradation showed a clear influence of the composition of the gut microbiota of individual volunteers. Results allowed us to propose potential catabolic steps occurring during microbial conversion of CGA in the colon.

The impact of consuming date fruits on the colon health and reducing cancer biomarkers

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Research indicates that fruit, vegetable and the insoluble fibre and polyphenols they contain, may have an impact on the gut microbiota to enhance colon health. We first carried out the chemical analysis of different date varieties, showing that Ajwa dates (AJD) were the richest in both polyphenols (phenolics acids, flavonoids glycosides and anthocyanins) (22.87mg/100g) and insoluble fibres (6.1g/100g). Secondly we carried *in vitro* experiment, utilizing pH-controlled batch cultures, using digested date fruits DDE (1.5g) and date polyphenols extract (150mg/ml). DDE induced a significant increase in bifidobacteria, bacteroides and total bacteria along with increases in SCFAs (Acetate, propionate and butyrate). Whereas, the impact of DPE (150mg/ml) was less profound, where only bifidobacteria growth was enhanced. DPE & DDE were also shown to exert anti-proliferative effects on Caco-2 cell growth, with DDE (0.2mg/ml) inducing significantly more inhibition than DPE (0.2mg/ml). Lastly, we conducted a randomized cross-over human study on twenty-two volunteers, consuming 50g AJD dates (7peices) or 32g of a placebo for 21d. Date consumption significantly reduced the DNA damage in colon cells ($p<0.05$) and ammonia concentrations ($p<0.05$), following AJD consumption in relative to the control. Date consumption also led to a significant increase in stool frequency and flatulence when compared to the control, with limited alterations in the growth of intestinal microbiota. Our findings show that date consumption may enhance colon health, without inducing large changes in bacterial growth.

Impact of dietary flavonoids on blood flow and vascular function – from clinical to nutrigenomic studies
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Prospective cohort studies suggest that higher intakes of flavonoid-rich foods may be protective against cardiovascular diseases (CVD). A number of short-term, small-scale human intervention studies tested the effect of flavonoid-rich foods on well-characterized clinical markers of CVD, namely cholesterolemia, blood pressure, arterial stiffness, platelet and endothelial functions. They suggest that relatively high intakes of some flavonoid-rich foods and beverages may improve these markers. These vascular effects may also play a role in determining brain blood flow and changes in cognitive performance.However, it is not yet known whether flavonoids themselves are protective and whether these short-term improvements will result in long-term reduction in CVD risk. To determine if flavonoids are the causal agents in mediating vascular benefits, some key limitations with regard to the experimental design of most of the trials performed with flavonoid-rich foods must be addressed. Furthermore, to progress in the understanding of the molecular mechanisms underlying the vascular protective effects of the flavonoid compounds, their multifaceted bioactivity should be considered using holistic nutrigenomics approach and cell experiments using plasma metabolites at physiologically-relevant concentrations. This integratedapproachwill be illustrated with the resultsfrom our clinicalandmechanistic studies focused on the vascularеffects ofthe citrusflavanones.

Flavonoids and neuro-cognitive improvements: The involvement of the vascular system as a mediator of benefits
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Evidence suggests that dietary phytochemicals, in particular flavonoids, may exert beneficial effects on the central nervous system by protecting neurons against stress-induced injury, by suppressing neuroinflammation and by improving cognitive function. Historically, they were believed to do this via an ability to express classical antioxidant activity in the brain. However, their poor brain bioavailability and extensive metabolism means that this is unlikely. Instead, their actions on the brain appear to be mediated by effects on both the peripheral and cerebro-vascular system that lead to changes in improve blood flow to the brain capable of inducing angiogenesis, neurogenesis and changes in neuronal morphology. Such vascular effects may lead to the activation of critical protein and lipid kinase signalling cascades in the brain, leading to a suppression of neuroinflammation and the promotion of synaptic plasticity. This paper will focus on the acute effects of flavonoids and flavonoid-rich foods on human executive function (attention, sustained attentiveness and task responsiveness) and how such effects may be mediated by changes in peripheral and cerebrovascular blood flow, measured using flow-mediated dilatation and fMRI. Through such a mechanism, the consumption of flavonoid-rich foods throughout life holds the potential to limit neurodegeneration and to prevent or reverse age-dependent loses in cognitive performance. In addition, flavonoids may represent important precursor molecules in the quest to develop a new generation of brain enhancing drugs.

Polyphenols found in berry fruit improve cognitive function in inflammatory models of aging
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Research has demonstrated that, in both humans and animals, cognitive functioning decreases with age, to include deficits in processing speed, executive function, memory, and spatial learning. The cause of these functional declines is not entirely understood; however, neuronal losses and the associated changes in the activity of neurotransmitters, secondary messengers, and their receptors may be caused by long term increases in and susceptibility to oxidative stress and inflammation. Therefore, one approach to improving neuronal functioning might be to alter the neuronal environment to reduce the impact of oxidative and inflammatory stressors. Research conducted in our laboratory has shown that consumption of fruits and vegetables high in polyphenolics can prevent and even reverse age-related cognitive deficits. The polyphenolic compounds found in these foods may exert their beneficial effects indirectly, through their ability to lower oxidative stress and inflammation, or directly, by altering neuronal structure and signaling involved in neuronal communication. Therefore, dietary interventions with polyphenolic-rich foods may be one strategy to forestall or even reverse age-related neuronal deficits.

Natural product approaches to increasing stem cell function in the aged
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Aging is a complex process that involves cellular senescence, a gradual loss of tissue homeostasis and declines in organ function. Denham Harman first proposed the free radical theory of aging in 1956. This theory now includes the mitochondrial theory of aging. Mitochondria, as the power source of the cell, have important control over metabolic function, which has been a major focus of aging research in recent years. Disruption of mitochondrial energy metabolism is linked to diverse aspects of aging including brain aging and Alzheimer's disease.Emerging evidence suggests that stem cell niches within the body may be particularly sensitive to aging and may, in part, be an underlying cause of aging as stem cells are important in maintaining and repairing tissue and organ function. We show that dietary approaches are capable of increasing stem cell function in aged animals. One of high interest is NT-020 a proprietary blend of blueberry, green tea, carnosine and Vitamin D3. Our previous studies have shown that this intervention increases neurogenesis in the aged rat brain and improves cognitive behaviors. We now further show that some of these changes can lead to increased health spanand the effect may be mediated by increased mitochondrial energy metabolism in the stem cell niche. We will discuss a recent human study in normal elderly. In this highly functioning elderly population we observed a significant improvement in two timed tests. This reflects an improvement in processing speed, a measure sensitive to aging and thought to underlie performance on other higher order cognitive test

Neuroprotection by quercetin: facts and pitfalls
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A great diversity of pharmacological effects has been described for Quercetin, a ubiquitous flavonoid in nature. Thus, numerous *in vitro* and *in vivo* experimental studies have provided evidence for a neuroprotective capacity of quercetin. Nevertheless, in neurons in culture, the protective concentration range is narrow (25-50 µM) and higher concentrations are toxic. In acute ischemia experimental models, the protective effects are seen in a time range administration after ischemia not longer than one hour. In neurodegenerative experiments such as those of experimental Parkinson s Disease, while liposomal quercetin protected Substantia Nigra (SN) neurons when administered 1h or 24 h after the toxic insult, no protection – as higher number of surviving neurons -is observed after repeated administrations (24 to 72 h after the insult) and there is even an increase of neuronal death in SN. All these examples are for acute utilization. Oral chronic administration appears to be safer although no firm and systematic evidence of quercetin presence in the brain has been provided and effects of metabolites have been proposed. Although neuroprotective activity of quercetin cannot be neglected, the narrow concentration range for positive effects *in vitro* and the short therapeutic window *in vivo* are aspects that should be taken into account for future clinical studies.

Consumption of a flavanone rich beverage is associated with acute benefits in objective and subjective measures of cognitive function
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A growing body of evidence suggests acute cognitive benefits are observed following consumption of flavonoids in the form of grape juice and blueberries. However, no studies have examined whether consumption of flavanone-rich juices are associated with cognitive benefits. We present data from two separate studies with crossover designs. Study one examined the effect of a flavanone-rich breakfast juice and a lemon barley squash control drink on cognitive performance, blood pressure and cerebral blood flow with 24 adults aged 18-30 years. Cognitive processing speed and blood pressure were significantly improved 2 hours following the flavanone rich juice compared to the control. There was a non-significant trend of increased cerebral blood flow following flavanone rich juice consumption. Study two examined the effect of a high flavanone orange puree with an energy matched water based control on cognitive performance and mood at 2hrs and 6hrs in 22 healthy adults aged 30-65. The orange puree was associated with significantly increased subjective alertness and processing speed at 2hrs and 6hrs and significantly improved attention at 2 hours. These data show that a single acute dose of flavanone-rich beverages can produce detectable changes in cerebral blood flow, blood pressure and cognitive function in young healthy adults.

Polyphenols, endothelial function and cardiovascular disease

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Polyphenols are found in many natural products, among them cocoa, and have many biological properties, i.e. anti-oxidant effects and activate specific intracellular pathways such as endothelial nitric oxide synthase (eNOS). In cells in culture and in isolated blood vessels, intact organisms and the human cardiovascular system polyphenols stimulate the expression and activity of eNOS and the release of nitric oxide (NO) and improve endothelial function. Healthy smokers with endothelial dysfunction, dark chocolate containing polyphenols, but not white chocolate, improves endothelial function. Furthermore, dark chocolate also improves the metabolic syndrome and *ameliorates* insulin resistance. We showed that similar effects can be seen in the forearm circulation of patients with heart failure. Finally, such effects also occur in the coronary circulation of patients after heart transplantation in whom oxidative stress is increased; dark chocolate improves endothelial function as assessed by the cold pressor test.
Among the polyphenols epicatechin is the most important. After ingestion of cocoa plants or chocolate, respectively, plasma epicatechin levels are markedly increased. Preliminary in vitro and in vivo studies strongly suggest that there is not only a temporal relation between the increase in epicatechin levels and improvement of endothelial function, but indeed a direct causal relationship in that this polyphenol directly stimulates eNOS.
These vascular effects of polyphenols and epicatechin are clinically relevant because endothelial dysfunction is a precursor of atherosclerosis. Epidemiological studies suggest that chocolate consumption is inversely related to myocardial infarction and death. Several studies suggest that cocoa consumption decreases blood pressure by 3 mmHg. However, chocolate also may have untoward effects such as high calorie intakeand a considerable fat and sugar countent. Thus, attempts have been made to provide epicatechin together with other polyphenols as a food additive to improve beneficial cardiovascular health.

The effect of dietary polyphenols on vascular function and blood pressure

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Population studies suggest cardiovascular health benefits of consuming fruits and vegetables which may be due in part to their high content of polyphenolic compounds. While many plant derived polyphenols, such as flavonoids, display potent antioxidant activity *in vitro*, the *in vivo* effects of these compounds is more likely the result of specific actions on key enzymes in the vasculature rather than total antioxidant effects.
Certain dietary flavonoids such as quercetin and epicatechin can acutely augment nitric oxide production and reduce endothelin-1 in human volunteers. Quercetin and epicatechin derived from apples can also increase nitric oxide production and improve endothelial function in healthy subjects. We have also shown in a long term (6 months) randomised controlled trial that black tea polyphenols can significantly reduce blood pressure in subjects with high-normal BP.
Incorporation of quercetin into the diet of the apoE knockout mice can reduce lesion formation significantly by a combination of anti-inflammatroy, antioxidant and improvements in vascular function over 26 weeks. Quercetin also increased the expression of heme oxygenase-1 (HO-1) in aortic lesions. In mouse aortic rings, quercetin protected vessels from oxidant (HOCl)–induced endothelial dysfunction *ex vivo.* HOCl is a physiological oxidant generated in atherosclerotic lesions. In addition, quercetin and its metabolites are able to improve vascular function by inducing endothelial nitric oxide synthase (eNOS) by phosphorylation of AMPK in human aortic endothelial cells. We conclude that certain dietary polyphenols may be beneficial for cardiovascular health and that both isolated compounds and those incorporated into food or beverages are also effective.

Cardiovascular benefits of cocoa flavanols in the healthy general population: relevance to dietary recommendations

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Accumulating evidence from dietary intervention studies suggest that diets rich in flavanols are causally related to cardiovascular health benefits in humans. In order to consider flavanols as potential candidates for inclusion in future dietary recommendations, it is necessary to assess the efficacy, relevance, and applicability to the general public. Therefore, the pan-European research consortium FLAVIOLA aimed at investigating the cardiovascular effects of cocoa flavanol (CF) intake in various cohorts representing a broader segment of the healthy population. In particular, we investigated the efficacy of CF from a gender- and age perspective in healthy people. Our data demonstrate that CF intake exerts beneficial effects on various accredited cardiovascular endpoints in healthy men and women across all the ages and groups investigated. Taken together, we provide direct evidence for population-based health benefits, thus supporting a potential role for cocoa flavanols in primary cardiovascular disease prevention and future dietary guidelines.

Flavanols and health: epidemiological considerations

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Dietary intervention studies have shown a vasculoprotective effect of flavan-3-ols, and data from anthropological studies suggest that a similar effect can be observed at population level. However, data from epidemiological studies conducted previously are more ambiguous and have failed to establish a clear association between flavan-3-ol intake and the risk of cardiovascular disease. Possible explanations for this are the lower habitual intake of flavan-3-ols in the study populations and methodological limitations, in particular the use of food-frequency questionnaires (FFQ) as dietary assessment instruments.
We have investigated habitual flavan-3-ol intake in fourteen countries of the European Union and in more than 25,000 participants of the Norfolk cohort of the *European Prospective Investigation into Cancer and Nutrition* (EPIC) for whom 7-day food diaries were available. The average intake ranged from 25mg/d to 200mg/d in the general public, and 235mg/d in EPIC Norfolk. The main dietary sources of flavanolswere tea and pome fruits. The intake of flavan-3-ols was therefore higher than in observational epidemiological studies conducted previously.
Using this data, we can compare habitual intake with those used in dietary intervention studies. This will allow us to investigate if and how the findings from these studies can be translated into dietary recommendations.

Grape seed extract delivered in a beverage: effects on blood pressure and metabolic endpoints in individuals with pre-hypertension

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Hypertension (HTN) is a major risk factor for heart disease and stroke. Extracts derived from grape seeds have been shown to reduce blood pressure (BP) when delivered in capsules. The aim of this study was to investigate the effect of grape seed extract (GSE) delivered in a beverage on BP in individuals with pre-HTN. Secondary endpoints included markers of endothelial function, oxidative stress and metabolic indices. BP and secondary endpoints of middle-aged men and women were measured in a randomized, double-blind, 10-week parallel design trial before and after 6 weeks GSE (n=18, 300 mg/d) or control (n=18, 0 mg/d GSE) beverages and again after 4-week follow-up. Significant reductions in systolic BP (GSE -7.4±2.3 vs control 1.6±1.9 mmHg, p=0.003) and diastolic BP (GSE -3.8±1.6 vs control 0.7±1.3 mmHg, p=0.03) were observed after 6 weeks intervention. The effectiveness of GSE was improved in subjects with higher starting BP ($p<0.05$). Changes in insulin were marginally significant (p=0.07). The present study supports the use of GSE as a functional ingredient in a low calorie beverage to reduce the risk of HTN and may aid in glucose control through improved insulin sensitivity; the latter requires further investigation. Supported by Polyphenolics Inc., Madera, CA.

(-)-Epicatechin reduces blood pressure and endothelial dysfunction in genetically hypertensive rats by improvement of vascular nitric oxide bioavailability

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This study investigated the effect of dietary (-)-epicatechin (EPI) on blood pressure and vascular function in spontaneously hypertensive rats (SHR). Adult SHR males were divided into control group and EPI-treated group, n=6 in each. EPI was administered in solid diet for 6 daysin the daily dose approximately 250 mg/kg.
Six-day EPI-treatment reduced blood pressure and elevated aortic NO synthase activity by about 13% and 173%, respectively, as compared to control ($p<0.05$). Maximal acetylcholine (ACh)-induced endothelium-dependent relaxation of the femoral artery (determined in vitro by myograph in isometric conditions) was about 58% of serotonin-induced pre-constriction and EPI improved it to 84% ($p<0.05$). This improvement was associated with a significant elevation of NO-dependent component of ACh-induced relaxation by about 48% vs. control. SNP-induced endothelium-independent relaxation was unaffected by EPI. Additionally, EPI-treatment partially reduced noradrenalin-induced constriction.
In conclusion, dietary EPI administration reduced blood pressure and improved vasorelaxation in genetically hypertensive rats by the increase of vascular NO bioavailability. These results give a rationale to prospect nutritional or pharmacological approaches by using (-)-epicatechin to treat hypertension in humans.This study was partially supported by the grants Nos. APVV-0523-10, VEGA 2/0084/10 and CONICET.

New insights into metabolic regulation by protein-tyrosine phosphatase 1B

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Protein-tyrosine phosphatase 1B (PTP1B) is a physiological regulator of glucose homeostasis and adiposity and is a drug target for the treatment of obesity and diabetes. Herein, we identify pyruvate kinase M2 (PKM2) as a novel PTP1B substrate in adipocytes. PTP1B deficiency leads to increased PKM2 total tyrosine and Tyr105 phosphorylation in cultured adipocytes and in vivo. Substrate-trapping and mutagenesis studies identified PKM2 Tyr105 and Tyr148 as key sites that mediate PTP1B-PKM2 interaction. Moreover, in vitro analyses illustrate a direct effect of Tyr105 phosphorylation on PKM2 activity in adipocytes. Importantly, PKM2 Tyr105 phosphorylation is nutritionally regulated, decreasing in adipose tissue depots after high fat feeding. Furthermore, decreased PKM2 Tyr105 phosphorylation correlates with the development of glucose intolerance and insulin resistance in rodents, non-human primates and humans. Together, our findings identify PKM2 as a novel substrate of PTP1B, and provide new insights into the role of adipose PKM2 in metabolic regulation.

New insights into the anti-inflammatory mechanisms of dietary flavonoids: involvement of mitochondrial dysfunction and autophagy

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Dietary flavonoids have long been recognized to protect blood vessels from atherogenic inflammation by yet unknown mechanisms. We have previously discovered the specific localization of quercetin-3-O-glucuronide (Q3GA), a phase II metabolite of quercetin, in macrophage cells in the human atherosclerotic lesions, but the biological significance is poorly understood. We have now demonstrated the molecular basis of the interaction between quercetin glucuronides and macrophages, leading to deconjugation of the glucuronides into the active aglycone. It is of interest that the deconjugation of Q3GA catalyzed by macrophage-derived β-glucuronidase was significantly enhanced upon mitochondrial dysfunction in macrophages, characterized using antimycin-A (a mitochondrial inhibitor) and siRNA-knockdown of Atg7 (an essential gene for autophagy). The deconjugated aglycone, quercetin, acts as an anti-inflammatory agent in the LPS-stimulated macrophages by inhibiting the JNK activation, whereas Q3GA acts only in the presence of extracellular β-glucuronidase activity. Furthermore, we found that quercetin and related analogs significantly induced the autophagic degradation, presumably inhibiting the inflammatory responses in macrophages. These results showed that mitochondrial dysfunction and autophagy in macrophages could be the key players in the anti-inflammatory actions of dietary flavonoids. This study may provide new insights into the anti-inflammatory/anti-atherosclerotic mechanisms of dietary flavonoids within the inflammation sites.

Quercetin reverses monocrotaline-induced pulmonary hypertension

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Quercetin is a dietary flavonoid which exerts vasodilator, antiaggregant and antiproliferative effects and reduces blood pressure, oxidative status and end-organ damage in humans and animal models of systemic hypertension .We hypothesized that oral quercetin treatment might be protective in a rat model of pulmonary arterial hypertension, a rare but life-threatening disease.Methods and Results: Three weeks after injection of monocrotaline, administration of quercetin for 10 days significantly reduced mortality. In surviving animals, quercetin decreased pulmonary arterial pressure, right ventricular hypertrophy and muscularization of small pulmonary arteries. Other biomarkers of pulmonary arterial hypertension such as the reduced expression of BMPR2 or Kv1.5, increased survivin expression or Erk1/2 phosphorylation, endothelial dysfunction and hyperresponsiveness to 5-HT were unaffected by quercetin. In vitro, quercetin induced pulmonary artery vasodilator effects which were more potent under hypoxic conditions and inhibited pulmonary artery smooth muscle cell proliferation and induced apoptosis. In conclusion,quercetin reduced the mortality and the hemodynamic and vascular and cardiac anatomical changes induced by monocrotaline. The protective effect of quercetin against pulmonary hypertension may involve a direct vasodilator effect and inhibition of proliferative pathways.

Potent and specific inhibition of VEGF signalling via slow tight-binding of polyphenols to VEGF: a novel paradigm for explaining the health benefits of dietary polyphenols

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Background: In a previous study, we provided evidence that the predominant effect of a low dose of an apple procyanidin extract on gene expression in cultured human vascular endothelial cells was inhibition of angiogenic functions and associated signalling pathways [1].Since vascular endothelial growth factor (VEGF) is the dominant driver of vascular angiogenesis, and VEGF signalling plays crucial roles in diseases for which flavonoids have been shown to be protective, we further investigated the interactions of polyphenols with VEGF signalling. Methods and Results: We present data to show that the polyphenols such as EGCg (green tea) andprocyanidin oligomers (cocoa, apples) potently inhibit VEGFR2 signalling in HUVEC by directly binding to VEGF-A and preventing ligand-receptor binding. The most potent polyphenols inhibited VEGF-induced VEGFR2 activation by 50% at concentrations <100 nM, but many polyphenols including (-)-epicatechin were completely ineffective. The polyphenol-VEGFA complexes were formed as a result of slow, tight-binding reactions from which VEGF activity could not be recovered by dialysis.Remarkably, even though VEGFR-2 signalling was completely inhibited at 1 μM concentrations of EGCg and procyanidins, endothelial NO synthase (eNOS) was shown to still be activated via the PI3K/pAKT signalling pathway. Conclusion: These datashow that polyphenols can potently inhibit VEGFR-2 signalling while retaining the pro-endothelial functions that occur downstream of PI3K/Akt. Future directions: Excessive concentrations of VEGF-A drive angiogenesis and cause complications such as age-related macular degeneration and increased growth of tumours and atherosclerotic plaques. Current anti-VEGF-based therapies may be effective, but are associated with hypertension and other complications because the pro-endothelial and vasodilatory functions of VEGF receptor-2 signalling are compromised. There is tremendous scope to develop polyphenol-rich foods that effectively dampen hyper-VEGF-mediated pathogenic angiogenesis but do not negatively affect endothelial function. This research was funded by the Biotechnology and Biological Sciences Research Council UK (BBSRC: CASE studentship to CWAM, and an Institute Strategic Programme Grant 'Food and Health'BB/J004545/1 to IFR), the European Union 7th Framework Programme (BACCHUS-312090 and VegFenol-274885), and Coressence Ltd (UK). 1 Garcia-Conesa MT et al. (2009). *MolecNutr Food Res***53**, 266-276.

A grape extract containing resveratrol exerts a moderate immunomodulatory effect on peripheral blood mononuclear cells of patients with coronary artery disease

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Resveratrol (RES) exerts anti-inflammatory effects *in vitro* and in animal models but human trials evidencing these effects are limited and the molecular mechanisms triggered *in vivo* following the intake of RES are not yet understood. We have investigated the molecular changes in peripheral blood mononuclear cells (PBMCs) associated with the one-year daily intake of a RES enriched (8 mg) grape extract (GE-RES) in hypertensive male patients with type 2 diabetes mellitus (T2DM). We analyzed expression changes in genes and microRNAs (*miRs*) involved in the inflammatory response modulated by the consumption of the GE-RES in comparison to a placebo and GE lacking RES. We also analyzed several serobiochemical variables, inflammatory and fibrinolytic markers. Supplementation with GE or GE-RES did not affect body weight, blood pressure, glucose, HbAlc or lipids, beyond the values regulated by gold standard medication in these patients. We did not find either any significant change on serum inflammatory markers except for a significant reduction of ALP and IL-6 levels. However, the 12-months supplementation with the GE-RES downregulated the expression of key pro-inflammatory cytokines (*CCL3*, *IL-1β* and *TNF-α*) in PBMCs with the involvement of inflammation-related *miRs (miR-21*, *miR-181b*, *miR-663*, *miR-30c2*, *miR-155* and *miR-34a*).Our results provide preliminary evidence of a beneficial immunomodulatory effect of low doses of RES in humans.

Oleuropein and/or rutin consumption decreases the spontaneous development of osteoarthritis in Hartley guinea pig

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The objective was to assess the potential protective effect of oleuropein and rutin, two polyphenols found in olive oil, fruits and vegetables, on cartilage breakdown in a model of spontaneous osteoarthritis (OA) development.
Sixty 4 week-old Hartley guinea pig were randomized in four groups and received during 7 months either a standard guinea pig diet (control group) or a standard guinea pig diet supplemented with oleuropein (0.025%), or rutin (0.5%) or rutin/curcumin mix (0.5 and 0.25%, respectively). Blood was collected every 6 weeks and at sacrifice (week 35). Blood biomarkers for cartilage breakdown and inflammation were measured at each time point. Histological assessments of knee cartilage and synovial membrane were performed after sacrifice.
Guinea pigs from the control group spontaneously developed important cartilage lesions with mild synovial inflammation. Histological scores of cartilage lesions and synovitis were significantly correlated with measured biomarkers. All treated groups exhibited a decrease of cartilage degradation, and oleuropein induced also a significant decrease of synovitis. Oleuropein decreased inflammation and cartilage degradation biomarkers, while rutin combined or not with curcumin decreased only breakdown markers.In conclusion, oleuropein and rutin significantly slowed down the progression of OA lesions in guinea pig. However, oleuropein seemed to limit cartilage breakdown also through anti-inflammatory mechanisms.

Natural product-derived polyphenols enhance the cardiovascular protective endothelial function in health and diseases by targeting eNOS, oxidative stress and the angiotensin system

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It is well established in experimental animals and humans that endothelial cells, which cover the luminal surface of all blood vessels, have a pivotal role in the control of vascular homeostasis. The protective effect of endothelial cells is mostly due to their ability to respond to hormones, autacoids, blood- and platelet-derived factors by inducing vasodilatation via the release of nitric oxide (NO) and protacyclin (PGI_2), and the induction of endothelium-derived hyperpolarization. The most important one of these mechanisms is the release of NO, which is generated from L-arginine by the enzyme termed endothelial NO synthase. In addition to inhibiting vascular tone, NO is also a potent inhibitor of platelet activation and it has anti-thrombotic and anti-atherosclerotic properties. An endothelial dysfunction characterized by a reduced generation of these endothelium-dependent vasodilator mechanisms associated with vascular oxidative stress and the formation of endothelium-dependent contracting factors such as contractile prostanoids is often observed in most types of cardiovascular diseases including hypertension, hypercholesterolemia, diabetes, and during physiological ageing in both experimental animals and humans. Nutrition-derived polyphenols such as grape products, tea catechins, cocoa, and berries have been shown to increase the endothelial formation of NO by causing the Src/PI3-kinase/Akt-dependent activation of endothelial NO synthase (eNOS) and to enhance eNOS expression leading to a sustained formation of NO. Moreover, polyphenols have also been shown to both improve an established endothelial dysfunction and delay the onset of the induction of an endothelial dysfunction in several experimental models of cardiovascular diseases and in ageing. The protective effect is mostly due to their ability to reduce vascular oxidative stress by inhibiting the overexpression of NADPH oxidase and the angiotensin system. Thus, nutrition-derived polyphenols may be an interesting approach to delay the onset of risk factor- and ageing-related endothelial dysfunction and, hence, the initiation and development of cardiovascular diseases.

Cranberries, flavonoids, and heart disease

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The fruit and undiluted juice of Vaccinium macrocarpon (American cranberry) are too tart to consume alone but many foods, beverages and dietary supplements made from them that vary in the bioactive constituents are widely consumed in the US and elsewhere. The signature flavonoids in cranberries are the flavan-3-ols, the proanthocyanidins (PACs), flavonols and anthocyanidins. Other bioactive constituents include phenolic ursolic acids, potassium, dietary fiber and carbohydrates. We briefly review the evidence from epidemiological and human intervention studies on associations between cranberries, flavonoids, and heart health. In epidemiological studies, other dietary components present make causal inference of associations between cranberries, flavonoids and heart disease complicated. In recent cohort studies,associations between total dietary flavonoid intakes and cardiovascular disease mortality and morbidity were mixed, but generally positive. A meta-analysis of the associations of flavonols with heart disease, which found only weak associations, did not include two recent positive studies. In the Framingham study, flavonol intakes were associated with decreased incidence of type 2 diabetes, which is a cardiovascular risk factor. Evidence from human intervention studies is mixed but more consistent for beneficial effects of flavonoids on surrogate (intermediary) biomarkers such as blood pressure, endothelial function, and arterial stiffness. Research now in progress should clarify these findings.

The link between tea and tea flavonoids and cardiovascular health, with focus on vascular function

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Most epidemiological studies have reported an association between consumption of green and black tea and a reduced cardiovascular incidence. Meta-analyses on clinical data indicate that glucose and cholesterol metabolism may be affected by tea. Other possible mechanistic pathways to explain the epidemiological association between tea and improved cardiovascular health could be the effects of tea on endothelial function and blood pressure. There is strong clinical evidence that green and black tea improve endothelial function. Endothelial-dependent flow-mediated vasodilation is improved acutely as well as after long-term intervention with tea. This parameter represents the ability of arteries to dilate and the value predicts future cardiovascular events. Moderate, but consistent effects on systolic and diastolic blood pressure have been reported in recent tea intervention studies with humans. In vitro and in vivo experiments direct to the tea flavonoids as the active components, but the magic bullet, if at all existing, has not been identified so far. Interestingly, a limited number of tea servings per day seem to be sufficient to exert the beneficial effects. Considering the widespread global consumption of tea, this beverage may contribute significantly to human health.

Molecular basis of the effects of red wine polyphenol on cardiovascular diseases associated with alterations of angiogenesis

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Paradoxical effects of red wine polyphenol extract (RWPC) treatment occur in a rat model of post-ischemic neovascularization, where a low-dose is pro-angiogenic while a higher dose is anti-angiogenic. RWPC exert endothelial NO release via the activation of the estrogen receptor-alpha (ERα). NO and ERα are key regulators of mitochondrial capacity, and angiogenesis is a highly energetic process associated with mitochondrial biogenesis.
The molecular mechanisms of RWPC on the different aspects of angiogenesis are investigated at cellular level and in an experimental model of neovascularisation with special interest to the role of ERα and the mitochondrial capacity.
Evidence is provided for a role of mitochondria in the regulation of angiogenesis by RWPC by assessing mitochondrial respiration, expression of mitochondrial biogenesis factors and mitochondrial DNA content. Using ERα deficient mice and recovery after hindlimb ischemia, we depicted the subtle effects of RWPC on blood flow and capillary density in conjunction with NO pathway activation and VEGF expression. Of particular interest are the demonstration of the activation of ERα and other axis leading to an upregulation of some transcriptional co-activators of mitochondrial factors. A corner stone of novel pathways for RWPC to correct cardiovascular diseases associated with alterations of angiogenesis will be discussed.

Red wine polyphenols: beneficial effects in the cardiovascular and renal systems

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Red wine polyphenols have been reported to exert beneficial effects in prevention of different diseases, but the molecular mechanisms of their actions were studied much less. We have focused on the effects of red wine extract (Provinols™) on cardiovascular and renal systems in relation to the molecular and biochemical mechanisms of this compound. Treatment with Provinols™ partially prevented development of spontaneous and NO-deficient hypertension and accelerated decrease of blood pressure in already established hypertension. The effects of Provinols™ include prevention and/or attenuation of myocardial fibrosis, reduction of vessel wall thickness and improvement of vascular functions. Furthermore, Provinols™ had cardio- and reno-protective effects in ischemic, hypertensive and CsA-induced nephrotoxic conditions. Its administration was associated with decreased tubular injury and interstitial fibrosis. These functional and structural alterations were associated with significant increase of NO synthase activity and eNOS protein expression associated with increase in calcium signaling and the activation of tyrosine kinase pathway within endothelial cells. Moreover, Provinols™ reduced increase of iNOS and NF-kappaB (p65) expression via decreasing the level of ROS and acting on intracellular kinases expressions. In conclusion, the cluster of multiply molecular mechanisms seems to be responsible for protective effect of Provinols™.
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Flavan 3-ols bioactivities on metabolic syndrome– a new angle of observation

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There is evidence that flavan 3-ols rich chocolate have the potential to contribute to the risk reduction of cardiometabolic disorders according to recent epidemiological or intervention studies. However, the detail of alteration on the metabolic syndrome risk factors by flavan 3-ols remains unclear. We attempt to elucidation of flavan 3-ols acute or repetitive bioactivity against circulating and energy metabolism system using several experiments. In circulation, a single oral administration of flavan 3-ols elevated blood flow and recruitment of capillaries in skeletal muscle detected by the in vivo intravital microscopy with an immediate elevation of blood pressure, in contrast, repeated ingestion of flavan 3-ols reduced mean blood pressure. In addition, these effects were diminished by the pretreatment of adrenalin receptor blocker. In energy metabolism, repeated treatment of flavan 3-ols showed alteration of energy expenditure estimated by an indirect calorimetric method. We found that the average of respiratory exchange ratio was significantly reduced with elevation of mitochondrial copy number in skeletal muscle and induction of the expression of uncoupling protein and β oxidation relative enzyme. According these results, a part of alteration on metabolic syndrome risk factors by the ingestion of chocolate may be induced by sympathetic-mediated responses.

Polyphenols and cardiovascular health: gene expression relationship

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Epidemiological, clinical and animal studies support a role of polyphenols consumption in prevention of various diseases, particularly cardiovascular diseases. Recent findings suggest that polyphenols could interact with cellular signalling cascades regulating the activity of transcription factors and consequently affecting the expression of genes. However, molecular targets of polyphenols are still not well deciphered.We have shown, using nutrigenomic approach, that polyphenols can modulate expression of large number of genes, both in animal models as well as in humans, that are involved in regulation of inflammation andthe initial steps of atherosclerosis development. Nutrigenomics analysis in in-vitro studies indicated that polyphenol metabolites, present in plasma and used at physiologically-relevantconcentrations can affect the expression of genes in endothelial cells, genes that regulate endothelial function and integrity. Furthermore, animal and cellular studies suggest that the mechanisms underpinning their ability to modulate expression of genes are linked to the potential of metabolites to interact with and modulate cell-signalling pathways. Together with “classical” signalling pathway regulation, we also revealed that polyphenols can modulate the expression of microRNA that act as post-transcriptional modulators of genes also regulating inflammation and atherosclerosis development. Taken together, these data provide a global and integrated view of molecular mechanisms of polyphenols underlying their health properties and show their multi-targetedmode of action.

Cocoa flavanols modulate the transcription of genes involved in atherosclerosis pathways with complex epigenetic changes of their DNA methylation state

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Epidemiological studies show that a flavanol-rich diet (cocoa, grape) is associated with a decreased risk of cardiovascular disease (CVD). In how far biological effects occur in endothelial HUVEC cells exposed to flavanols in vitro or in humans upon flavanol supplementation is yet unclear. In the Flaviola-consortium (www.flaviola.org), potential biological processes modified by flavanols were evaluated at the transcriptomic and epigenomic level in HUVEC cells exposed to specific flavanol metabolites or in leukocyte samples collected from diet intervention studies. In vitro experiments in HUVEC cells demonstrate that flavanol metabolites significantly decrease monocyte cell adhesion, concomitantly with changes in gene expression and DNA methylation in cell adhesion pathways. Furthermore, in a randomized, double-blind, placebo controlled diet intervention trial with cocoa flavanols, we further evaluated DNA methylation changes in blood samples, measured by Illumina 450K CpG array. Furthermore, PCA analysis identified various differentially methylated CpGs in atherosclerosis patients versus healthy controls or diet versus placebo setup related to cell adhesion, estrogen-inflammation and drug detoxification pathways. Altogether, flavanols may elicit cardioprotective effects by decreasing cell adhesion pathways at the transcriptomic and epigenomic level.

Nutri-epigenetics and cancer prevention - an overview

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Within the past decade, epigenetic mechanisms and their modulation by natural products including polyphenols have gained major interest in the chemopreventive community. The term “epigenetics” refers to modifications in gene expression caused by heritable, but potentially reversible, changes in DNA methylation and chromatin structure. Major epigenetic mechanisms include DNA hyper- and hypomethylation, histone acetylation and methylation, and non-coding (micro) RNAs. Given the fact that epigenetic modifications occur early in carcinogenesis and represent potentially initiating events in cancer development, they have been identified as promising new targets for prevention strategies. Polyphenols from various dietary sources, including green tea, soy, coffee, apples, red wine, raspberries, turmeric, onions, pomegranate, cashew nuts and others were shown to directly target enzymatic activities or modulate expression of enzymes involved in epigenetic gene regulation, including DNA methyltransferases, histone acetyltransferases, deacetylases and demethylases, as well as sirtuins. Also, many polyphenols were shown to alter miRNA expression in cell culture. These activities might contribute to their effects on signal transduction mediated by nuclear receptors and transcription factors such as NF-κB, cell proliferation and cell cycle progression, cellular differentiation, DNA repair, apoptosis induction, cell motility, metastasis formation, and cellular senescence. Future research will need to identify best strategies for chemopreventive intervention to target the epigenome. References: (1) Huang J et al. Curr Drug Targets 2011; 12: 1925-56. (2) Gerhauser C. Top Curr Chem. 2013; 329: 73-132.

Resveratrol mimics the effect of calorie restriction on transcriptional targets of healthspan in white adipose tissue

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Although calorie restriction (CR) slows the aging process in diverse species, CR is not a pragmatic strategy to attenuate aging in free-living humans. Accordingly, there is growing interest in identifying CR mimetics—compounds that elicit the salutary effects of CR without a reduction in energy intake. Because increased white adipose tissue (WAT) is associated with accelerated onset of many age-associated diseases, and because a reduction of WAT is a consistent feature of CR, diet-induced changes in WAT bioactivity may be attractive targets for assessing the efficacy of CR mimetics. We analyzed gene expression datasets from WAT of obese mice and humans and found a significant modulation of 22 pathways indicating decreased mitochondrial function and an up-regulation of the immune response and lysosomal structure. The change with obesity was abrogated by long-term CR in WAT of both mice and rats for 13 of the 22 pathways. For those 13 pathways, consumption of the polyphenol resveratrol both opposed the effect obesity and mimicked the effect of CR for 11 and 10 pathways in human and mouse adipose tissue, respectively. Numerous studies have shown that resveratrol delays diverse aspects of the aging process, suggesting that our observed transcriptional response in WAT is indicative of increased healthspan. We propose that these pathways may be used as a framework to screen for additional polyphenols that may act as CR mimetics.

Opposite effects of daidzein and genistein supplementation on whole genome gene expression profiles in adipose tissue of postmenopausal women

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During menopause, body fat distribution and cardiovascular risk profile change. Isoflavones might modulate these changes; we investigated effects of two different isoflavone supplements on whole genome gene expression in adipose tissue, in a randomized placebo-controlled cross-over trial with postmenopausal women. Both supplements provided ~100 mg isoflavones/day (aglycone equivalents); daidzein and genistein content of supplement D were 56% and 16% and of supplement G 49% and 41%, respectively.
Isoflavone plasma concentrations after supplement D and supplement G were respectively 1.40 and 1.15μM daidzein and 0.44 and 1.19μM genistein. After intake of supplement D 1245 genes were statistically significant expressed compared to placebo (n=24) and after supplement G 528 genes (n=31). Gene set enrichment analysis revealed opposite changesby the two supplements; fatty acid and triglyceride synthesis, PPAR target genes and glucose metabolism were downregulated after supplement D and upregulated after supplement G. Furthermore, supplement D induced downregulation of oxidative phosphorylation, while supplement G induced upregulation of estrogen signalling, cell cycle and protein processing.
Eight weeks of isoflavone supplementation resulted in isoflavone-specific effects on adipose tissue gene expression, implying that isoflavone supplement composition directs effects in adipose tissue. This abstract contains preliminary results; results on underlying mechanisms, impact of equol-producing phenotype and relevance for adipose tissue health are expected in September 2013.

An integrated approachto understand the metabolic effects of a rosemary (*Rosmarinus officinalis* L.) extract rich in carnosic acid: critical differences between lean and obese phenotypes

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A mutation in the leptin receptor gene (*fa*) causes a major metabolic deregulation that affectsthe ability of the organism to respond to dietary changes and results in obesity. Using the Zucker rat model we have investigated the potential mechanisms contributing to the anti-obesity effects of a rosemary extract rich in carnosic acid (CA) as well as the bioavailability of its main diterpenic constituents.
Diet supplementation with the CA-enriched RE moderated weight gain and decreased serum lipids in the lean rats (*fa/-*). CA and carnosol were detected in the stomach where pre-duodenal carboxylesterase activity was inhibited. Fecal volume was also augmented and the microbiota significantly altered. These results suggesteda reduction of the digestion and absorption of fat and were concomitant with a metabolic and anti-inflammatory regulatory response which involved: induction of xenobiotic metabolism, activation of hepatic key energy regulatory genes (*PPARGC1A*) and regulation of circulating pro- and anti-inflammatory molecules. The RE-supplemented obese animals (*fa/fa*) displayed similar changes in body weight, carboxylesterase activity, fecal size, and microbiota but serum lipids and the pro-inflammatory state remained unmodified. Notably, in the obese rats, the hepatic transcriptional regulator*PPARGC1A* was not activated and the levels of pAMPK were decreased in the adipose tissue after the intake of RE. The results demonstrate the ability of a rosemary extract rich in CA to affect metabolic pathways and highlight the impact of genetic makeuponthe responseto the intake of natural extracts with anti-obesity effects.

Standardized phenolic compounds attenuate Alzheimer's disease by preventing pathological β-amyloid misfolding and promotion of amyloid clearance in the brain.

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Background: There is a growing interest in the development of polyphenolic compounds for prevention and treatment of chronic and degenerative diseases, such as cardiovascular disorders, cancer, and neurological diseases, including Alzheimer's disease (AD). Based on the previous evidence that grape seed extract (GSE) can prevent β-amyloid (Aβ) oligomerization, resveratrol can promote Aβ clearance, and Concord grape juice extract (juice extract) can promote alpha secretase-mediated non-amyloidogenic amyloid precursor protein (APP) processing, we hypothesized that the application of multiple polyphenolic preparations simultaneously targeting multiple mechanisms may provide synergistic effect in attenuating β-amyloid (Aβ) mediated neuropathology and cognitive impairments.Methods:In this study we treated J20 mice that express human APP with both the Swedish (K670N/M671L) and the Indiana (V717F) mutations (*APPSw-Ind*) under a neuronal specific promoter with either resveratrol, GSE, juice extract, or a combination of the three, starting at 3 months of age and continuing for 6 months. The Morris water maze test was used to evaluate the effects of treatment on cognitive function and neuropathology. Results: We found that all four groups of mice on polyphenol treatment showed improved cognitive function compared to the non-treated control transgenic mice. However, mice on combinatory polyphenols exhibited much lower amyloid neuropathology in the brain, both in total amyloid peptide content and in plaque burden, compared to those treated with individual polyphenol. Conclusion:Our study demonstrated that combinatory polyphenols with heterogeneous and partially redundant bioactivities provide synergistic protection against Aβ-mediated neuropathology and preservation of cognitive function in an animal model of AD, and provide impetus for the application of combination therapy in human clinical trials.

Bioavailability and bioefficacy of chlorogenic acids in humans.

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Epidemiological data suggest regular consumption of (poly)phenol rich foods and beverages is associated with a reduced risk of certain pathological conditions. Chlorogenic acids and derivatives like phenolic acids are potentially bioactive commonly found in many foods. Although some recent findings have helped improve the understanding of how chlorogenic acids are absorbed, metabolized and excreted by the human body, data on the bioavailability of these compounds are still lacking. By using a validated liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) method we were able to quantify methylated, sulfated and/or glucuronidated metabolites of chlorogenic and phenolic acids in human plasma after the consumption of coffee. Moreover, it was previously suggested that some metabolites can be incorporated into low-density lipoproteins (LDL) protecting them against oxidative damage. Consequently, we aimed to identify some of these metabolites. By matching gas- and liquid chromatographic retention time, accurate mass of the molecular ions, electron ionization mass spectrums and electrospray product ion spectrums with reference standards we were able to identified chlorogenic acid metabolites bound to cholesterol in human plasma samples. While the mechanism of action of phenolic acids still hasn't been clearly demonstrated, altogether these data could help to elucidate the bioactive compounds after consumption of foods containing chlorogenic acids.

Influence of resveratrol and of red wine polyphenols in the inhibition of digestive tract cancers *in vitro* and *in vivo*

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Moderate wine consumption is considered to be good for health, particularly against atherosclerosis (1), but also possibly against cancer. It is common to attribute beneficial effects to polyphenols notably resveratrol. Resveratrol is well known for its antiproliferative action (2), its pro-apoptotic effect on transformed cells (3), its pro-differentiating mechanism (4) and its ability to modulate pro-oncogenic or tumor-suppressor miRNAs (5). The objective of our work focused on the possible effect of wine polyphenols towards the biological action of resveratrol. Thus, we evaluated the efficacy of red wine extract (RWE) and of resveratrol on colorectal established cancer cells and on intestine precancerous state. For this, we prepared different RWE extracts from different wine preparation but coming from the same grape collection of the same vineyard. The polyphenol content was analyzed by HPLC in order to compare with their efficacy towards colon tumor cell line proliferation. The inhibitory effect of RWE was compared to resveratrol, a well standard used in such studies. The effect of RWE was tested as well as quercetin, an abundant polyphenol of wine in the resveratrol uptake. In addition we performed preclinical study on the effect of RWE against the development of azoxymethane (AOM)-induced aberrant crypt foci in male CF-1 mice by measuring preneoplasia foci. The results are the following: 1°) - RWE shows *in vitro* antiproliferative activities in a dose-dependent and qualitative- dependant manner on human colorectal cell line SW480 and on human liver hepatoblastoma HepG2.2°) - A combinaison of wine polyphenols with resveratrol was stronger antiproliferative than separated exposures. A synergy has been also observed between resveratrol and quercetin. 3°) - The efficiency of red wine polyphenols extracts depends on their richness and the quality of polyphenols. 4°)- The *in vitro* inhibition of cell proliferation by red wine polyphenols is independant of richness in resveratrol. 5°) - One of the mechanism which can be involved in the synergy can be a modulation of cell transmembranar of polyphenols especially resveratrol. 6°) - The *in vivo* diet absorption of red wine polyphenols slowers the progression of polyps neoplasia (crypt aberrant foci) especially concerning the largest ones in azoxymethane-treated mice. Interestingly, the synergistic effect of resveratrol with other wine polyphenols in the prevention of pathologies would help to clarify the apparent discrepancy between limited plasmatic level of wine polyphenols and their biological effect. (Supported by UNESCO Chair, Heritage and Wine tradition). (1) Rifler JP et al. Mol Nutr Food Res, 2012, 56: 345. (2) Delmas et al., Int J Mol Med, 2002, 10: 193. (3) Colin et al., Cancer Prev Res, 2010, 4:1095. (4) Kaminski J et al., Biochem Pharmacol, 2012, 84:1251. (5) Tili E et al., Carcinogenesis, 2010, 31: 1561.

Phenolic compounds as skin photoprotective agents
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The skin is constantly exposed to solar radiation. Among wavelengths of solar spectra ultraviolet radiation (UV) is considered the most deleterious and has been responsible for many skin disorders including ageing and cancer. Direct DNA damage and increase in reactive oxygen species production are among the basic mechanism underlying UV induce skin cells lesion. Because exposure prevention to solar light is not an easy task, the use of photoprotective agents constitutes one of the best strategies to counteract UV light exposure and its deleterious effects. Polyphenols and phenolic acids, due to their antioxidant properties, have been proposed as important components of the diet to protect the skin against UV induced lesion. Besides that, phenolic acids can absorb UV radiation indicating that topical application of these compounds can bring beneficial effects against UV radiation. To exert both properties after topical application, formulation characteristics constitute a very important point to be considered in order to guarantee that the compounds will reach the lower layers of the skin. Updated information on the mechanism of action of these compounds will be presented that support their potential use as skin photoprotective agents. Supported by FAPESP – grant 2011/21087-1.

Resveratrol rescues insulin sensitivity in "obese" adipocytes
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Resveratrol is a cell permeable *trans*-stilbene derivative that can scavenge intracellularly produced HOCl (J. Biol. Chem, 285:20062/71). Our DIO model in B6 mice showed that epididimal AT expresses myeloperoxidase (MPO) in AT macrophages (ATM) that also express M1 markers (e.g., IL-6, iNOS). MPO expression was observed in ATM located in the typical crown-like structures; and also inside adipocytes. Tissue fractionation showed MPO mRNA in the ATM, but not in adipocyte fraction. Treatment of these isolated adipocytes with H_2O_2 blocked glucose uptake. Based on these findings, we hypothesized that MPO interferes with AT insulin signaling by producing HOCl and causing oxidation of intracellular components involved in insulin-triggered signaling. To test this hypothesis we differentiated human adipocytes and loaded them with human MPO. Treatment of MPO-loaded adipocytes with H_2O_2 caused intracellular production of HOCl, reduced insulin-triggered GLUT-4 translocation to the membrane and glucose uptake. Furthermore DMPO-based immuno-spin trapping and MS-tandem showed radicalization of specific components of the insulin signaling (e.g., IRS-1/2, SOCS3, GLUT-4). These effects were prevented by resveratrol, but not by taurine or methionine because these cannot pass across the membrane. Together our findings suggest that resveratrol can protect insulin sensitivity in the obese AT by scavenging intracellularly produced HOCl.

Quantitation of novel glycine conjugates of chlorogenic acid from coffee after consumption by volunteers
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Chlorogenic acid is found in high abundance in coffee and habitual consumption can contribute up to 1 g of total phenolics to the daily diet. Upon absorption, chlorogenic acid is cleaved and the resulting caffeic acid is metabolised into various forms including sulphate, glucuronide and glycine conjugates prior to renal excretion. Whereas data regarding other conjugates has been appearing in the literature over the last few years, information regarding glycine forms is limited and the overall pathway unexplored.
Glycine conjugates were chemically synthesised and quantified in urine of volunteers (n=5) using external calibration based on optimised multiple reaction monitoring. After consumption of a cup of commercially available coffee (722 µmol chlorogenic acids), samples were collected at 0-4, 4-8, 8-12, 12-24 and 24-36 hours and prepared with liquid-liquid extraction prior to LC-QqQ-MS analysis.
The major glycine conjugates excreted 0-36 hours post coffee consumption were feruloylglycine (38.8 µmol +/- 17.2 µmol) and vanilloylglycine (33.0 µmol +/- 16.2 µmol) comprising ~10 % of the ingested dose, whereas dimethoxycinnamoylglycine, dihydroferuloylglycine and dimethoxybenzoylglycine were only found in trace amounts. Our recent findings point to the importance of this metabolic pathway for a more detailed understanding of the bioavailability of coffee bioactives.

Isoflavone and glyceollin modulation of gene expression *in vivo*

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Soy isoflavones (IF) have similarities to mammalian estrogens including the ability to interact with estrogen receptors.Stressedsoybeans can produce glyceollinswith antiestrogenicand other properties. Consumption of these agents may produce variable effects depending upon systemic estrogen levels. Dietary IF haveprimarily estrogen antagonist effects on the breast in a high estrogen environment, and minimal effects in a low estrogen environment. Here we evaluated the effects of IF alone or in combination with glyceollinson breast responses to estrogen. Monkeys received diets containing estradiol (1 mg/day) and one of three protein sources for 3 weeks: (1) controlcasein/lactalbumin (Control), (2) soy protein containing 194 mg/day IF (SOY), or (3) glyceollin-enriched soyprotein containing 189 mg/day IF + 134 mg/day glyceollins (GLY). Estradiol increased breast cell proliferation in Controls but not in SOY or GLY, and GLY prevented increases in expression of estrogen responsive genes in the breast. GLY modulated mammary gland expression of greater numbers of genes (>2x) than SOY, influencing pathways involved in lipid and carbohydrate metabolism, PPAR-γ and AMPK signaling, and adipocytokine expression. The findings demonstrate genomic effects of dietary soy isoflavones which are dependent upon the estrogen environment and modulated by glyceollins.

ADME of isoflavonoids after soy intake

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Soy is the major source of dietary exposure to isoflavonoids (IFLs). Accumulating evidence supports a role for soy and IFLs in the protection against many chronic diseases including cancer. In order to understand IFL effects we examined the bioavailability of IFLs and found that the biphasic IFL appearance pattern is due to absorption of IFLs during the first 2 hours after soy intake in the small intestine (ca. 10%) and during 6-8 hour after soy intake in the large intestine (ca. 90%). While the time for the disappearance from the circulation is different for each IFL we found excellent correlations between urinary and circulating IFL values and developed algorithms to convert urinary excretion values into circulating levels. We refer to 'apparent bioavailability' when using urinary excretion to describe IFL exposure.

We discovered that IFL bioavailability is influenced by gut bacteria, oral antibiotic therapy (OABX), and individuals' age and health status. While daidzein (DE) and genistein start to be absorbed minutesafter intake equol (EQ) appears in plasma at least ca. 8 h after soy intake owing to the required transit time to the colon where the conversion of DE to EQ takes place by intestinal microbiota. We have also shown that the apparent IFL bioavailability is higher in children than adults, higher in healthy versus non-healthy individuals, and decreased and increased during OABX in children and adults, respectively. Finally, we used a urinary EQ/DE ratio in combination with a DE threshold in order to identify EQ producers and observed that EQ production is inconsistent over time in 15-30% of premenopausal and also postmenopausal women.

Nanometabolomicsusing SWATH-MS on a 5600 TripleTOFmass spectrometer for the study of polyphenol uptake and metabolism in small animal models

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Most analyses of known and unknown polyphenols and their metabolites utilizeLC-electrospray ionization-mass spectrometry. However, even with targeted multiple reaction ion monitoring on a triple quadrupole mass spectrometer (MS), the limits of sensitivity for an initial sample (blood/urine) size of 0.2 ml is typically 2-10 nM. Since the binding constants of some polyphenols and their metabolites to estrogen receptor beta are less than 1 nM, a much more sensitive method of analysis is needed that (1) can capture the entire MSMS spectrum of a particular compound rather than one of its allegedly "diagnostic" product ions and (2) to be able to measure precursor and product ions with high mass accuracy (2-3 ppm). All of these capabilities can be met by using a combination of nanoLC and a 5600 TripleTOFMS which can collect 20 high mass accuracy MSMS spectra of selected precursor ions per second. Using this approach, polyphenol metabolites can be quantitatively measured down to 0.1 nM(with signal-to-noise >10:1) starting with as little as 50 microliters of serum in combination with full mass spectra to assure their identification. A new approach (SWATH-MS) permits comprehensive (non-data or operator-selected acquisition) collection of MSMS spectra of all compounds in a sample allowing simultaneous discovery and quantification of each compound.

Urinary isoflavone variability in postmenopausal women during a three-year intervention study
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The effects on urinary isoflavone excretion of two isoflavone doses (80 or 120 mg/d) were evaluated during 3 years in healthy postmenopausal women (80 mg, n=58; 120 mg, n=66). Urine collections at 0, 6, 12, 24, and 36 months were analyzed for genistein, glycitein, daidzein, dihydrodaidzein (DHD), *O*-desmethylangolensin (ODMA), and equol. The 120 mg/d group excreted higher amounts of genistein (*P*=0.01), glycitein (*P*=0.02), and ODMA (*P*=0.03) than the 80 mg/d group. Equol producers excreted lower amounts of DHD (*P*≤0.0001) and ODMA (*P*=0.003) compared to non-producers. Approximately 30% were equol producers, and this tended to increase over time. Intra-individual variability (30-149%) in the excretion of each metabolite was lower than inter-individual variability (40-212%). Equol producers tended to have lower variability compared to non-producers, suggesting that they exhibited a more stable profile of intestinal microflora. 10.5% of subjects were inconsistent with respect to their equol-producing status, apparently related to antibiotic use. Antibiotic use significantly (*P*=0.004) decreased equol excretion. Isoflavonesmay exert a more pronounced and predictable response in equol producers, likely dependent upon antibiotic use. This concept is worth investigating because of implications with respect to biological responsiveness to isoflavone treatment.

Sociodemographic and lifestyle correlates of urine isoflavone concentrations in the US population
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We used correlation analyses, simple linear regression, and multiple linear regression modeling to study sociodemographic (age, sex, race-ethnicity, education, and income) and lifestyle variables (smoking, alcohol consumption, BMI, physical activity, and dietary supplement use) in relation to spot urine concentrations of plant isoflavones (daidzein [DAZ], genistein [GNS]) and enterogenous DAZ metabolites (equol [EQU] and O-desmethylangolensin [DMA]) in the US population ≥20 y (NHANES 2003–2006). We observed many statistically significant (*P* <0.05) associations that withstood covariate adjustment. Urine DAZ, GNS, EQU and DMA were estimated to be 16–40% lower in non-Hispanic blacks vs. non-Hispanic whites, and EQU and DMA were estimated to be 36–55% lower in Mexican Americans vs. non-Hispanic whites. Smoking was a significant correlate of urine DMA with concentrations at least 25% lower in smokers vs. nonsmokers. Consumers of 1 daily alcoholic drink vs. none were estimated to have 18–21% lower urine EQU and DMA concentrations. Dietary supplement use was not significantly associated with any of the urine isoflavones. Overall, we found that relationships between sociodemographic and lifestyle variables and urine isoflavone concentration were highly compound- and class-specific.

Daidzein metabolizing phenotypes influence metabolomic responses in individuals with cardiometabolic risk factors
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Consumption of polyphenol-rich diets, including soy, is associated with beneficial health effects and chronic disease prevention. However, large variability exists in individual responses to soy. Equol, a daidzein metabolite produced by some colonic microbiota, contributes to this variability. The metabolomic profile associated with Equol producing individuals compared to those who do not, is unknown. Therefore, this pilot study utilized untargeted metabolomics to assess effects of a soy intervention on metabolites in participants at cardiometabolic risk. 17 individuals completed a randomized, controlled, crossover study receiving 70 g soy nuts (100 mg aglycone equivalents) or control food daily for four weeks, separated by two week washout. Serum and urine were used for clinical biomarkers and NMR analyses. Statistical analyses included multivariate PCA and PLS-DA. Metabolomic responses related directly to phenotypes based on gut microbial products of daidzein and were not distinguished by pre/post-soy changes. Three metabolomic phenotypes were identified: Equol+ODMA, ODMA only, and non-producers. The Equol+ODMA phenotype had significantly lower levels of multiple metabolites including TMA, creatinine, and aromatic and branched-chain amino acids, which typically increase in obesity and diabetes. Paradoxically, this phenotype also had significantly higher pro-inflammatory cytokines (TNF-α, IL-6, IL-18). Conclusion: Metabolic profiling identified phenotypic responses in at-risk individuals.

Cardiovascular effects of anthocyanins: evidence from intervention trials
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The physiological benefits of high anthocyanin intake are being increasingly established. Recent findings from prospective cohort studies highlight the potential beneficial impact of habitual intakes of anthocyanins on a range of health outcomes including heart disease, hypertension, type 2 diabetes and cognitive decline. However proof of efficacy from longer-term human randomised controlled trials (RCTs) is distinctly lacking. To date, few carefully controlled human intervention trials have examined the effects of anthocyanins on insulin resistance and vascular health; those that have are of relatively short duration (< 2 months). In one trial both systolic and diastolic blood pressure (BP) decreased, while in another study, berry consumption (two portions daily) resulted in favourable changes in platelet function, BP and HDL-cholesterol levels. In another intervention, daily intakes of anthocyanins over 4 weeks resulted in a significant decrease in pulse wave velocity, a measure of vascular function. To date, only one study has examined the impact of anthocyanins on insulin sensitivity as assessed by the hyperinsulinemic-euglycemic clamp technique, and of the three studies that assessed fasting glucoseand HbA1c levels, two observed beneficial effects. To date no long term studies have investigated the dose-response effects of anthocyanin-rich foods on insulin resistance and vascular function.

Anthocyanins and disease - Strong evidence from epidemiological studies
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Strong compelling evidence suggests that a diet rich in fruits and vegetables is beneficial to markers of metabolic diseases. Results from large prospective studies of hard clinical outcomes also suggest that fruits and vegetables lower the risk of cardiovascular disease and some cancers. Current data point towards the specific importance of berry fruits and other foods high in the polyphenol class of anthocyanins as the potential bioactives responsible for the observed inverse associations with hypertension, vascular function, diabetes CHD, and cognitive decline. Our recent prospective study reported a 15% reduction in risk of diabetes comparing the highest to lowest quintiles of anthocyanin intake and a 23% reduction in risk with increased intake of blueberries (>2/week vs. <1/month). For hypertension, comparing the extreme quintiles of anthocyanin intake the RR=0.92 (95% CI 0.86-0.98). Blueberry intake explained most of the inverse association observed with a high anthocyanin intake. To date, all prospective studies on fruit and vegetable consumption have assessed intake either at one time point, which assumes intake is stable, or have used a cumulative average during the study period. Neither of these methods best represents evidence that could be used to make clinical recommendations. Studying changes in intake over time may be more relevant in terms of both biological efficacy and translation into prevention strategies. Thus future epidemiological studies should be 1) large prospective studies of change in anthocyanin consumption over time, and 2) long term clinical trials on anthocyanins or foods containing this highly bioactive compound to understand better the biological pathways that lead to better health.

Absorption and metabolism of anthocyanins: insights from ^{13}C labelling approaches
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Labelling or 'tracer' studies are paramount to our understanding of human nutrition, and have been particularly useful when studying the bioavailability, metabolism and pharmacokinetics of flavonoids, whose metabolites are often shared with those derived from other nutrients and phytochemicals. Establishing the bioavailability of anthocyanins has proven particularly challenging, as they are chemically unstable relative to other flavonoids. Previous studies feeding human participants unlabelled anthocyanins have generally failed to recover greater than 1% of the dose consumed. We recently completed an intervention feeding 8 healthy male volunteers 500 mg of ^{13}C-labelled cyanidin-3-glucoside (6,8,10,3',5'-$^{13}C_5$-C3G). Blood, breath, urine and faecal samples were taken over a 48h period and analysed by isotope-ratio MS and HPLC-ESI-MS/MS. 44±26% of the label was recovered in the urine, breath and feces over 48h, representing a relative bioavailability of 12±1%. More than 30 unique labelled metabolites were identified and confirmed using commercial and synthetic standards, including C3G, its degradation products, phase II conjugates and colonic metabolites. Individual metabolites were identified at maximal concentrations of up to 2.0±1.4 µM and some peaked as late as 30.1±11.4h post consumption. Hippuric acid, ferulic acid and vanillic acid were found in the highest concentrations and peaked in serum at 16±4h, 8±4h and 13±12h respectively. The results indicate that anthocyanins undergo significant biotransformation, where they form benzoic acids, phenylacetic acids, and phenylpropenoic acids. We conclude that anthocyanins are extensively metabolised and their metabolites are found in the circulation at much higher concentrations than their parent structures. These metabolites should be the focus of future research exploring anthocyanin bioactivity.

The mechanisms of colour

de Pascual-Teresa S

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Anthocyanins are the main group of natural hydrosoluble pigments in plants. They introduce colouring to foods, with colours ranging from blue to red and orange. Nowadays, their importance for the Food and Pharmaceuticalindustriesis mainly based in the existing scientific work evidencing their beneficial effects on the prevention of cardiovascular diseases and visual conditions. Different mechanisms have been shown to be involved in those effects. The most consistent onesare related to their antihypertensive and endothelium protective activities, antiatherogenic activity and their interaction with the estrogenic receptor. In some of the existing work, studies on structure-activity relationship have been done showing that modifications on the structure of anthocyanins, besides having an effect on their colours, have a clear incidence on their interaction with different steps in the principal pathways related to these diseases. Therefore, different colours might show different molecular mechanisms. However, in a normal diet most of these compounds are present simultaneously and, thus, they can act by different mechanisms but give rise to a common final action. Design of new food products or food supplements should take this potential for synergies into consideration.

Strawberry-derived polyphenols and their metabolites in human plasma: Detection and identification by LC/MS Q-TOF

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Strawberry intake is associated with a number of health benefits, including reducing the risk of cancer, atherosclerosis, diabetes, obesity and age related memory decline. Recent in vivo data from our laboratory has shownthat strawberry intake attenuated postprandial oxidative- and inflammatory- stress with improved postprandial insulin action. The relationship between the bioavailability of strawberry polyphenols and the kinetics of the metabolite profiles relative to their health promoting action during the postprandial state is not well understood. Traditionally, these studies employ a targeted analysis using LC-MS/MS to quantify a predicted set of metabolites from a set of abundant polyphenols present in the food.We were interested in determining the utility of combining non-targeted (NT) with targeted (T) approaches to characterize and quantify metabolite components of interest after strawberry consumption. In a dose-response study with strawberry (0, 10, 20 40 g freeze-dried strawberry prepared in a beverage), we analyzed plasma collected over 6 h post-strawberry ingestion from insulin resistant subjectsby Q-TOF (quadrupole-time-of-flight) LC/MS (NT) and LC-MS/MS (T). Thirty-three compounds were tentatively identified by Q-TOF LC/MS; of which 7 had not been previously reported. Pelargonidin-glucuronide (PG) was the primary metabolite owing to the dominance of pelargonidin-based anthocyanins in strawberry. Area under the curve (AUC) and concentration maximum (C_{max}) increased with dose, but absorptive efficiency decreased. Relative bioavailability of pelargonidin glycosides was between 1 and 2% inversely related to dose. LC/MS-Q-TOF technology provides data for the qualitative identification of polyphenolic compounds and allows for the targeted and quantitative analysis of polyphenolic compounds and their metabolites in human plasma.

Anthocyanins profile of new red-flesh apple varieties: a comparative study

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Malus x domestica is a popular and important fruit in human diet, e.g. in Europe every year around 10 millions tons of apples are consumed. Anthocyanins are a particular class of flavonoid compounds causing red, blue, or purple color to fruits, flowers and vegetables. These compounds show important antioxidant activity, and are widely accepted to promote human health. The color of the skin of red and bicolor apples is due to these compounds. It's known that there are various genotypes of apple that biosynthesize anthocyanin in additional fruit tissues like the flesh and the core. In the last years red-flesh apples have became quite popular especially for the development of new varieties and for the so-called "functional products" (e.g. fresh juices). However, in contrast with traditional white flesh apples, the anthocyanins structures involved as well as the chemodiversity among the various red-flesh genotypes are poorly studied. In this study, the polyphenol profile of 5 red-flesh apples, originated in recent breeding program, was analyzedin pulp and peel and compared to previous analysis of approximately 100 "true-to-type" apple varieties. In order to enable a direct comparison of the apple varieties, all varieties were grown in the same experimental field and four harvest years were compared.

Handle with care: flavonoids are different than essential nutrients
Balentine DA

ILSI North America: Dietary Bioactives Committee and Unilever North America

Food and beverages are important sources of macro and micro nutrients considered essential to health. Research has shown that many non-essential food components or bioactives are important elements of diets for optimum health and/or reducing risk of chronic diseases. While the DRI process has been applied to non-essential nutrients, such as fiber; the majority of bioactive components await a recommended intake. One barrier to setting recommendations is data quality caused by inconsistent study design, inconsistent terminology, lack of defined analytical methods and limited food composition databases. Flavonoids and polyphenols provide some good examples. A second challenge has been the limited availability of surrogate end point biomarkers of chronic disease risk and lack of agreed biomarkers for health and wellness. Data quality is improving, food composition databases are now available and analytical methods to standardize experimental materials and study bioavailability have been developed for many bioactives. However, for recommended intake levels and dietary guidance to be set for bioactives, an agreed evidence based framework is required to guide studies needed to fill evidence gaps, agree clinical endpoint markers for health and wellness and guide authoritative review panels who are tasked with reviewing the evidence and making recommendations.

Reporting requirements for flavonoid research: a critical component in enhancing our understanding
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Despite significant progress in research highlighting the potential role for flavonoids in human health, development of dietary recommendations for these “non-essential” food components has not yet been possible. The diversity of flavonoid forms, dietary sources and preparations, methodological variations, and inconsistent use of databases have combined to create errors in reporting of flavonoid dosing/consumption levels. Furthermore, a gap exists in the general recognition of reporting requirements for studies that aim to elucidate flavonoid benefits in humans. A better understanding of these limitations is therefore required to facilitate development of recommendations for design and reporting of future research consistent with development of dietary recommendations for flavonoids. This presentation will describe ongoing efforts to outline specific limitations observed in published flavonoid research including: inconsistent use of nomenclature and flavonoid databases, application of inappropriate analytical methodologies, and inconsistent practices in design and quality control of flavonoid materials used in intervention trials. Progress in the development of general guidance on the design and reporting requirements for flavonoid research will be described for observational, pre-clinical and intervention trials. Reaching consensus and adoption of specific reporting requirements for flavonoid research will aid in development of dietary recommendations for flavonoid intake.

Flavonoids: from data to databases to adequate intakes
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Emerging science reveals non-essential dietary constituents, such as flavonoids, may play an important role in the promotion of health and reduction in the risk of chronic disease. Where sufficient data on intake, status, and outcomes like physiological function or intermediary biomarkers are available for flavonoid subclasses, establishing reference values could better inform new dietary guidelines and stimulate development of innovative food products. Current dietary assessment tools and food composition tables have several limitations but can still provide useful evidence about flavonoid intake. The status of flavonoids in plasma and urine are now being determined by targeted and metabolomic approaches though more data from chronic studies with usual dietary intakes would be useful. While an index for prevention of deficiency syndromes is not possible for flavonoids, a new paradigm (and new terminology) may be necessary to define intakes to achieve specific functional outcomes. However, a reasonable analogy can be made to the Adequate Intake or recommended consumption based on observed or experimentally determined estimates of mean intakes from healthy people. Moving forward to create a framework for recommended intakes of flavonoids requires an appreciation that complete and perfect information from each of these elements are not necessary to begin the process.

Flavonoids and cardiovascular health - what progress has been made towards public health recommendations for flavonoids?

Keen CL

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Epidemiological studies suggest that diets rich in flavonoids are associated with reductions in the risk for vascular disease. While these studies are encouraging, intervention trials with defined diets are needed to properly assess the health attributes of this class of plant compounds. The potential vascular health effects of nutrients are typically characterized by their influence on a few select parameters (e.g. blood pressure, and plasma lipids), but it can be argued that the impact of diet on vascular health can be better defined by its effect on a larger array of biomarkers. To study the effects of flavonoids on vascular disease risk, a number of endpoints that are influenced by cardiovascular risk factors have been employed. Endpoints thought to reflect key pathophysiological events in the development and progression ofcardiovascular disease include, but are not limited to, measures of vascular function, blood pressure, components of the immune response, platelet reactivity, and circulating progenitor cells. Depending on the specific measurement, information on the influence of individual flavonoids, as well as combinations of flavonoids, on specific physiologic pathways can be assessed. Examples of recent studies that have examined the effectsflavonoid-rich foods on cardiovascular endpoints will be reviewed.

The evolving path towards dietary guidance for flavonoids: Challenges, gaps and priorities moving forward.

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It has been two decades since the Food and Nutrition Board of the US National Academy of Sciences launched its efforts to develop Dietary Reference Intakes (DRIs). From the beginning there was an intention of addressing dietary recommendations for non-essential food components within the DRI framework. This is still an unfulfilled goal. Despite considerable research progress on determining flavonoid content and bioavailability from foods, metabolism of these compounds and clinical trials to evaluate their bioactivity, a variety of factors have prevented development of DRIs for flavonoid classes. The current framework for DRIs is appropriate for essential nutrients but less so for food components such as flavonoids. The Adequate Intake (AI) and Upper Levels (UL) designations might be appropriate. However, there are inherent limitations in running clinical trials with high flavonoid-containing foods. This presentation will review the challenges and gaps in knowledge that must be addressed before public health messages can be developed for flavonoids. In addition, there will be a discussion of the standards and types of evidence that might be required. Lastly, the implications of not moving forward will be discussed. Many scientists feel that some accreditation of certain flavonoid classes would be most appropriate for enhancing public health.

POSTERS

P1.1-01

Antioxidant activity and total phenolic compounds in grape juices produced in small scale

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The aim of this work was to determine the total phenolic compounds and the antioxidant capacity of grape juices obtained by small scale extraction methods. Grapes cv. Isabella were used to prepare juices by using steam extractor, extractor machine, juicer, and blender. Besides these, three brands of commercial grape juices were analyzed. Analyses included total and soluble phenolic compounds content, antioxidant capacity by DPPH and FRAP total soluble solids and total acidity. Data were submitted to ANOVA, and Tukey's test. There were significant differences in the content of phenolic compounds and in the antioxidant capacity among the juices extracted by different methods. Juices prepared using steam extractor and juicer had higher content of soluble phenolic while those obtained by using juicer and extractor had higher total phenolics content. The amount of these compounds varied between 194 to 1072.9 and 2726.7 to 3514.3 mgEq gallic acid.L-1, respectively. Antioxidant capacity by DPPH and FRAP were higher for juices obtained by steam extraction (2560 and 4539 µM Eq Trolox, respectively). The data were compared to those obtained for commercial juices. The results demonstrate that phenolic compounds and antioxidant capacity of grape juices produced in small scale are influenced by the extraction method applied. Support: FAPERJ.

P1.1-02

Determination of flavones and flavanones in Brazilian citrus peels

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The present work aimed to evaluate the flavonoids contents in citrus peels from fruits grown in Brazil. Two samples of each of four varieties of *Citrus sinensis*; two tangerine species ─ *Citrus reticulata*; *Citrus deliciosa* and four lemon species─*Citrus limon*; *Citrus limettioides*; *Citrus latifoliatanaka* and *Citrus limonia*─ were investigated. Peels were dried in oven with forced aeration, at 55°C. The main flavones and flavanones were quantified by HPLC-DAD-reverse phase. Results were compared by ANOVA/Fisher LSD test ($p<0.05$). Regarding major compounds, hesperidin contents varied between 143-260 mg100g-1 in oranges, 77-141 mg100g-1 in lemons, 8-153mg100g-1 in tangerines. Narirutin contents varied between 45-230 mg100g-1 in oranges, 9-26 mg100g-1 in lemons, and 35-240mg100g-1 in tangerines; rutin contents varied between 25-35 mg100g-1 in oranges, 6-65 mg100g-1 in lemons and 7-17 mg100g-1in tangerine. Among the three citrus species evaluated, oranges presented the highest flavanones contents (68,9±5,8mg100g-¹), while tangerines presented the highest flavones contents(9,4±8,8mg100g-¹). Pera variety was the best source of hesperidin (243±23,1mg100g-¹); Murcott tangerine, of narirutin (234,0±8,1mg100g¹) and naringenin (18,2±0,5mg100g-¹); Sicilian lemon, of rutin (64,2±2,2mg100g-¹); Ponkan tangerine, of nobiletin (24,0±1,4mg100g-¹) and of tangeritin (29,0±3,0mg100g-¹); Rangpur lime, of diosmin (6,0±0,7mg100g-¹);Tahiti, of naringin (6,5±0,1mg100g-¹);Valencia, of scutellarein (4,8±0,8 mg100g-¹) and sinensetin (3,4±0,0mg100g-¹); Persian lime, of hesperitin (4,9±0,5mg100g-¹). Keywords: Flavones; flavanones; Citrus Flavonoids

P1.1-03

Flavonoids from sugar cane juice

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Saccharum officinarum (Poaceae), known as sugarcane, is one of the most important crop plants in Brazil. In traditional medicine, culms are used as treatment for anemia, thrush, constipation, itching, infections and bronchitis. And the decoction of leaves is used for hypertension therapy. Among the metabolites found in S. officinarum, the main flavonoids are O- and C-glycosylflavones, derived from apigenin, luteolin and tricin.

The aim of this study is the isolation and identification of flavonoids present in sugarcane juice.

First, 2L of sugarcane were chromatographed on a Diaion HP-20 column. The elution was started with 1L of H_2O for complete sucrose removal. Then, the adsorbed fraction on Diaion HP-20 resin was eluted in MeOH 100%. This methanol fraction exhibited a high content of flavonoids, according to the TLC analysis, and it was chromatographed on Sephadex LH-20 column in a step gradient mixture of H_2O and MeOH(1:0, 8:2, 6:4, 4:6, 2:8 and 0:1). Fractions eluted in H2O 100% presented a single flavonoid.

Through many analysis, such as HPLC-UV, ESI and RMN (one- and two-dimensional), the isolated flavonoid was identified as tricin-7-O-b-(6"-pmethoxycinnamate)-glucoside.

P1.1-04

Phenolic compounds profile in solutions obtained by microfiltration processof purple cactus pear (*Opuntia ficus-indica*) pulp

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The intake of polyphenols has been inversely correlated with the chronic diseases incidence, such as several types of cancer and cardiovascular diseases.It is known that purple cactus pear is a fruit that containsbetalains (natural red pigment) and can also be a natural source of polyphenols, providing health benefits through its antioxidant properties.

The objective of this work was to evaluate the phenolic compounds profile in solutions obtainedby microfiltration process of purple cactus pear (Opuntia ficus-indica) pulp.

A monotubular ceramic membrane was used with cutt-off 0.2 µm (transmembrane pressure 0.64 bar/ 20°C / 240 min), and the feed solution was pulp:water(1:1).

Total polyphenol content (Folin Ciocalteau method) and profile (HPLC method) were determined in feed, permeate and retentate solutions from the microfiltrationprocess.The total polyphenol contentsin feed, permeate and retentate solutions were 590.1, 218.2 and 241.5 mg GAE (gallic acid equivalent), respectively. The phenolic compounds profile showed mainly isorhamnetin glycosides derivatives, being similar inall solutions. The permeate correspond a clarified solution (without mucilage), which could be used as a source of polyphenols. Acknowledment: FONDECYT-Chile N° 1110126 and CONICYT Chile for the scholarship N° 24110060 and 21090694.

P1.1-05

Chlorogenic acids and lactones in toasted leaves of *Ilex paraguariensis*

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Mate (*Ilex paraguariensis*) is a herb widely consumed in South America, in the form oftea made from dried green or toasted leaves. While in South Brazil consumption of green mate is higher, in Southern Brazil, toasted mate is more consumed. Innumerous health benefits effects have been attributed to this beverage, especially due to its chlorogenic acids (CGA) content. However, data on CGA profile in mate leaves are scarce. This study aimed to investigate the content of CGA and their lactones formed during toasting of commercial dried mate leaves. Thirteen samples were investigated by HPLC-DAD-reverse-phase. Eight CGA compounds were identified in all samples: caffeoylquinic acids (3-CQA, 4-CQA and 5-CQA), dicaffeoylquinic acids (3,4 di-CQA, 3,5 di-CQA and 4,5 di-CQA) and feruloylquinic acids (4-FQA and 5-FQA).Total mean content of CGA was 3.0±1.2g/100g, with CQA, diCQA and FQA, corresponding to 75.5%, 21.6% and 2.9% of total CGA, respectively. 5-CQAwas the major isomer (30% of CGA).For the first time, two cinammoyl-1,5-ã-quinolactones (3-CQL and 4-CQL) were quantified in toasted mate leaves, totaling 0.2 ± 0.1 g/100g. 3-CQL alone contributed with 61.5% of total lactone. The present results indicate that toasted mate leaves are important sources of CGA and CGL.

P1.1-06

Chlorogenic acids'profilein commercial green dried leaves of *Ilex paraguariensis*

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Mate (*Ilex paraguariensis*) is a herb widely consumed in South America, in the form of teas made from dried green or toasted leaves. Green mate is known to be an excellent source of chlorogenic acids (CGA), which potentially exert various health benefits to humans. However, data on the content and distribution of CGA in mate leaves are scarce in the literature. The aim of the present study was to investigate the profile and content of CGA in commercial dried samples of green mate leaves available in the South and Southern regions of Brazil. Eight samples were investigated by HPLC-DAD-reverse phase.Eight CGA compounds were identified in all samples: caffeoylquinic acids (3-CQA, 4-CQA and 5-CQA), dicaffeoylquinic acids (3,4 di-CQA, 3,5 di-CQA and 4,5 di-CQA), and feruloylquinic acids (4-FQA and 5-FQA), totaling, on average, 11.4 ± 1.7g/100g. CQA compounds corresponded, on average, to 58.2% of total CGA, with 3-CQA being the major isomer (3.7±0.9g/100g); DiCQA represented 41.3% of total CGA, with average contents of 4.7±1.0g/100g and with 3,5diCQA being the major diCQA isomer (3.1±0.8g/100g); FQA represented 0,5% of total CGA, with mean of 0.05±0.04g/100g. The present results showed that *Ilex paraguariensis* leaves are the best source of CGA in nature.

P1.1-07

Phenolic compounds from the South American *Prosopis* pods syrup "algarrobina"

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The sweet pods of *Prosopis* species, known as "algarrobo" in South America,have been used as human foodstuff from prehispanic times. At present, algarrobo pods are locally used to produce a syrup known under the name "arrope de algarrobo" and "algarrobina" in Argentina and Perú, respectively. Native fruitsyrups are part of a culinary tradition still present in some places of the continent and are now incorporated into gourmet preparations with regional characteristics. Three commercial samples of algarrobo syrup were purchased in Peru and Argentina and analysed for total sugar, moisture, ash, acid, total phenolic and flavonoid as well as antioxidant activity. The Amberlite-retained fraction of the *arrope* was analyzed by HPLC-DAD and HPLC-ESI-MS to assess the phenolic composition of the samples and the possible association with antioxidant effect. The phenolic constituents identified in the Peruvian syrups were isoschaftoside / schaftoside (6-α-arabinopyranosyl-8-β-glucopyranosylapigenin or its isomer) and Vicenin II. The high similarity of the phenolic profiles of arropes from Peru suggest that the same algarrobo species is used for algarrobina production in northern and central Peru, while a less clear picture is observed for the Argentinean sample. Acknowledgements: FONDECYT 1120096

P1.1-08

Comparison of antioxidant and anti-inflammatory activities of peach (*Prunus persica* (L.) Batsch) and products *in vitro* and *ex vivo*

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To access the antioxidant and anti-inflammatory effects of peaches and its products we conducted some experiments in vitro and in slices of kidney, liver, heart and cortex of rats. The parameters evaluated in vitro demonstrated that the peach and the peel have similar antioxidant capacity. The peach in syrup despite having this capability is evident to have less antioxidant capacity. In addition to inhibiting about 40% of glycation the peach and the peel demonstrate great amounts of carotenoids and flavonoids. Liver, Kidney, Cortex and Heart slice systems were prepared from rats, and incubated in Kreb's Ringer Hepes medium with different products of peach. Anti-inflammatory parameters were evaluated in medium and antioxidant effects were assessed in tissues showing that the production of cytokines and parameters of stress were significantly decreased in kidney, liver and cortex, proving beneficial effects of peach and its products. We concluded that peach and peach's products have a high antioxidant and anti-inflammatory effect in vitro and in slice methods indicating a possible protector effect to human diseases.

P1.1-09

***In vitro* model of Brazilian craft beers antioxidant properties in comparison to large-scale beers**

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The Brazilian´s craft beer market has been growing annually. Hops are responsible for 100% of the antioxidant properties in final product due to the presence of flavonoids as xantohumol and isoxantohumol in its composition. We evaluated the non-enzymatic antioxidant protection potential *in vitro* of craft beers compared to large-scale beers. Samples of 04 different craft beers, as well their respective concentrations of ethanol (tripel= 11%; Red Ale= 8%; ESB= 6%; blonde= 5%) and hops extract (tripel = 1.80 g hops/L; Red Ale= 2.80 g/L; ESB= 3g/L and blonde = 1.08 g/L) were compared to 04 large-scale beer and a system using TRAP assay. Possible differences were evaluated using the area under the curve (AUC; P<0.0001). Craft beers presented a protective ability significantly higher than those from the large-scale beers tested, possibly by the greatest amount of hops used in recipes. All beers differed significantly in relation to the system. Large-scale beers were not different from each other in antioxidant non-enzymatic potential. The ethanol concentrations showed no difference compared to the system. All hop extracts differed in relation to the system, except the blonde ale. These data suggests that craft beers have higher capacity of antioxidant capacity than large-scale beers.

P1.1-10

Contributions of LC-NMR analysis for the quality control of green tea (*Camellia sinensis*)

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Tea of *Camellia sinensis* is the second most consumed beverage in the world after the water, and green tea is one of the most important varieties. Catechins (phenolics compounds)are the representative metabolites of green tea, with several pharmacological properties reported, such as antioxidant and metabolism accelerator. In this study a green tea infusion was prepared as consumed by the population aiming the identification of the metabolites ingested. LC-NMR was used to analyze the lyophilized extract of green tea infusion and enabled identify several metabolites. As expected, catechins (Epigallocatechin gallate - catechin found naturally and almost exclusively in green tea, Epicatechin and Epigallocatechin) and caffeine were the main metabolites identified. Furthermore, LC-NMR analyses showed metabolites not yet reported for green tea, which co-eluted with peaks that has been characterized only by LC-DAD. Theseresults demonstrate the potentialof this technique for complex mixture analyses, since the NMRdetector iswider thanthe DAD. There is no standardized technique for controlling the quality of green tea. Thecombined use ofNMRandDAD as LC detector could be the techniques of choice for a detailed structural characterization of the natural mixture.

P1.1-11

Bioactive coumarins and HPLC-PDA-ESI-ToF-MS metabolic profiling of edible queule (*Gomortega keule*), an endangered endemic Chilean species

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Antioxidant guided fractionation of the methanolic extract from the edible fruits of the endangered endemic Chilean species *Gomortega keule* (Molina) Baillonled to the isolation of eight antioxidant compounds, including rare highly oxygenated coumarins, besides the more common antioxidant compounds rutin and chlorogenic acid. The isolation of the compounds was achieved by preparative C_{18}-HPLC and the structural elucidation was performed using ^{1}H- and ^{13}C-NMR techniques. A metabolomic fingerprint was generated and 32 compounds were detected and analyzed on the basis of HPLC-PDA and HR-ESI-ToF-MS. Main compounds as well as total phenolic and flavonoid content were also quantified by spectroscopic methods. The major compounds, chlorogenic acid (51.80 ± 1.49 mg/100 g dry weight) and dimethyl-fraxetin (9.57 ± 0.11 mg/100 g dry weight), as well as total phenolic and flavonoid content were also quantified by spectroscopic methods. This is the first report of antioxidant capacity and polyphenolic content of the edible queule fruits.

P1.1-12

Identification and quantification of polyphenols in Chilean propolis and evaluation of its antimicrobial activity on *Streptococcus mutans*

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Streptococcus mutans is accepted as the main cariogenic agent. Today, there is a great interest in alternative treatment, opposite to non-specific mechanical plaque removal or application of broad-spectrum antibacterials that are currently used. The aim of this study was to identify and to quantify the main polyphenols presents in the Chilean propolis and to evaluate its antimicrobial activity on *S. mutans*. The polyphenols were identified by means LC-MS-MS and quantified by HPLC. The minimum bactericidal concentration (MCB) was determined by the dilution method on microplates. Were identified flavons (apigenin), flavanones (pinocembrin), flavonols (galangin, quercetin, myricetin, kaempferol, rutine, and cafeic phenylester acid - CAPE) and phenolics acids (clorogenic, galic, caffeic, cumaric and chatecol). The main compounds identified were apigenin, pinocembrin and CAPE. The ethanolic extract of propolis and the commercial polyphenols mixtures showed an important antimicrobial activity on *S. mutans*. This effect can be attributed to the chemical composition of this natural product and its higher content of polyphenols. However, furtherstudies are necessary to elucidate the molecular mechanism involved in the antimicrobial activity mediated by polyphenols from Chilean propolis.Financial support: CONICYT (Fellowship) and DIUFR0 (DI12-TD02)

P1.1-13

Flavonoids and antioxidant activity of *Cassia australis* (Fabaceae, Leguminosae)

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Cassia australis is a small-medium bush and may reach up to 2 meters and e 2,5 meters of diameter.Occurs at sandbankof the Brazilian coast, mainly in Rio de Janeiro, Espirito Santo, Bahia, Sergipe, Alagoas and Pernambuco states. So far there is no information published concerning the phytochemistry of the species. This work has the goal to study the composition and content of flavonoids and evaluate the antioxidant activity.
The more polar partitions (ethyl acetate and n-butanol), obtained from hydro-methanolic extract, shown by HPLC/UV analysis the presence of flavonoids like flavones and flavonol glycosylated, respectively.
Two new flavones were isolated and identified, described for the first time in the genus, from the ethyl acetate partition: tricetin-4'-methoxy-3'-β-D-glucosyde and isoscutellarein-6-C-β-glucosyde. The flavones were identified by spectroscopic methods such as ^{1}H NMR, ^{1}H-^{1}H COSY, HSQC, HMQC e HMBC.
Total phenolic contents were determined by the Folin–Ciocalteau method and the antioxidant activity was evaluated by DPPH. The results showed an excellent activity, even with the glycosydes, for the ethyl acetate and n-butanol partition (EC_{50} = 5,55µg/mL e 16,8 µg/mL, respectively) and a high phenolic content (620 uAG/mg and 332 UAG/mg, respectively).

P1.1-14

Flavonoids of commercial flower of Asteraceae family sold in Rio de Janeiro, RJ, Brazil

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Tagetes is a genus containing about 55 species, many of them commonly used as ornamental flowers. There are some information about the composition of flavonoids previously described. So far there is no information published concerning the flavonoids of the speciescommercialized in the state of Rio de Janeiro. This work has the goal to study the composition and content of flavonoids of commercial flower of Asteraceae family sold in Rio de Janeiro, RJ, Brazil.
The more polar partitions (ethyl acetate and aqueous residue), obtained from hydro-methanolic extract of the flowers, shown by HPLC/UV analysis the presence of flavonoids like flavones and flavonol glycosylated.
Three flavonoids were isolated and identified: A flavonol were isolated from the aqueous residue (Kaempferol-3,7-di-O-rhamnoside) and two flavones were isolated from the crude extract (Apigenin-7-O-glucoside and Luteolin7-O-glucoside). The flavonoids were identified by spectroscopic methods such as ^{1}H NMR, ^{1}H-^{1}H COSY, HSQC, HMQC e HMBC, and by mass spectrometry (ESI). The flavones were separated by semi-preparative HPLC.
Many tests are being done with the purpose of show a new biological activity of flowers like viral activity and analgesic activity. The results are promising and are being finalized.

P1.1-15

Comparative evaluation of antioxidant capacity in fractions of anthocyanins and flavonol-phenolic acids from calafate (*Berberis microphylla*)

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Calafate (*Berberis microphylla*) is an underexploited endemic fruit growing in Chile and Argentine Patagonia. A recent research demonstrated their great nutraceutical potential, due to their high concentrations of anthocyanins and hydroxycinnamic acids. In this research, antioxidant capacity in different fractions of anthocyanins and flavonols–phenolic acids of calafate were tested. The results were compared with fractionsof same families of compounds from *Vitis vinifera L.* "Red Globe" and "Pink Globe".
Concentration of calafate fractions varied between 0.60–31.68 µmol/g of total anthocyanins, 0.26–1.78 µmol/g of total flavonols and 0.68–4.94 µmol/g of total hydroxycinnamic acids.
CUPRAC and ABTS antioxidant capacity were higher for anthocianins fractions of calafate (between 13 to 50% and 4 to 30% respectively) than flavonols-phenolic acids fractions (between 7 to 20% and 3 to 10% respectively). A tendency of higher antioxidant capacity was observed for the most concentrated anthocyanins fractions. The sum of both fractions represents 20-66% of $TEAC_{CUPRAC}$, meanwhile represents 8-32% of $TEAC_{ABTS}$, demonstrating that other bioactive or reducing molecules are present in the whole calafate extract. Comparatively, the values obtained are between 2 and 10 times higher than those for *Vitis vinifera L* berries.

P1.1-16

Phenolic profile of beverages obtained by gluconic fermentation of strawberry

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Strawberry is a source of bioactive compounds as (poly)phenols, carotenes, melatonin. Nevertheless it is a very perishable fruit with a short term life and commercialization period. As a consequence surplus production is often thrown away. The transformation into derived products is an alternative to prevent food loss and agronomic wastes.
This work focuses on the elaboration of drinks from strawberry by a selected fermentation process. The selected *Gluconobacterjaponicus* transforms glucose into gluconic acid while fructose remains unaltered providing the product sweet properties. Furthermore, this derived product is intended to retain the bioactive compounds and the additional healthy value of use by diabetic patients.
Phenolic profile has been determined by LC-MS/MS describing up to 22 compounds. The composition has been determined throughout four fermentation cycles including starting point, final point and after pasteurization. Results proved there are not significant changes after the elaboration process. Therefore, this drink maintains the bioactive composition and healthy properties.
We are grateful for the funding provided by the Spanish Government (Project MICINN AGL2010-22152-01), and to HUDISA Desarrollo Industrial S.A. (Spain), the University of Cordoba (Spain) and the University of RoviraiVirgili (Spain) for providing the samples.

P1.1-17

Optimization of extractionof phenolic compounds frommurici (*Byrsonimacrassifolia* (L.) *Kunth*)by response surface methodology

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Considering that the extraction of the active ingredient is essential if they are to be of prophylactic or therapeutic value, this study aimed to optimize the extraction conditions for phenolic compounds from murici (*Byrsonimacrassifolia*(L.) Kunth) using response surface methodology (RSM).A central composite rotatable design (CCRD) was applied to investigate the effects of: solvent composition (%), extraction temperature (°C), time (min) and the solvent to solid ratio (mg/mL) on total phenolic content (TPC) from murici.Among the tested solvents initially (water, ethanol, methanol and acetone), acetonewas more efficient for extraction of polyphenols. Acetone concentration, time and temperature significantly affected the TPC measured by Folin reagent, whereas the solvent to solid ratio did not ($p > 0.05$). The TPC of the extracts varied from 12.83 mg gallicacid equivalents (GAE)/ 100 mg to 27.04 mg GAE/ 100 mg. Regression analysis showed that more than 84% of the variation was explained by the mathematic model. The optimum extraction conditions were found to be acetone concentration 43.4 % for 50.8 min at 28.9 °C. The experimental values of TPC agreed with those predicted, thus indicating suitability of the model employed and the success of RSM in optimizing the extraction conditions.

P1.1-18

Soybean cake as a potential source of bioactive compounds

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Soybean cake is a byproduct of soybean oil processing often used as feed. However, it has been shown to contain significant amounts of bioactive compounds. In this study we evaluated the potential of soybean cake as a source of isoflavones and soyasaponins. Soybean cake from soybean oil industry (SCI) and produced under laboratory conditions (SCL) were analyzed by spectrophotometric assays for phenolic compounds, flavonoids and saponins. Antioxidant capacity (AC) was evaluated by FRAP, TEAC and ORAC assays. Isoflavones and soyasaponins were also analyzed by LC-DAD-MS. Both cakes showed high levels of phenolic compounds (2.82 mg gallic acid equivalents/g), flavonoids (1.00 mg genistein equivalents/g), saponins (32.47 mg/g) and AC (FRAP: 9.38 µmol Fe^{+2}/g; TEAC: 16.81 µmol Trolox equivalents/g; ORAC: 3.33 mmol Trolox equivalents/g). Despite their similar flavonoid contents, LC-DAD-MS analysis revealed different isoflavone profiles between samples. SCI profile was similar to that of whole soybeans, with higher levels of □-glycosylated and malonylglycosylated isoflavones (0.90 and 0.25 mg/g) than SCL (0.16 and 0.03 mg/g). Aglycone isoflavones, which are the form absorbed by humans, were present in higher levels in SCL (1.00 mg/g) than in SCI (0.23 mg/g). Our results show that soybean cake is a potential source of bioactive compounds with AC.

P1.1-19

Flavanolignans from *Silybum marianum* L. - chemistry and characterization as primary phyproof® reference substances

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Since ancient times milk thistle (*Silybum marianum* L.) fruits are known to have medicinal properties. Recognized medicinal application areas are the supportive treatment of chronic inflammatory liver disorders and cirrhosis of the liver as well as the intravenous treatment of toxic liver damage (e.g. Amanita mushroom poisoning). Completely new areas of application opened up in the 1990s after it was discovered that silymarin and isolated flavanolignans inhibited the spread of tumor cells. The active principle is a mixture of various flavanolignans known as silymarin. According to the Pharmacopoea Europaea silymarin is a mixture of silybin A and B, isosilybin A and B, silychristin and silydianin. So far, the pure diastereoisomers were not commercially available, and thus the isomeric pairs were used for analytical purposes as well as in pharmacological research. For the first time, the isolated flavanolignans silybin A and B, isosilybin A and B, silychristin, silydianin as well as 2,3-dehydrosilybin A and B were characterized as primary reference substances according to international guidelines. These compounds, now available in a certified quality for the first time, can be applied in the quality control of milk thistle preparation as well as in pharmacological research.

P1.1-20

Contribution of bioactive compounds (phenolics and flavonoids) of single-flower honeys of *Euclyptusspp*

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Honey is a complex mixture of carbohydrates and minor compounds produced in nature, its chemical composition varies with its floral origin. They have reported that phenolic compounds could be a potential indicator of biological quality for its antioxidant properties. In several studies of European honeys, there has been demonstrated that the honeys have a notable phenolic profile constituted by several acids and ésteres organic aromatics and for the agliconas of flavonoids.

In this work we analyzed the phenolic composition of honeys from *Eucalyptus spp*. and development a methodology for high performance liquid chromatography which allowed a good separation and identification. The honeys analyzed showed between 80.6 and 98% pollen of *Eucalyptus spp.* and showed profiles of flavonoids in pollen-nectar as major phenolic compounds and phenolic acids scarce. Flavonoids included the flavones (as pinocembrin and pinobanksin, luteolin and apigenin) monohydroxylated flavonols in ring B (only traces of kaempferol in some samples), coumaric acid and quercetin. In conclusion, the results suggest that the honeys of *Eucalyptus spp*. might be a source of compounds biactivos natuarles and these would manage to be constituted in indicators of his biological value.

P1.1-21

Obtaining phenol compound from agroindustrial remains

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Several research works highlight the importance of maintaining balance between free radicals and endogenous and exogenous antioxidants in a healthy organism. It is also well-known the existing suspicion of the carcinogenic effect of synthetic antioxidants, being the benefits of natural antioxidants enhanced. Due to this reason, nowadays, there is a great interest in searching new sources of biological substances with antioxidant activity.
Polyphenol compounds are antioxidants constituting a kind of secondary metabolites biosynthesized by the plant kingdom. The olive tree, as many other plants, increases polyphenols production as a response to environmental factors translated as stress for tissues.
From 1990, there was an expansion of modern olive growing in the country and Catamarca became the main olive oil producer, generating, in consequence, a great amount of agroindustrial remains. These have high phenol content as well as an excellent antiradical activity, so they may become a natural and completely renewable raw material for phenol compounds. These facts would result in competitive advantages for the sector, health improvement and environmental protection. This paper aims at disseminating the results obtained in so far so as to enhance olive industry byproducts for its potential use in the scope of health.

P1.1-22

Inhibition of pancreatic lipase in vitro from polyphenols from camu-camu (*Myrciariadubia H. B. K. McVough*) and their antioxidant capacity

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Brazil is a country that has a natural resource of plants. Although, many of them are unexplored.Camu-camu is a native Amazonian bush from the Myrtaceae family and this fruits have shown a potencial source of bioactive compounds. Theaims of this study was to evaluate the inhibition of pancreatic lipase, antioxidant capacity and polyphenols contents, mainly flavonoids, of camu-camu pulp. The extractions were made by two different solid phases: Polyamide (PA) and C-18. The results showed pancreatic lipase inhibitory effect range IC_{50} mg/ml extract 11±0,01 (C-18), 25±0,01(PA); IC_{50} mg eq. catechin/ml extract 2,18±0,01 (C-18), 0,907±0,02 (PA). In addition, the fruit has high antioxidant capacity and the most flavonoids found in Polyamide and C-18 was ellagic acid, miricetin, hidroxybenzoic acid and syringic acid.The results of this study give scientific support on the potential health benefits and to incentive for further research into bioactive compounds of camu-camu.

P1.1-23

Purification of bioactive phenolic compounds of Cupuassu (*Theobroma grandiflorum*) by activated carbon

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Brazilian native fruits are important food sources of phytochemicals, especially polyphenols, which are considered bioactive compounds with health-promoting activities. Among these, cupuassu stands out for its unique flavor. The objective of this work was to study the purification of phenolic compounds fromcupuassuby adsorption on activated carbon in order to allow further characterization of their biological potentials.
Adsorption of cupuassu to activated carbon (0, 2 g to 10 g) was tested in water and 70% aqueous methanol extracts (1 g lyophilized fruit powder/20 mL), for one hour at 4ºC. Total phenolic compounds (SINGLETON et al., 1999) and proanthocyanidins (PAC) contents (PORTER et al., 1986) were determined after activated carbon removal by filtration. The results were expressed as % of phenolic compounds adsorbed.
Adsorption of both total phenolics and proanthocyanidins was higher in water compared to 70% methanol. Amounts of 1 g of activated carbon or higher, per 20 mL extract, were able to adsorve more than 60% of total phenolic compounds and more than 45% of PAC. In this way, activated carbon can be considered an efficient way to purify phenolic compounds from cupuassu.

P1.1-24

Anthocyanins contents of Jussara (*Euterpe edulis*) juice microcapsules obtained by spray drying depend on the encapsulating agent

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The fruit of Jussara palm tree (*Euterpe edulis*) is recognized by its functional properties mainly related to the contents of anthocyanins. However, these phenolic compounds may be degraded by several processing factors. Microencapsulation is a preservation method in which sensitive compounds are trapped within a coating material. The aim of this study was to obtain microcapsules of Jussara juice using Capsul® and a mixture of Capsul® and inulin (1:1) as encapsulating agents and to evaluate anthocyanins in these microcapsules. The spray dryer was fed with a mixture of juice and the encapsulating agent (1:1) at an inlet temperature of 140 °C. Anthocyanins were analyzed by HPLC-UV. In all samples, we identified both cyanidin 3-glucoside and cyanidin 3-rutinoside. Microcapsules prepared with a mixture of inulin and Capsul® showed higher contents of both cyanidin 3-glucoside (7.14g/kg) and cyanidin 3-rutinoside (8.98g/kg) than those prepared with Capsul® (5.00 and 5.73g/kg of cyanidin 3-glucoside and cyanidin 3-rutinoside, respectively). Good anthocyanins retention (73%) was achieved when the mixture of encapsulating agents was used. Our results show that Jussara juice microencapsulation was viable and that inulin was a more effective encapsulating agent, probably due to its higher hydrophilicity in comparison to Capsul®. Financial Support: CAPES, CNPq, FAPERJ, UFRJ.

P1.1-25

Evaluation of antioxidants compounds of fermented *jabuticaba* (*Myrciaria cauliflora*) alcoholic beverage

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Through the increasing concern for human health, the exploration for new research on compounds with antioxidant activity have increased daily that are discovered or new food products, which have the ability to match free radicals and prevent diseases. In this context, the aim of this study was evaluate the antioxidant potential of fermented alcoholic *Jabuticaba* (*Myrciaria cauliflora*) produced a winery in the city of Varre-Sai-RJ. To conduct this study were acquired three types of fermented *Jabuticaba* at a winery City Varre-Sai-RJ, were evaluated under the soluble solids, pH, antioxidant capacity by DPPH method and the determination of flavonoids and polyphenols content. Results showed the alcoholic beverage *Jabuticaba* reds had a higher antioxidant capacity compared to fermented alcoholic *Jabuticaba* white probably due to the fact that they present about 15 folds (7.5µ mols of catechin) more flavonoids and 10 fold (800 mg Eq A.G/100g M.F) of polyphenols than white, which is produced only with fruit pulp, portion containing a low concentration of polyphenols. Thus, we conclude that the study of the antioxidant potential of fermented alcoholic *Jabuticaba* produced handcrafted in Varre-Sai-RJ is important to better target the applicability of these beverages with functional properties, in preventing and combating various chronic diseases.

P1.1-26

Effects of protein on the antioxidant capacity of guarana (*Paullinia Cupana*) under conditions of *in vitro* digestion

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The information concerning the antioxidant capacity of polyphenols in the presence of dietary nutrients are limited. The aim of this work was to evaluate the effects of protein on the antioxidant capacity of guarana powder (*Paullinia cupana*) under conditions of *in vitro* digestion. Samples of guarana and a mixed of guarana and casein were subjected to an in vitro enzymatic digestion with pepsin, pancreatin and bile salt. These samples were analyzed for total phenolics (TP) by Folin-Ciocalteu, for procyanidins (PC) by butanol-HCl hydrolysis, for Trolox equivalent antioxidant capacity (TEAC) assay with ABTS radical anion and oxygen radical absorbance capacity (ORAC) using fluorescein as the fluorescent probe. Analysis of variance (ANOVA) and significance test ($p<0.05$) were realized using the SAS software. The mixed of guarana and casein showed the highest values for TP and PC. Also, the antioxidant capacity was increased with the addition of casein, by ORAC this was 19.9% higher than only guarana and by TEAC this increased was 55%. The interaction of protein-polyphenol could be affected by several parameters such as the temperature, pH, type and concentration of protein, and the type and structure of phenolic compounds. The protein increased the antioxidant capacity and the bioaccessibiliy of polyphenols from guarana powder. Key Words:Antioxidant capacity, in vitro digestion, guarana, protein, phenolic compounds.

P1.1-27

Determination of phenolic acids by HPLC-DAD of Cape gooseberry pulp (*Physalis peruviana L.*)

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Cape gooseberry (*Physalis peruviana L.)* is a potential candidate for processing new functional foods because of their nutritional properties as well as their biologically active components.

This study aims at the identification and quantification of phenol acids from physalis pulp. It includes the extraction of free and bound phenolics. The knowledge of free and bound phenolics in fruits and vegetables could provide us some insight into their potentials of improving human health.

The identification and quantification methodology was made by High Performance Liquid Chromatography analysis (HPLC) with a diode array detector. The extraction of free phenolic acids (PAs) was made with acetone, and the bound phenolic acids include a basic hidrolysis with NaOH, then a neutralization and a liberation of PAs with ethyl acetate.

Five phenolic compounds were detected and quantified both in free and bound fractions from the pulp. Namely, syringic, vanillic, p-coumaric, trans-ferulic and cinnamic acids. Both free and bound phenolic contents ranged from 2.36 to 4.44 mg /100 g d.m. and from 0.56 to 3.0 mg/100 g d.m., respectively. Acknowledgments.The authors gratefully acknowledge the financial support of project Regular FONDECYT N°1120102.

P1.1-28

Antioxidant properties of polyphenol-enriched extract from *Syzygium cumini* leaf

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Syzygium cumini (L.) Skeels – SC (Jambolão) is popularly known for its hypoglycemic and hypolipidemic properties. In Northest Brazil, differing from other countries, leaf is the main part applied for remedies preparation. Thus, in the present work, we aimed to evaluate antioxidant properties and characterize polyphenolic composition of a polyphenol-enriched extract prepared from SC leaf. Air-dried and powdered leaves were macerated in 70%EtOH (1:6 w/v) to render the hydroethanolic crude extract (HCE), which was further partitioned with chloroform and ethyl acetate to render the polyphenol-enriched extract (PEE). Polyphenolic content was assessed by Prussian Blue Method and expressed as gallic acid equivalents (GAE). PEE (71.8± 8.6 GAE/100g) had a threefold higher polyphenolic content than HCE (22.3±1.1 GAE/100g). Concerning antioxidant activity, PEE was found to be significantly more potent than HCE in both $DPPH^{\bullet}$ (IC_{50}: 54.9±1.2 *vs.*140.8±1.2 GAE/100g) and $ABTS^{\bullet+}$ (IC_{50}: 2.5±1.2 *vs.* 9.2±1.2 ug/ml) assays. Noteworthy, PEE IC_{50} value for $ABTS^{\bullet+}$ did not differ from quercetin (IC_{50} 1.8±1.2 ug/ml). Preliminary HPLC-MS/MS, indicate the presence of ellagitannins like hexahydroxydiphenoyl-hexoside ($[M-H]^-$at m/z 481), galoil-hexahydroxidiphenoyl-hexoside ($[M-H]^-$at m/z 633) and pedunculagin I ($[M-H]^-$at m/z 783). Taken together, our data reinforce the importance of *S. Cumini* leaf as an important source of polyphenols and antioxidants. *Financial Support:* FAPEMA, CAPES and UFMA.

P1.1-29

Identification and quantification of di-C-glycosylapigenins in carob germ flour

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Carob germ flour is prepared from seeds of locust bean (carob, *Ceratonia siliqua*) and is used as a food color additive in Japan. The color of carob germ flour is light yellow but turns to yellow when the flour is kneaded into noodles mixed with alkaline salts. This change is due to bathochromic shift of flavonoids in the flour under alkaline condition. While flavonoids composition in carob leaf, pod and cotyledon has already been reported, however, the identification of flavonoids in carob germ remains unknown. Therefore, alcoholic extract from carob germ flour was analyzed using UPLC/TofMS and flavonoids were identified with NMR experiments. Major flavonoids are schaftoside (6-*C*-α-D-glucopyranosyl-8-*C*-β-L-arabinopyranosylapigenin) and its Wessely-Moser isomer, isoschaftoside. As minor flavonoids, anomeric and/or structural isomers of arabinose moiety in schaftoside and isoschaftoside were identified. Furthermore, 4′-*O*-glucosides of these di-*C*-glycosylapigenins were also detected. These di-*C*-glycosylapigeninderivatives were quantified using UPLC with apigenin as astandard and the total contentin the flour was1.13% (w/w).In addition, fresh carob germ and endosperm, which was raw material for locust bean gum, were prepared from the beans and analyzed. The results showed that di-*C*-glycosylapigenin derivatives content of germ was approximately twenty times higher than those content of endosperm.

P1.1-30

High hydrostatic pressure preserves anthocyanins in Jabuticaba (*Myrciaria cauliflora*) juice

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Jabuticaba (*Myrciaria cauliflora*) is an anthocyanin-rich fruit native to Brazil. However, the short crop period and high perishability of this fruit restrain its wide commerce and consumption. High hydrostatic pressure (HHP) is a non-thermal food processing method that preserves the sensory and nutritional qualities usually lost during traditional thermal processes. In this context, we evaluated the effect of HHP on anthocyanins contents in Jabuticaba juice. After vacuum packing, juice was pressurized (in duplicate) at 200 MPa for 5 and 10 min. Pressurized and control (untreated) juice samples were centrifuged, filtered and analyzed by HPLC-UV (530nm) using a C18 column and gradient elution with water and acetonitrile, both added with formic acid. Juice pressurized for 5 min showed a higher cyanidin-3-O-glucoside content (1.57mg/L) than both control (1.11mg/L) and 10 min pressurized (0.89mg/L) juices. No difference was observed between control and 10 min pressurized juices. The increase in cyanidin-3-O-glucoside content after HHP treatment for 5 min may be related to changes in membrane permeability and disruption of cell walls favoring the release of this anthocyanin from tissue and, in consequence, improving its extractability. Our results suggest that HHP is a suitable alternative preservation technique for Jabuticaba juice processing in the food industry. Financial Support: CAPES, CNPq, FAPERJ, UFRJ.

P1.1-31

Calafate, a superfruit from South Patagonia

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Calafate (*Berberis microphylla*) is an endemic non cultivated shrub with dark reddish-blue berries that grows in the Chilean and Argentinian Patagonia. These berries are very rich in anthocyanins and have been consumed since prehispanic times, while their roots are used as colorants due to the presence of berberine. Anthocyanins, hydroxycinnamic acids (HCAs) and flavonols are important groups of phenolic phytochemical compounds in berries, being considered to be nutritionally important because of their antioxidant activities, which are correlated with important biological activities.

In this research, the study of these beneficial compounds in a large number of calafate samples was addressed. Their antioxidant capacity was also studied by TEAC method. Quali-quantitative results for HCAs, flavonols, anthocyanins and berberine were 3.57, 1.37, 19.28 µmol/g and 1.00 mg/100 g respectively, being obtained by HPLC-DAD-ESI-MS/MS and by NMR for correct identification. The antioxidant capacity was 58.7 µmol/g, thus suggesting that calafate could be considered as a superfruit from the point of view of their nutraceutical composition. These results from different geographic origins were analyzed by chemometric tools and were compared with their genetic diversity data (AFLP and SSR), concluding that the observed differences in phenolic profiles would be better explained by environmental than by genetic factors.

P1.1-32

Study of degradation kinetics of anthocyanins and color in elderberry and blackcurrant pulps

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Anthocyanins, responsible for color in most berries, easily degrade following various reaction mechanisms affected by pH and temperature principally. The objective of this work was to study the degradation kinetics of anthocyanins content (TAcy) and color parameters during heat treatment at different temperatures, for elderberry and blackcurrant pulps from "El Bolsón" Río Negro. Pulps were heated at constant temperatures (70, 80 and 90°C). For each sample removed at regular time intervals, TAcy was measured at 520nm with pH-differential method. In addition, evolution of percent polymeric color (%PC) was evaluated. Color parameters were obtained with CIELab method using a Minolta Spectrophotometer. TAcy and color degradation followed first order kinetics. Rate constants for TAcy were 0.0264h^{-1}, 0.0863 h^{-1} and 0.4337 h^{-1} for elderberry and 0.0333h^{-1}, 0.0969 h^{-1} and 0.2588 h^{-1} for blackcurrant at 70, 80 y 90 °C respectively. In the same order of temperatures, half-lives times ($t_{1/2}$)for TAcy degradation were 26.25 h, 8.03 h and 1.6 h for eldelberry and 20.81h, 7.15h, and 2.68 hfor blackcurrant. Reaction rates for color parameter a* were 3 times lower than for Tacy. However, the temperature dependence was similar for both reactions, obtaining E_a values of 147 kJ/mol for elderberry and 110 kJ/mol for blackcurrant.

P1.1-33

Effect of extraction solvents on the phenolic compounds and antioxidant activity of jatobá-do-cerrado (*Hymenaea stigonocarpa* Mart.) flour

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The Brazilian Savannah has an extensive biodiversity, but it is underexplored. Among the native vegetables, the jatobá-do-cerrado (*Hymenaea stigonocarpa* Mart.), a leguminouswith great potential for exploration because it's an excellent source of dietary fiber and carotenoids. However, there are not studies about the contents of phenolic compounds (TPC) on this legume. The aim of this study was to evaluate the effects of three selected solvent systems at ambient temperature for 60 min on the TPC and antioxidant activities (AA) of jatobá. The solvents systems included water, 60% acetone (v/v) or 60% methanol (v/v). The TPC was evaluated by Folin reagent and AA were tested using ORAC, radical scavenging activities against DPPH·, ferric reducing antioxidant power (FRAP) and Rancimat®. The major TPC was obtained using acetone 60% (536.15 mg gallic acid equivalents-GAE/100g), followed by water (315.36 mg GAE/100g) and methanol 60% (124.97 mg GAE/100g). The main result for AA was also observed in acetone extract (ORAC-119.79 µM Trolox equivalents/g; FRAP-106.40 µM Fe(II)/g; DPPH-IC_{50} 1.44 mg/mL). However, all extracts were ineffective for inhibiting the oxidation of fat in the system Rancimat®. The solution 60% acetone is a recommended solvent for extracting phenolic and other antioxidants from jatobá for analytical purposes.

P1.1-34

Grape cane extracts: a rich source of stilbenes with antioxidant capacity

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The evidence of the health benefits of *trans*-resveratrol has increased during the last twenty years. This fact has triggered the interest in studying the levels of this and other stilbenes in grapes, wine and winemaking residues. In Chile, more than 100.000 tons of winemaking residues are produced each year, including pomace, seeds, stalks and canes. In this research, profiles and levels of stilbenes in these waste products are studied by using liquid-solid extraction with ethanol-water 80% and HPLC-DAD-ESI-MS/MS in negative ionization mode. Main results show that ε-viniferin is the main stilbene in these matrixes, followed by *trans*-resveratrol, except for canes, where *trans*-resveratrol was higher. Total stilbenes (expressed as *trans*-resveratrol equivalents in dry matter) were 22.81 ± 10.59 mg/kg; 5.17 ± 2.02 mg/kg; 0.93 ± 0.44 mg/kg and 4485.87 ± 200.28 mg/kg in stalks, pomace, seed and cane, respectively. Considering these results, a stilbene rich extract was produced at pilot scale (7 L reactor) using canes, obtaining a dry extract which contained 15 % of stilbenes. The antioxidant activity of this extract is being studied using cell free and cell based antioxidant assays (TEAC, ORAC, CUPRAC and HUVECs).

P1.1-35

Total phenol content in seeds of *Cucurbita spp* Chaco province

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The objective of this study was to evaluate the phenolic content and antioxidant activity in four varieties of Cucurbita spp seeds: Tetsukabuto (hybrid between C. moschata and C. maxima Duchesne ex Lam.), C. Pangalo mixed (striped pumpkin), C. moschata (Duchesne ex Lam.) Duchesne ex Poir. (Coreanito) and C. maxima Duchesne (pumpkin Lead). Extracts were obtained from decreasing polarity 10 grams of seed. Total phenols were determined according to the Folin-Ciocalteu method and their content was: Tetsukabuto 41.37 ± 1.15, 68.77 ± 1.10 striped pumpkin; Coreanito 29.69 ± 1.14, 53.22 ± Lead 1.61 (mol GAE / g sample). The antioxidant activity was calculated on the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and is expressed as median inhibitory concentration (IC50) showing the following values; Tetsukabuto 29.98 ± 1.53; Striped Pumpkin 19.94 ± 0.344; Coreanito 27.89 ± 0.20; Lead (mg / ml)22.63 ± 1.32. Fisher test (LSD) revealed that there are two homogeneous groups, formed by C. Pangalo mixed and C. maxima Duchesne which are those with higher phenolic content and antioxidant activity. The results obtained show that these seeds can be considered a source of natural antioxidants.

P1.1-36

Study of the antioxidant properties and stability of *Eucalyptus globulus* and *Calendula officinalis* extracts for synthetic additive replacement

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Plant extracts have emerged as sources of natural antioxidants for food formulations to replace synthetic additives that are currently being questioned by consumers. Eucalyptus leaves, oil, fruits and barks and marigold flowers have been used in traditional medicine due to their anti-inflammatory, antibacterial and analgesic properties. The aim of this work was to determine the experimental conditions to maximize the polyphenols extraction from eucalyptus dried leaves *(Eucalyptus Globulus*) and from marigold dried flowers *(Calendula officialis*) and also to analyze the relationship between polyphenols content and antioxidant activity.Extracts were obtained by stirring in water-methanol and water-ethanol solutions. After vacuum evaporation of solvents, extracts were freeze-dried and stored under nitrogen atmosphere. UV-Vis spectra of samples were determined in the range 800-200 nm. Total phenolic content (TPC) was evaluated by Folin–Ciocalteu and the antioxidant capacity (AOC) by HPLC with electrochemical detector and by DPPH assay. The maximum polyphenol content was achieved in methanol-water 70:30. A direct correlation between the amount of TPC and AOC was observed for all the analyzed extracts. Eucalyptus showed higher absorbance at 300-270 nm, and higher TPC contents and AOC than marigold extracts. Eucalyptus extracts could be sources of antioxidants to employ in the formulation of functional ingredients.

P1.1-37

Antocyanins, tocopherols and antioxidant capacity of the oil from Jussara (*Euterpe edulis*) fruit depend on the extraction solvent

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Jussara (*Euterpe edulis*) is an exotic fruit native to Brazil rich in lipids and anthocyanins. The aim of this work was to obtain oils from Jussara fruit by cold extraction with either petroleum ether (JPO) or ethanol (JEO) and to evaluate anthocyanins and tocopherols by HPLC-UV, total phenolic compounds and total flavonoids by spectrophotometric methods and antioxidant capacity (AC) by TEAC assay in these oils. Although JPO extraction yield was higher (48.9%) than that of JEO (37.7%), JEO showed higher contents of all evaluated compounds, as well as higher AC. Cyanidin 3-rutinoside and cyanidin 3-glucoside contents were 24-fold higher in JEO (22.1 and 21.1mg/100, respectively) than in JPO. Tocopherols contents were 1.6-fold higher in JEO (150.9mg/100g) than in JPO. Alpha- and delta-tocopherols corresponded to 56% and 40% of total tocols, respectively. Total phenolics and total flavonoids contents were 1.8 and 3.1-fold higher in JEO (12.8mg gallic acid equivalents/100g and 30.8mg catechin equivalents/100g, respectively) than in JPO, respectively. AC was also higher (24-fold) in JEO (8.4mmol Trolox/kg) than in JPO, probably due to the higher contents of antioxidant compounds in JEO. Our results show that JEO is rich in functional compounds with potential applicability in the food and cosmetic industries. Financial Support: CAPES, CNPq, FAPERJ, UFRJ.

P1.1-38

Effect of roasting on melanoidins, chlorogenic acids and antioxidant capacity of mate (*Ilex paraguariensis*)

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Mate's roasting leads to reduction on chlorogenic acids (CGA) and formation of melanoidins. However, little is known regarding the contribution of these components to mate's antioxidant capacity (AC). Thus, we evaluated the effect of roasting on CGA and melanoidins of mate's infusions and related these components to AC. Additionally to green mate, roasted samples were collected during industrial processing and classified according to color (light, medium, dark and very dark). CGA were analyzed by LC-DAD-MS. Clarified infusions (CI) were prepared using Carrez's solutions to eliminate melanoidins. Melanoidins fractions (MF) were obtained by ultrafiltration. AC was evaluated by the FRAP assay in whole infusions, CI and MF. A maximum of MF was observed in light mate (341mg/L), decreasing in darker samples. Considering green and very dark samples, the observed percent decrease in CGA content (91%) was more pronounced than that of whole infusion AC (37%) and similar to that of CI AC (94%). On the other hand, a 5-fold increase in AC between green and very dark MF (0.55 to 2.76 mmol Fe^{2+}/mg) was observed. These results indicate that besides CGA, melanoidins are important contributors to mate's AC, probably due to the incorporation of CGA into melanoidin's backbone during roasting.
Financial support: CAPES, CNPq, FAPERJ, UFRJ.

P1.1-39

Grape Canes: Influence of post-pruning storage on stilbene levels

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The main aim of this research was to determine the effect of post-pruning storage on stilbene levels in grape cane. Stilbenes were extracted with an ethanol/water mixture 80:20 (v/v), using an ultrasonic bar for 5 min. Then, the extracts were analyzed by HPLC-DAD-ESI-MS/MS. In all samples, the predominant stilbene was *trans*-resveratrol, followed by ε-viniferin. In whole canes (cv. Pinot Noir) stored after pruning at room temperature, stilbenes presented a significant increase after two months. The yield raised up 5.2 times, reaching 4.777 mg/kg dry matter. This effect does not occur in frozen, lyophilized or milled material. Branches obtained directly from the plants and remaining on the field without storage after pruning, showed no significant change in stilbene levels, containing only 611 mg kg^{-1}dry matter. One possible explanation is that storage at room temperature and injury caused by pruning triggers stilbene biosynthesis, but the involved biochemical mechanism is still unknown for grape cane. Another alternative is that in fresh non-aged grape cane stilbenes are bound to other components of canes and thus are less extractable. In both cases, storage of whole canes at room temperature for at least 3 months is advisable to increase the extraction yield. Grant: FONDECYT 1110767

P1.1-40

Antioxidant capacity and metabolic profiling of the Chilean "copao" fruit (*Eulychnia acida*, Cactaceae)

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Copao (*Eulychnia acida* Phil.) is endemic Cactaceae specie occurring in the Coquimbo region, Chile. The fruits are collected and commercialized by local harvesters within the Elqui and Limari valley. This activity is relevant for the small agricultural communities in the semi-desertic areas from the Limari to Copiapo river valleys. We now report the total phenolic and flavonoid content, antioxidant activity and phenolic fingerprint from "copao" fruits collected during 2012-2013 in different location from the Elqui and Limary valley.
Fruit pulp and epicarp were separately analyzed for comparison of constituents and antioxidant activity, including bleaching of the DPPH free radical, ascorbic acid content and FRAP. The identity of phenolic compounds from the Amberlite-retained methanolic extract of the fruit pulp and epicarp was assessed by HPLC-DAD-MS/MS. Two main groups of compounds were identified according to their retention time and UV-spectrum, namely simple phenolics and glycosyl flavonoids. The main compounds were isorhamnetin rutinoside and quercetin derivatives. The HPLC pattern allows a clear differentiation of samples from the different valleys. The compounds identification, associated with the antioxidant activity, adds relevant information for the development of this native fruit into the nutraceutical products business. Acknowledgements: FONDECYT 1120096 and CONICYT-PCHA/Doctorado Nacional/ 2013-21130048 for financial support.

P1.1-41

Use of thyme essential oil to improve the nutritional profile of minimally processed lettuce

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Thyme essential oil (TO) (*Thymus vulgaris*) is recognized for its flavor and aroma and as antimicrobial and antioxidant agent. The high volatility and reactivity of TO and its strong odor limits its application in foods. Encapsulation of TO in β-cyclodextrin (β-CD) can diminish these problems and also improve the nutritional profile of foods. The effect of different TO treatments (0,0.5,1 and 1.5g/L) and TO-(β-CD) capsules (0,19.2,38.5 and 57.7g/L) on total phenolic content, total flavonoids, antioxidant capacity (DPPH and TEAC) and sensorial quality was investigated during storage of minimally processed lettuce at 5ºC. Concentrations tested of TO and TO-(β-CD) were equal on thymol content. The most effective concentrations of free TO (1 and 1.5g/L) significantly increased total phenolic content and DPPH of lettuce during the first 5 days of storage. However, at the end of the storage, these values were similar to those found for the control and the produce did not present acceptable sensorial quality. TO-(β-CD) capsules (38.5 and 57.7g/L) showed the highest total phenolic content, antioxidant status and sensorial quality throughout the storage. Results demonstrate the disadvantages of applying free TO and the effectiveness of the β-CD as a carrier of TO antioxidants to produce healthier fresh vegetables.

P1.1-42

Polyphenols, curcuminoids, and antioxidant capacity of turmeric and curry powder

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The main of this work was to determine the polyphenols content and the antioxidant activity of three different brands of both turmeric and curry powder. Soluble (SP) and hydrolysable (HP) polyphenols content were determined using the Folin-Ciocalteu's reagent. The antioxidant capacity was determined by the methods DPPH and FRAP and curcuminoids content was determined by HPLC. The polyphenols content in curcuma ranged from 1.4 to 1.8 mgGAE/g (SP) and 2.7 to 3.7 mgGAE/g (HP), while in curry powder it ranged from 1.5 to 1.8 mgGAE/g (SP) and 2.7 to 3.7 mgGAE/g (HP). The HP extracts presented the highest antioxidant capacity than SP extracts. The content of curcumin in turmeric ranged from 0.98 to 1.57 mg/g and in curry it ranged from 0.20 to 0.98 mg/g. We concluded that the content of polyphenols, curcuminoids, and the antioxidant capacity did not vary amongst the different brands of spices. However, curcumin content was lower in curry most likely because this condiment is a mixture of spices. Supported by: FAPERJ, CAPES, and FAF.

P1.1-43

Assessment of the photochemoprotective proprieties and *in vitro* and *in vivo* skin penetration of an Amazonian plant incorporated topical formulation

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The aim of this study was to investigate the ability of an enriched polyphenol fraction obtained from *Byrsonima crassifolia* (*Bc*) leaves, to avoid the UVA induced oxidative damage in L929 fibroblast cell line and to evaluate *in vitro* (pig ear skin) and *in vivo* (hairless mice skin) penetration of this fraction incorporated in a gel formulation. We observed that *Bc* fraction ranging from 5 to 2.5 µg/mL reduced the intracellular ROS formation, besides being able to avoid lipidic peroxidation and GSH level depletion.In addition, the *in vitro* and *in vivo* penetration exhibited was, respectively, 1.65 and 21.36 µg of the fraction for cm^2 of skin. The majoritary phenolics compounds retained as pig skin as hairless mice skin, respectively were: galic acid, catechin (22.31, 20.60 µg/ cm^2), quercetin 3-o-glucopiranoside (2.61, 0.93 µg/ cm^2) and one non identified compound (retention time 29') (≈ 3.3, 3.7 µg/cm^2). The results demonstrated the ability of this fraction in decreasing the UVA induced oxidative stress in L929 fibroblast cell line, it this fact can be due to the high antioxidant activity of the phenolic compounds present in the fraction which showed good retention on pig ear/hairless mice skin. Keywords: Ultraviolet radiation, *Byrsonima crassifolia*, Reactive oxygen species, L929 fibroblast cell line, Skin penetration. Acknowledgements: CNPq, CAPES and FAPESP.

P1.1-44

Determination of the anthocyanin content and antioxidant capacity of the Maqui pomace

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Introduction: The Maqui is a southern Chilean berry, wich has recognized antioxidant properties derived from a high concentration of both, poliphenols and anthocyanins. The Maqui industry generates solid remains or pomace, wich are economically undervalued, inspite its composition wich might include several compounds that are of biological interest. Objective: Evaluate the antioxidant properties and the anthocyanin content of a Maqui pomace extract, as a first step into a possible pharmacological potential. Methods: The extraction was performed by heating (50° C for 6 hours) 1g of Maqui pomace in 20mL solvent (water: methanol 50:50). This mixture was filtered and the liquid recovered was subjected to total Phenolic compounds analysis (TPC, Folin-ciocalteu method). Antioxidant activity was assessed by free radical captation methods DPPH, iron reduction method FRAP and ORAC. Anthocyanin content was evaluated by HPLC. Results: The TPC showed 26,18g galic acid equivalent/100g Total Solids (TS). DPPH method for antioxidant activity resulted in 31g trolox equivalent (TE)/100g TS. FRAP showed 27,07g ascorbic acid equivalent /100g TS. ORAC resulted in 11016 µmol TE/100g TS. The anthocyanin content found was 23.48g/100g TS.Conclusion: These results show that the Maqui pomace has a great antioxidant capacity, alowing a re-valorization of this industry subproduct.
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P1.1-45

Flavonoids from commom bean (*Phaseolus vulgaris*, l.) and its effects in human health

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Flavonoids are secondary metabolites of plants and have antioxidant, anticarcinogenic and antimutagenic properties, involved in the reduction of cardiovascular disease, cancers and diabetes mellitus. We aimed to identify the flavonoids present in raw and cooked white beans, soaked or not, and highlight their biological effects. The flavonoid's profile was analysed by High Performance Liquid Chromatography. Kaempferol was detected only in soaked and cooked samples. Kaempferol-3-rutinoside had its concentration increased by cooking, with or without soaking, and quercetin-3-glucoside and catechin increased with cooking preceded by soaking. Kaempferol-3-glucoside and quercetin weren't significantly influenced in their concentrations after heat treatment. It is known that kaempferol is associated with reduced risk of developing cardiovascular diseases and cancer, and that the glycosylated forms have a widerange of pharmacological activities (antioxidant, anti-inflammatory, antimicrobial, cardioprotective, neuroprotective, antidiabetic, estrogenic/antiestrogenic, analgesic and antiallergic activities). Quercetin is the predominant flavonoid in food and donates hydrogen atoms easily, what gives it significant antioxidant and anticarcinogenic activities. Catechin is associated with anticancer, anti-obesity, anti-atherosclerotic, antidiabetic, antibacterial and antiviral properties. In general, heat treatment preceded by soaking had increased concentrations of the phenolics measured, what is important since raw beans have antinutritional factors and therefore need to be cooked to serve as food.

P1.1-46

Polyphenols recovering from olive oil residue

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A variety of phenolic compounds have been identified in *Olea europaea*. Among them, oleuropein and hydroxytyrosol have been intensively studied for their potential effect on human health.

This study was undertaken to determine the phenolic composition and the antioxidant activity of extracts recovering from olive oil residue, *alperujo*, using different extraction conditions.

Extraction methods consisted basically of *alperujo* incubation with solvents at pH 2 for two hours by continuous stirring at indicated temperatures.

1 - Ethanol, room temperature

2 - Ethanol, reflux

3 - Water, reflux

Extracts were analyzed with regard to total polyphenol contents, phenolic profiles and antioxidant activities by Folin-Ciocalteu assay, capillary zone electrophoresis (CZE) and scavenging of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, respectively.

Only hydroxytyrosol was recovered using water as extraction solvent; whereas both hydroxytyrosol and oleuropein in comparable amounts were recovered using ethanol, where the temperature increased the extraction yield.

The antioxidant activity, referred to hydroxytyrosol content, was higher in the aqueous extract than the ethanol extract, but comparable between ethanol extracts recovering at different temperatures. Other compounds unidentified by CZE can be contributed to the aqueous extract antioxidant activity.

These results demonstrate the interest of *alperujo* as a natural, inexpensive and concentrated source of high-added-value polyphenols.

P1.1-47

Quantitative analysis of secondary metabolites in *Berberis buxifolia* Lam: A potential source of bioactive polyphenols

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Berberis buxifolia Lam is a plant used in traditional medicine mainly to combat gastrointestinal diseases and asthma. Furthermore, have antioxidant and antimicrobial activities. Antioxidants are secondary metabolites, that prevent disease and aging, among them are the: phenols, tannins and flavonoids which by its properties, can reduce oxidative damage. Thus, the aim of the present work was to determine the total content of these phytochemical constituents in root of *Berberis buxifolia* by spectrophotometric methods. Our results show that this plant presents: 2.8 mg 100 g^{-1} of phenols, 994.7 mg 100 g^{-1} of tannin and 41.4 mg 100 g^{-1} of flavonoids. Therefore, we can conclude that *Berberis buxifolia* presents chemical compounds that could be applied in herbal medicine. Further, the molecular basis of the biological activities of the chemicals compounds present in *Berveris buxifolia*, potentially responsible for health promotion, need to be investigated. Financial support: CONICYT-Chile.

P1.1-48

Polyphenols retention in microencapsulated cashew (*Anacardium occidentale*, L.) juice by spray drying

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The cashew (*Anacardium occidentale*, L.) is a tropical fruit that possess high antioxidant potential, due to the presence of polyphenols and vitamin C. However, its perishability has motivated the development of processes that create more stable products. The microencapsulation by spray drying is a method more economically feasible for production of food graded microparticles. The present study aimed the evaluation of impact of processing on the retention of total polyphenols from microencapsulated cashew juice (CJ). Solutions containing CJ and the encapsulation matrices (EM) – maltodextrin (M) and capsul (C) - were prepared in a with 10% of total solids content. Five different formulations were produced changing the ratio of EM components for the encapsulation process. The solutions were dried in a mini spray-dryer Büchi 290 and microparticles obtained were analyzed for the total polyphenols retention using the Folin-Ciocalteau's reagent. Results showed that polyphenols retention ranged between 65% and 92%. One of the formulations, conduced with 40% M and 60% C, showed lower polyphenols loss when compared to the other treatments. The treatment conduced with 100% of M showed the highest polyphenols loss. The polyphenols retention took advantage of an EM composed by M and C than applying M in an isolated manner.

P1.1-49

Gunnera tinctoria Mol.: a new source of polyphenols with medicinal properties

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Gunnera tinctoria Mol. (Gunneraceae) is a native plant from Chile. In spite of the traditional use of aqueous extracts from petiols and leaves of this species to treat inflammation, no scientific studies are available.[1]

This study aimed to corroborate their presumed antioxidant and antiinflammatory activity, identify polyphenols content and validate their traditional use.

A phytochemical and pharmacological screening was performed to determine the presence of secondary metabolites. The determination of total polyphenols was measured spectrophotometrically using the Folin-Ciocalteu method. All samples, were subjected to topical assays for the inhibition of inflammation elicited by arachidonic acid (AA) or phorbol ester (TPA), inducing inflammation on the mice ear.[2] The presence of secondary metabolites in the phytochemical screening was detected principally in aqueous, methanolic and ethyl acetate extracts. Ethyl acetate extract had already been shown to exhibit strong anti-inflammatory effects in both models AA and TPA, 55.0% and 54.7%, respectively. References: (1) Estomba D., Ladio A., Lozada M. *J. of Ethnopharmacol*. 2006, 103: 109-119. (2) Rodríguez-Díaz M, Delporte C, Cassels BK, González P, Silva X, León F, and Wessjohann L. *J Pharm Pharmacol*2011; 63: 718-724.

P1.1-50

Chilean _Prosopis_ mesocarp flour: polyphenolic content, antioxidant activity and fingerprint analysis

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Algarrobo pods were gathered by Native Americans as a food source in prehispanic times. Later, *Prosopis* pods were relevant as livestock feed and sometimes were used to produce a syrup used in the local cuisine. While human groups collected *Prosopis* pods based on size and flour yield, taxonomic studies in Chilean species did not clearly differentiate the taxa. Six samples of algarrobo pods were collected in valleys and places where prehispanic settlements are known and the composition of flour phenolics was compared by spectroscopic and spectrometric means. Large differences in colour, shape, flour yield and phenolics were found. One of the samples, with deep purple pods was shown to contain anthocyanins. Main phenolics in the Amberlite-retained methanol extracts from the pods were flavonoids. The morphological differences in the pods are also observed in the chemical constituents and indicated the need for a more careful taxonomic classification of the northern Chile *Prosopis* species. Acknowledgements: FONDECYT 1120096 and CONICYT-PCHA/Doctorado Nacional/ 2013-21130048 for financial support.

P1.1-51

Purification, structural elucidation, antioxidant capacity and neuroprotective potential of the main polyphenolic compounds contained in _Achyrocline satureioides_ Lam D.C. (Compositae)

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The aim of the present study was to purify, identify and determine the antioxidant properties and neuroprotective potential of the main polyphenolic compounds contained in the South Americannativeplant *Achyrocline satureioides*. For this purpose, an aqueous decoction of the plant aerial parts was made and then purified by HPLC-DAD and semi-preparative column. Peaks with characteristic UV spectrum of polyphenols were collected and identified by mass spectrum and H^1-NMR. Their chemical structure were elucidated to be: 3,5 -dicafeoil quinic acid; 4,5-dicafeoil quinic acid; isoquercitrin; quercetin; luteolin and 3-O-methylquercetin. The rank of antioxidant capacity of the compounds obtained by *in vitro* antioxidant capacity assays ($ABTS^{\cdot+}$ and lipoperoxidation) was as follows: quercetin>luteolin>3-O-methylquercetin> 4,5-dicafeoil quinic acid >3,5-dicafeoil quinic acid > isoquercitrin. Furthermore analysis of the interaction between the oxidized forms of these compounds and endogenous antioxidants (glutathione and ascorbate) showed that neither acids nor luteolin were reactive, suggesting that the 3- OR substitution in C ring is important for their reactivity with these endogenous antioxidants. Results of neuroprotective effects of these compounds in a model of oxidative damage in primary cultures of cerebellar granule cells showed that quercetin prevented neuronal death. Although, neither luteolin nor 3-O-methylquercetin showed this effect.

P1.1-52

Dicaffeoylquinic acids – occurrence, structure elucidation, and characterization as primary phyproof®-reference substances

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Dicaffeoylquinic acids (diCQA) are a widespread subgroup of polyphenolic compounds. They occur in artichoke, coffee, mate and coneflower, but have been detected in many other genera such as *Eleutherococcus*, *Lonicera* and *Artemisia*. In coffee beverage, the dicaffeoylquinic acids have been shown to be responsible for a large proportion of its antioxidant activity. Six isomeric diCQA exist, i.e.1,3-, 1,4-, 1,5-, 3,4-, 3,5- and 4,5-dicaffeoylquinic acid, which makes isolation as well as structure elucidation (e.g. by NMR and their mass spectrometric fragmentation pattern) a challenging process. For analytical purposes of medicinal herbs and food products well characterized reference substances are required but so far only cynarin (1,3-diCQA) has been commercially available. Selection of the correct reference substance is further complicated by the fact that different nomenclature systems exist. As a result the ring numbering of the quinic acid core can differ from IUPAC nomenclature and is inconsistent not only in the scientific literature but also in the product description of commercial suppliers. Especially cynarin is sometimes described as 1,3- or 1,5-diCQA, applying different CAS numbers in the product description as well. The poster describes the process of isolation, structure elucidation and characterisation of dicaffeoylquinic acids as primary reference substances for analytical purposes.

P1.1-53

Rapid comprehensive evaluation of (poly)phenolic compounds in complex matrices by UHPLC-MSn: the case of pomegranate (*Punica granatum* L.) juice

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The comprehensive identification of phenolic compounds in food and beverages is a crucial starting point for assessing their biological, nutritional, and technological properties. Pomegranate (*Punica granatum* L.) has been described as a rich source of (poly)phenolic components, with a broad array of different structures (phenolic acids, flavonoids, and hydrolyzable tannins) and a quick, high throughput, and accurate screening of its complete profile is still lacking. In the present work, a method for UHPLC separation and linear ion trap mass spectrometric (MS^n) characterization of pomegranate juice phenolic fraction was optimized by comparing several different analytical conditions. The best solutions for phenolic acids, anthocyanins, flavonoids, and ellagitannins have been delineated and more than 70 compounds have been identified and fully characterized in less than one hour total analysis time. Twenty-one compounds were tentatively detected for the first time in pomegranate juice. The proposed fingerprinting approach could be easily translated to other plant derived food extracts and beverages containing a wide array of phytochemical compounds.

P1.1-54

Extraction and microencapsulation of phenolic compounds from Brazil nut cake

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Brazil nut cake is a rich source of nutrients and bioactive antioxidant compounds, such as polyphenols, which could be valorized by microencapsulation. We aimed at microencapsulating Brazil nut cake extract, and evaluating the powder phenolic composition and antioxidant capacity. Phenolic compounds of the Brazil nut cake were extracted with ethanol:water (40:60, v/v) and the extract was dispersed was encapsulated with Capsul® (modified starch) by spray drying. Total phenolic compounds were assessed by the Follin-Ciocalteau reagent method (765nm). Antioxidant capacity was assessed spectrophotometrically by FRAP and TEAC assays. Phenolic composition was determined by HPLC-UV (280 nm) and HPLC-DAD-MS. Compounds' identity was determined according to retention times and mass spectra of standards, and external calibration was used for quantitative analysis. Microcapsules and the Brazil nut cake presented, respectively: 304.4±2.72 and 432±12,3 mg GAE/100g of total phenolics; 543.8±16.8 and 653.5±23.9 µM Fe^{+2}/100g (FRAP assay), 0.86±0.02 and 0.55±0.04 µM ET/100g (TEAC assay).The major phenolic compounds identified by HPLC-DAD-MS were phenolic acids: gallic, 3,4 dihydroxy benzoic, p-hydroxybenzoic, vanillic, syringic, *p*-coumaric, benzoic, and trace amounts of the flavonoids quercetin and myricetin. Spray-drying of the Brazil-nut cake extract promoted encapsulation of polyphenols with antioxidant capacity which might enable development of nutraceutical food products. Financial support: CAPES, CNPq, FAPERJ (Brazil).

P1.1-55

Comparison of polyphenol profiles of wines made from American, Eurasian, and interspecific grape varieties

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The mechanisms by which wine imparts human health benefits depend on the type of polyphenols ingested. In the eastern United States, wine is made from a wide range of grape species including *Vitis vinifera*, *Vitis labrusca*, *Vitis aestivalis*, *Vitis rotundifolia*, hybrids between these species, and hybrids with *Vitis riparia*. Under current climate change considerations, there has been a renewed global interest in interspecific hybrids due to their pest and temperature tolerance. The tremendous genetic diversity between Eurasian and American grape varieties results in wines with a similarly diverse range of polyphenols. We determined and compared polyphenol profiles of experimental red wines made from *Vitis vinifera*, *Vitis aestivalis*, *Vitis labrusca*, and their interspecific hybrids grown in research vineyards over nine vintages. This characterization provides a basis for selection of wines for future studies of transport, absorption, and metabolism of polyphenols in intestinal cell models, animal, and human studies. We confirmed that phenolic composition of varietal wines from different grape species is extremely heterogeneous. This fact must be taken into consideration in any study that investigates health effects of wine, or any research that optimizes the viticultural parameters for grapes farmed specifically for phytochemical production.

P1.1-56

High hydrostatic pressure preserves anthocyanins in Jussara (*Euterpe edulis*) juice

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Jussara (*Euterpe edulis*) is an exotic fruit native to Brazil. Although it shows good functional properties, Jussara is highly perishable, limiting its commercial use. High hydrostatic pressure (HHP) is a non-thermal food processing method that preserves sensory and nutritional qualities usually lost during thermal processes. We evaluated the effect of HHP on anthocyanins contents in Jussara juice. After vacuum packing, juice was pressurized (in duplicate) at 200 MPa for 5 and 10 min. Pressurized and control (untreated) juice samples were centrifuged, filtered and analyzed by HPLC-UV. In all samples, we identified both cyanidin 3-glucoside and cyanidin 3-rutinoside. The latter was the major anthocyanin present, corresponding to 77% of total anthocyanins. Juice pressurized for 5 min showed a lower total anthocyanins content (4.01g/kg) than both control (4.90g/kg) and 10 min pressurized (4.82g/kg) juices, which showed no differences. The decrease in anthocyanins content observed after pressurization for 5 min may be related to activation of polyphenol oxidases. In juices treated for 10 min this effect may have been counterbalanced by a higher extractability of anthocyanins due to changes in membrane permeability and disruption of cell walls. HHP treatment for at least 10 min is a suitable alternative preservation technique for Jussara juice. Financial Support: CAPES, CNPq, FAPERJ, UFRJ.

P1.1-57

Antioxidant activity, total phenolic and flavonoid contents of *Annona crassiflora* peel - a agroindustrial byproduct

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Exotic fruit consumption and processing is increasing worldwide due to the improvement in preservation techniques, transportation, marketing systems and consumer awareness of health benefits. However, the fruit processing industry deals with the large percentage of byproducts, such as peels and seeds, generated in the different steps of the processing chains. Therefore, in an attempt to contribute to valorize*Annona crassiflora*(araticum), a exotic Brazilian fruit from Cerrado biome, this study was conducted to quantify total phenols using Folin Ciocaulteau method, total flavonoids according to He *et al*. (2008) and to evaluate the antioxidant activity of *Annona crassiflora* (araticum) peel through the methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox Equivalent Antioxidant Capacity (TEAC) and Oxygen Radical Absorption Capacity (ORAC). Total phenolic content was 58406.48±1532.13 mg of gallic acid equivalents/100g of araticum peel and flavonoids content 17393.54±80.44mg of catechin/100g of araticum peel. DPPH, TEAC and ORAC values were respectively 36270.06±14.38 µmol Trolox equivalents (TE)/100g, 57337.32±38.27µmol TE/100g and 36977.71±81.14 µmol TE/100g of araticum peel. These results suggest a high total phenolic and flavonoids contents, and a significant *in vitro* antioxidant activity in araticum byproduct. As a result, the potentially valuable compounds could be used to make high-value products in antioxidant systems, for food and cosmetic industries..

P1.1-58

Critical considerations for the use of natural and model wines as a source of polyphenols for human health studies

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"Wine" per se is a highly variable and unstable source of polyphenols. Phenolics production in wine grapes is dependent on a large number of variables, including genetic predisposition, temperature profile of the growing season, pest pressure, light exposure, viticultural techniques that influence cluster and berry size, overall crop load as well grape maturity and hang-time. In addition, the winemaking process results in a greatly reduced concentration of phenolics in finished wine due mainly to changes in solubility and related copigmentation and polymerization phenomena. Due to inconsistency in viticultural and enological conditions, the phenolic profile of a wine can neither be predicted by genetic fingerprinting nor by appellation- and climate-based assumptions alone. In order to design reproducible feeding studies on uptake and availability, the accurate phenolic composition as well as the production and storage conditions of each wine must be defined and referenced, including compositional changes throughout the experiments. As an example of phenolic variability, this study assesses experimental red wines by grape variety, vineyard location, vintage, and wine age. Concentrations varying by an order of magnitude were observed with total phenolics ranging from 670 to 5,500 mg/L GAE, and total anthocyanins ranging from 3 to 160 mg/L C-3-G.

P1.1-59

Effect of roasting on chlorogenic acids contents in mate (*Ilex paraguariensis*)

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Mate (*Ilex paraguariensis*) is a plant rich in chlorogenic acids (CGA), which leaves are used to prepare infusions widely consumed in South America, such as *chimarrão* (green leaves) and *mate tea* (roasted leaves). Little is known about the influence of roasting on mate's CGA. Therefore, the aim of this study was to evaluate CGA contents in infusions prepared with mate of different roasting degrees. In addition to green mate, roasted samples were collected during industrial processing and classified according to color (light, medium, dark and very dark). CGA were analyzed by LC-DAD-MS. Ten CGA were identified in green mate infusion, totaling 1.52g/L. In this sample, 3-caffeoylquinic acid was the most abundant (512.5mg/L), representing 34% of total CGA. Roasting caused a progressive decreased in CGA content of mate infusions, ranging from 81% to 91% when compared to green infusion. For the first time, 3-*p*-coumaroylquinic acid was quantified in mate infusions (ranging from 46.6mg/L in green to 10.6mg/L in very dark). CGA distribution was also affected by the roasting process. During roasting, caffeoyl, feruloyl and dicaffeoylquinic acids contents shifted from 68.7%, 19.7% and 8.6% (green) to 54.6%, 14.3% and 23.1% (very dark) of total CGA, respectively. Financial Support: CAPES, CNPq, FAPERJ, UFRJ.

P1.1-60

Determination of total polyphenols and vitamin C in plasma of hypercholesterolemic rats treated with blueberry fruit (*Vaccinium ashei* R.)

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The fruits of the kind *Vaccinium* can be considered as the first AF described. The blueberry is a fruit species native to parts of Europe and North America, where it is much appreciated for its exotic taste and regarded as "source of longevity." It has high content of polyphenols such as flavonoids and anthocyanidins with high power antioxidant. The objective of the study was to evaluate the content of total polyphenols and vitamin C levels in plasma of rats treated with extract of blueberry fruit. We used 36 male Wistar rats, hypercholesterolemic, divided into 6 groups: group 1: control (NaCl 0.09%), group 2: simvastatin (10 mg/kg), group 3:bilberry extract (25 mg/kg); group 4: bilberry extract (50 mg/kg), group 5:bilberry extract (25 mg/kg) and simvastatin (10 mg/kg) and group 6: bilberry extract (50 mg/kg) and simvastatin (10 mg/kg). After 14 consecutive days of supplementation, the animals were euthanized and the whole blood removed for analysis of polyphenols and vitamin C using techniques classics. The results showed a statistically significant increase ($p < 0.05$) the content of polyphenols and vitamin C in the group treated with bilberry extract 50mg/Kg and simvastatin 10 mg/kg compared to other grups. The blueberry fruit extract (50 mg/Kg) with high content of antioxidants which helps to balance the redox living organisms.

P1.1-61

Bioactive polyphenols for COPD treatment from two edible Myrciaria fruits

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Jaboticaba (*Myrciaria cauliflora*) and false jaboticaba (*Myrciaria vexator*) fruits are two pleasant-tasting, dark-colored fruits, native to Brazil. They are rich sources of bioactive phenolic compounds, including anthocyanins, flavonoids, phenolic acids, tannins, as well as less well-known polyphenols like depsides. Jaboticabin, a depside originally we identified from jaboticaba fruits, is being explored a treatment for chronic obstructive pulmonary disease (COPD). These two *Myciaria* fruits are similar in morphology, but their taste profiles differ markedly. Using LC-MS-ToF, cyanidin-3-*O*-glucoside was found as the major anthocyanin in *Myrciaria* fruits. Delphinidin-3-*O*-glucoside was found to be the marker compound for jaboticaba, while cyanidin-3-*O*-galactoside and cyanidin-3-*O*-arabinose were two marker compounds distinguishing false jaboticaba via principal component analysis. In addition, two ellagitannins, iso-oenothein C and oenothein C, were isolated and identified from both of these fruits for the first time. Jaboticabin occurred in both fruits, and because of its potential to treat COPD, was synthesized in the laboratory for the first time. This work was supported by NIH-NHLBI, grant 5SC1HL096016.

P1.1-62

Flavonoids and alkaloids in *Passiflora* leaves

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Passiflora is the largest and most important genus of the family *Passifloraceae*, comprising about 500 species. The major constituents of *Passiflora* species are flavonoids and alkaloids. Teas from leaves of *Passiflora* are widely used in folk medicine in countries of America and Europe in the treatment of a wide variety of diseases. The aim of this study was to determine the flavonoids and alkaloids in leaves from six different cultivars of *Passiflora* (*Passiflora edulis* Sims cultivars Sol do Cerrado, Ouro Vermelho and Gigante Amarelo, *Passiflora alata* Curtis, *Passiflora setacea* DC and *Passiflora tenuifila* Killip). Identification and quantification of flavonoids were achieved using analytical reversed HPLC-DAD and the identification and quantification of alkaloids were achieved using a UPLC-MS.The flavonoids detected were homoorientin, orientin and isovitexin. Homoorientin was detected in all leaves and values varied from 8 to 42 mg/100g dry weight (DW), orientin was identified only in *P. altata* (20 ± 2 mg/100 g DW) and isovitexin was observed in leaves of *P. edulis* cultivars (11 – 47 mg/100 g DW). The alkaloids detected were harmine, harmaline and harmane. Harmane was detected in all the leaves and values varied from 0,005 to 0,03 mg/100 g D, harmine was identified in the same concentration (0,001 mg/100 g DW) for *P. edulis* cultivar Gigante Amarelo and *P. tenuifila*, and harmaline was not detected in the leaves analyzed.

P1.1-67

Bioactivity-guided isolaton of anti-inflammatory constituents from the fruits of sea buckthorn (*Hippophae rhamnoides*)

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Hippophae rhamnoides [sea buckthorn (SBT), Elaeagnaceae] is native to Eurasia, and has recently gained great attention worlwide because of its medicinal and nutritional properties (superfood). Different products prepared from the berries, seeds, leaves are used as therapeutic, prophylactic and heath promoting agents. All parts of the plant are rich source of biologically active substances, such as flavonoids, carotenoids, vitamins (C, E and K), tannins and fatty acid glycerides.

SBT berries has been applied in the traditional medicine for curing inflammatory disorders, such as asthma, skin diseases, gastric ulcers and for the treatment of different allegic symptoms. Accordingly this, antioxidant, cytoprotective, wound healing and tissue repair activites of SBT have been reported to date. Furthermore significant anti-inflammatory activity has been demonstrated for extracts of leaves and branches, but no such data were reported for the berries.

In the present study the anti-inflammatory activity of the berries was evaluated on rat paw edema test, and compounds responsible for the activity were identified using bioactivity guided isolation procedure. Triterpene acids, flavonoids, lignans and fatty acids were isolated from the extract of the peel by means of solvent-solvent partition, CC, VLC, RPC and HPLC. Some phenolic compounds were first descibed from this species.

P1.3-01

Differential stable isotopic coding of the metabolome to measure exposure to dietary polyphenols in epidemiological studies

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A large number of studies support a role of polyphenols in the prevention of chronic diseases such as cardiovascular diseases, diabetes or cancers, however epidemiological evidence is still limited. Robust methods are needed to reliably assess exposure to a large variety of dietary polyphenols which can be easily applied to epidemiological studies.
We report here the development of a semi-quantitative method to measure dietary polyphenols in urine, based on differential metabolite coding with ^{12}C- and ^{13}C-dansylation labeling and quantification of the coded metabolites by mass spectrometry.
Urine samples are first hydrolysed with a β-glucuronidase / sulfatase enzyme mixture and the resulting polyphenol aglycones extracted twice with ethyl acetate. Quantitative dansylation of phenolic hydroxyl groups is carried out with $^{13}C_2$-dansyl chloride (well-characterized reference pooled sample), or a non-marked ^{12}C-dansyl chloride (samples). ^{13}C-Dansylated reference sample and ^{12}C-dansylated samples are mixed and relative concentrations in samples over the reference sample are determined by UPLC-ESI-MS-MS.
The method is currently set up for the measurement of 40 different polyphenols, showing that relative quantification of the polyphenol metabolome can be achieved. This method provides a simple and robust mean for measuring polyphenols in urine, and overcomes the need of having expensive labeled standards for each compound.

P1.3-02

Metabolomic approach to identify bioactive compounds after long term consumption of Tropical highland blackberry (*Rubus adenotrichos*) juice

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Consumption of polyphenol-rich foods continues to be the focus of attention because of their putative impact on human health. Tropical highland blackberry (*Rubus adenotrichos*) juice is widely consumed from Mexico to Ecuador and represents an important source of ellagitanins and antioxidant substances for the population.Using blackberry as a model for other tropical fruits, we have shown how modern metabolomic profiling can be used to characterize the exposure to bioactive molecules and their metabolites in a nutritional human trial. Forteen subjects consumed blackberry juice or water in addition to a controlled diet, and urine were collected after one dose or 9 days supplementation. More than 60 ions discriminated the urine metabolomes analyzed by UPLC-QTof. Interestingly, the microbial metabolites of ellagic acid, urolithin A (UA)-glucuronide and urolithin B (UB)-glucuronide were the most important discriminants but other ions currently under identification could also contribute to blackberry juice health effects. Correlations will be searched between all discriminant metabolites and the individual capacity to produce UA and UB to investigate a possible inter individual variation in response to blackberry intake.

P1.3-03

Human plasma antioxidant capacity due to the polyphenols of yerba mate

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The infusions of yerba maté (*Ilex paraguariensis* St. Hil.) consumed in its traditional form of hot mate possess high antioxidant (AOC) due to its high concentration of polyphenols. The aim of the study was to evaluate in vivo changes in antioxidant capacity and polyphenol concentration in human plasma after an acute intake of 300 mL of an infusion of yerba mate for 120 minutes. We worked with 10 volunteers, performing determinations of total polyphenol concentration (TPC) and plasma AOC before ingesting the infusion and at 20, 40, 50, 60, 80, 100 and 120 minutes. The TPC was measured with the Folin-Cioclateu and the AOC with FRAP and ABTS methods. Were conducted similarly to controls, which were carried out with a intake of 300 mL of water. TPC was determined in plasma after acute ingestion of infusions mate, rose untill 120 minutes as the AOC, lasting over 120 minutes. It was found that the consumption of yerba maté in its traditional form increases the AOC plasmatic due to its polyphenol content.

P1.3-04

MSn and NMR analysis of (–)-epicatechin metabolites

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(–)-Epicatechin (EC) is a flavonoid present in many plants, and is particularly abundant in green tea, pome fruit, cocoa products, various berries, and red wine.A growing body of evidence demonstrates a potential role for dietary EC in the context of human health.Despite of this,the mechanisms of action (MOAs) that underlie the bioactivity of EC are not yet fully understood. One major prerequisite for the elucidation of potential MOAs is the complete structural identification and quantificationof EC-derived metabolites existingin humans. Recent technological advances in mass spectrometry (MS) and nuclear magnetic resonance (NMR) as well as the use of isotopically labeled compounds enabled the investigation of gut microbiome-derived EC metabolites, such as γ-valerolactones. In this study, urine samples, collected following the oral intake of (−)-[2-^{14}C]epicatechin by humans, were concentrated by solid phase extraction, using radioactivity to determine compound recoveries. Subsequently, HPLC-MS^n-fractionation was used to isolate intact γ-valerolactonesconjugates. These fractions were analyzed by direct infusion MS before being further investigated by NMR to elucidate their chemical structure.Using this approach we identified several γ-valerolactonesconjugates in human urine.

P1.3.05

Development and validation of a food frequency questionnaire for consumption of polyphenol-rich foods in pregnant women

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Previous studies have shown that maternal consumption of polyphenol rich foods after the third trimester of pregnancy may interfere with the anatomical and functional activity of the fetal heart. The aim of this study was to develop and evaluate the reproducibility and validity of a Food Frequency Questionnaire (FFQ), with 52 items, to assess the intake of polyphenol-rich foods in pregnant women in Brazil. This cross-sectional study included 120 pregnant women. The intake of polyphenol-rich foods estimated by the developed FFQ was compared with the average of two 24-hour recalls, with the average intake measured by a three-day food diary and with the urinary excretion of total polyphenols. The triangular method was applied to calculate Pearson's correlation coefficients, intraclass correlation, and Bland-Altman plots for the FFQ, using an independent biochemical marker, in addition to classification by quarters of consumption. Analysis of the reproducibility between the FFQ showed a very high correlation ($r=0.83$; $\alpha<0.05$). A significant association was observed between the FFQ and urinary excretion (0.23; $\alpha=0.01$). The association between the dietary survey methods was moderate to very high ($r=0.36$ to $r=0.83$; $\alpha<0.001$). In conclusion, this questionnaire showed reproducibility and validity for the quantification of consumption of total polyphenols in pregnant women.

P2.1-01

Improved oral bioavailability of curcumin incorporated into micelles

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Curcumin, a lipophilic polyphenol derived from the plant *curcuma longa* possesses numerous health-promoting activities. The oral bioavailability of curcumin is low due to its poor aqueous solubility, limited gastrointestinal absorption, rapid metabolism and excretion. Therefore, we tested, in a randomized crossover study, simultaneous application of phytochemicals and micellar solubilisation, alone and together, as strategies to enhance the concentration of curcumin in the body. Eleven women (22.5±2.6 y, n=6; 67.2±2.8 y, n=5) and 12 men (26.2±1.8 y, n=6; 62.2±2.7 y, n=6) took in random order 80 mg curcumin (C) alone, 80 mg curcumin plus the phytochemicals sesamin, ferulic acid, naringenin, and xanthohumol (CP), or identical doses of micellar curcumin (MC) or micellar curcumin plus phytochemicals (MCP) separated by one-week washout periods. Blood was collected at 0, 0.5, 1, 2, 4, 6, 8, and 24 h after intake. Preliminary analysis (n=9) showed that relative to native C, CP increased bioavailability 18-fold, MC 169-fold, and MCP 172-fold based on AUC. The full dataset and sex and age differences will be presented at the conference. In conclusion, based on these preliminary results, the incorporation of curcumin into micelles significantly and the simultaneous ingestion of phytochemicals numerically increases its bioavailability.

P2.1-02

Initial assessment of the intra- and inter-subject variability of the absorption, metabolism and excretion of cocoa flavanols

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Epidemiological and human intervention studies have provided evidence for the potential cardiovascular benefits of flavanol-containing foods. However, such biological effects are dependent on the bioavailability of flavanols and, as such, individual variations in absorption, metabolism and excretion are highly relevant. The present study was conducted to determine the intra- and inter-subject variability of (–)-epicatechin (EC) and its metabolites in absorption, metabolism and excretion following the intake of 10.7 mg of total cocoa flavanols/kg BW, in 20 healthy Caucasian males. Intra-individual variability was assessed in 7 subjects who orally ingested 10.7 mg of total cocoa flavanols/kg BW at two occasions separated by a 1 week wash-out. Flavanol metabolites in plasma and urine were analyzed by HPLC with fluorescence and electrochemical detection. The intra-individual variation expressed as coefficient of variation (CV%) with respect to $AUC_{(0\text{-}6h)}$ of total EC metabolites in plasma over time was 15.8%, with C_{max} being 16.6% and T_{max} being 13.9%, while the variation in total urinary excretion was 15.1%. The inter-individual variation based on the $AUC_{(0\text{-}6h)}$ was 38.1%, with C_{max} , T_{max} and total urinary excretion being 38.6%, 26.0% and 43.7%, respectively. Intra- and inter-variability of individual EC metabolites in $AUC_{(0\text{-}6h)}$, C_{max} andT_{max} ranged from 19 to 34% and from 23 to 54%, respectively. Our data supports future efforts to establish population-level cocoa flavanol intake recommendations.

P2.1-03

Effects of consumption timing on bioavailability of bilberry anthocyanins

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Anthocyanins are one of the most important flavonoid groups, and have beneficial effects including antioxidant activity. We previously examined bioavailability of anthocyanins existed in bilberry using mice model, and concluded that bilberry anthocyanins are absorbed into the body and distributed as intact forms in specific organs, particularly the liver, kidney, and testis (Sakakibara et al., 2009). Recently, many of the biological processes involving absorption and metabolism of consumed ingredients have been reported to follow diurnal rhythms recurring every 24 hours. In this study, we evaluated the effects of consumption timing on bioavailability of bilberry anthocyanins. After fasting, bilberry extracts were orally administrated in amounts of 100 mg/kg body weight at ZT0 (inactive phase) or ZT12 (active phase) to male C57BL/6 mice. Animals were anesthetized with ether at 0, 15, 30, 60, 120 min after the administration, and the plasma and gastrointestinal tract (stomach and ileum) were collected. The gastric emptying time of anthocyanins was significantly faster upon administration at the active phase than that of the inactive phase. Their amounts appeared in the plasma suggested different time-dependent property according to the consumption timing. Hence, bioavailability of functional food factors, at least anthocyanins, may vary with timing of consumption.

P2.1-04

Topical delivery of polyphenols, anthocyanins and essential lipids in nanosystems

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Ultradeformable liposomes (UL) are lipidic vesicles designed to penetrate across the stratum corneum (SC) driven by the transepithelial humidity gradient and to release their content to deeper skin layers. If loaded with antioxidants like polyphenols or anthocyanins, UL become potential tools for skin protection against damage from free radicals. An ethanolic extract from blueberry (*Vaccinium myrtillus*) was loaded into UL to obtain UL-B, which retained an 85 % of the antioxidant capacity of the extract, with low cytotoxicity.

On the other hand, Nanostructured Lipid Carriers (NLC) are nanoparticle systems obtained by mixing under certain conditions a solid lipid with a spatially incompatible liquid lipid. NLC increase humidity in the skin by occlusive effect due to their nanometric size. They also act as a depot for the slow release of actives into the skin. An essential oil from lemon was used as liquid lipid itself to form NLC-L. Essential oils from citrics are rich in Omega-3 and Omega-6 lipids, needed as precursors for reconstitution of plasma membrane of damaged skin cells and ceramides in the SC.

These two nanosystems were also combined in a single formulation, in order to provide their benefits together to the skin as a cosmetic/skin repair product.

P2.1-05

Multinational survey of flavonoids in human milk

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For infants, human milk is the primary source of nutrition and may serve as a source of flavonoids originating from the mother's diet. However, only limited information is available on the flavonoid contentof human milk globally. Samples were collected from 20 donors each in China, Mexico, and USA at 2, 4, 13, and 26 weeks postpartum. Flavonoid content was assessed by LC-TOF-MS following extraction and enzymatic deconjugation. Remaining samples were pooled for targeted flavonoid metabolite analysis. Epicatechin, epicatechin gallate, epigallocatechin gallate, quercetin, naringenin, kaempferol, and hesperetin were main flavonoids detected in human milk with total flavonoid ranges of 19.0-1007.4, 13.6-335.9 and 15.5-716.6 nmol/L in China, USA and Mexico respectively. Glucuronides of these flavonoids were detected as main metabolites in human milk. Anthocyanins were not detected in milk samples, despite a detection limit 0.04 nmol/L in milk. ANOVA was conducted on observations with detectable concentrations. Total flavonoid content differed significantly by donor (P=0.001) but not by lactation stage or country, suggesting milk flavonoid content may reflecttiming of collection and individual variation in dietary patterns and bioavailability. The frequency of flavonoid detection non-significantly varied between countries. These data expand our knowledge on flavonoid distribution in human milk globally.

P2.1-06

Spectroscopic determination of deprotonation constants flavonoid

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Although the mechanisms by which flavonoids exert benefical pharmacological effects are not fully understood, is accepted that these result from the radical scavenging ability to delay the oxidative stress. The chelating capacity of flavonoids, which has a crucial role in oxidative stress and in the treatment of metal overload diseases, is largely dependent on the number, position, and dissociation of their hydroxyl groups. Moreover, the dependence between the acidity constants of flavonoids can explain changes in the solubility and membrane permeability in biological processes. Therefore, the acidity constant of the hydroxyl groups is an important parameter controlling both, the anti-oxidative capacity and the chelating capacity of flavonoids.
In this work, the full deprotonation of flavanones containing the aglycones naringenin and hesperetin was studied in aqueous solutions by chemometrics methods obtaining UV-VIS spectra at different pH's.
We found that compounds with glycoside moieties are not significantly different but are significantly different between aglycones and glycosides. At physiological pH's in all case the neutral form and the first deprotonated form are the mainly species.

P2.1-07

Improving the properties of plant polyphenols

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We are developing polyphenol prodrugs, with the goals of increasing absorption from the gastrointestinal tract, and of providing temporary protection from Phase II metabolism. The rationale for this endeavour derives from the ability of polyphenols to interact with proteins such as signalling kinases, transcription factors and ion channels, and to modulate redox processes, such as those taking place in mitochondria. Biomedical applications of these natural compounds are however severely hindered by their low bioavailability and rapid metabolism, since only low concentrations are found in plasma even after a polyphenol-rich meal, and mostly in the form of metabolites.
We report here the synthesis, stability tests and pharmacokinetic studies of new derivatives of resveratrol, a natural phenol belonging to the family of stilbenoids, based on protection of the hydroxyl groups with capping groups linked via acetal- and carbamate ester-type bonds. Amino deoxy sugars, amino acids, and oligoethylene glycolcapping groups were used to modulate the chemico-physical properties of the resulting prodrugs. Some of the derivatives have shown near-optimal kinetics of hydrolysis in media mimicking physiological environments and in blood, leading to an improvement of intestinal absorption and protecting the phenol moieties from Phase II metabolic conjugation.

P2.1-08

The pharmacokinetics of anthocyanins and their metabolites in humans

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Anthocyanins undergo extensive biotransformation after consumption; however the pharmacokinetics of these metabolites has not been reported previously. The aim of the present study was to establish the pharmacokinetics of ^{13}C-labelled cyanidin-3-glucoside($^{13}C_5$-C3G) metabolites following a 500mg oral bolus dose of 6,8,10,3',5'-$^{13}C_5$-C3G(n=8 healthy males), and 48h of subsequent blood sampling. Serum samples were analysed by HPLC-ESI-MS/MS with elimination kinetics established using non-compartmental pharmacokinetic modelling. 18metabolites were identified, including $^{13}C_5$-C3G, its degradation products, protocatechuic acid and phloroglucinaldehyde, 10 phase II conjugates of protocatechuic acid, and 2 metabolites derived from phloroglucinaldehyde. The maximal concentrations of the phenolic metabolites (C_{max}) ranged from 0.01±0.01 µM to 2.0±1.4µM, between 1.8±0.2h and 30.1±11.4h (t_{max}) post consumption, with half-lives of elimination observed between 5.2±0.1h and 45.2±12.3h. The major phenolic metabolites identified were hippuric acid and ferulic acid, which peaked in serum at 15.7±4.1h (C_{max}12.0±1.4µM) and 8.2±4.1h (C_{max}0.8±0.4 µM) respectively. In conclusion, anthocyanin metabolites have highly variable elimination kinetics and establishing their unique serum kinetic profiles is a significant step towards understanding the relative importance of specific metabolites in the health benefits of anthocyanins.

P2.1-09

In vivo and ex vivo studies on structure-dependent absorption of polyphenols from coffee in the gastrointestinal tract

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We performed an *in vivo* study, where ileostomy volunteers consumed coffee with various CGA contents to investigate the dose-response and intestinal availability by appraisal of ileal fluid, urine and plasma. Secondly, we conducted absorption experiments using Caco-2 cells and pig jejunal mucosa in the Ussing chamber to evaluate our *in vivo* observations. During a human intervention study, we detected 78% of the ingested CQAs in the ileostomy bags. Additionally, higher CGA dosages led to a faster ileal excretion concomitant with a proportionally lower amount of CGA metabolized or hydrolyzed. We also identified sulfation as the major conjugation reaction whereas at higher CGA doses the glucuronidation increased. In contrast, the CGA metabolites in urine were not affected by the dose, whereas the ratios of sulfation:glucuronidation in urine showed a dose response. In our Ussing chamber studies, absorption was generally structure-dependent. We determined a higher absorption of the free acids as compared to the respective esters which was in line with our *in vivo* findings. The concentration-dependent absorption kinetics indicated a predominantly passive diffusion process.Overall, CGA absorption is affected by their physic-chemical properties.
These studies were supported by NRC (Lausanne, Switzerland).

P2.2-01

Investigation of colonic metabolisation of polyphenols in an in vitro gastrointestinal dialysis-model

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Introduction: The biological effects of polyphenols depend on their mechanism of action in the body. Recently, we modified and validated an *in vitro* continuous flow gastrointestinal dialysis-model (GIDM) with a culture of human colonic microflora to study the metabolisation and absorption of polyphenols, using chlorogenic acid as a model substance.
Methods*:* The GIDM simulates gastric and intestinal conditions in adults. The intestinal stage was performed using stirred cells, equipped with a dialysis membrane and a dialysis bag (1M $NaHCO_3$). The model is optimized with fecal slurry (homogenized human stool sample) cultured in an anaerobic atmosphere. Metabolisation of rutin, procyanidin A2 and chamaejasmin was investigated by QTOF mass spectrometry.
Results: The polyphenols showed to be poorly absorbed in the small intestine but are extensively metabolized by the human feces to phenylpropionic acids and phenylacetic acids. The different metabolites will be shown in more detail at the conference. The metabolites can be used as reference compounds for tracking the same products in blood after *in vivo* studies.

P2.2-02

Phenol-Explorer 3.0: exploring polyphenol research through a powerful and intuitive web interface

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Phenol-Explorer is the first comprehensive database on polyphenol content in food. Released in 2009, Phenol-Explorer contains detailed information and content values of over 500 polyphenols in 450 foods as well as information on polyphenol metabolism, with the latest update including information on polyphenol retention factors. With this update, the website has been significantly improved. New search, data visualization, and data extraction features enable users to explore this valuable resource in more powerful and intuitive ways. In terms of search, the ability to search polyphenols and polyphenol metabolites by chemical structure allows users to quickly identify similar structure and substructures. Advanced search has also been refined and is now easier to use, allowing the extraction of information on specific polyphenols and foods. Regarding data visualization, visitors can explore polyphenol content and metabolism data through the use of graphs, condensing and standardizing data from a variety of sources into a comparable synopsis. In addition a new taxonomy viewer has been developed. Finally, for data extraction, a web API has been added, enabling programmatic access and search. Here, a guided tour of these new features will be presented, demonstrating how to get the most out of this valuable resource on polyphenols.

P2.2-03

Bioavailability and colonic catabolism of flavanone-enriched orange juice by healthy humans

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Orange juice is rich source of flavanones, containing mainly hesperetin-7-*O*-rutinoside (hesperedin) and naringenin-7-*O*-rutinoside (narirutin), which have known beneficial effects against human diseases. We investigated the bioavailability and metabolism of flavanones, and their colonic transformation following acute ingestion of a flavanone-enriched orange juice by humans. Twelve healthy subjects consumed a flavanone-enriched orange or placebo juice, and urine samples were collected at different time points over 24h period post-ingestion. Urinary metabolites and phenolic acid catabolites were analyzed by HPLC-PDA-MS^2 and GC-MS. Fourteen flavanone metabolites were analyzed in urine collected after consumption. Hesperetin-*O*-glucuronides were the main flavanone metabolites, followed by naringenin-*O*-glucuronides and hesperetin-3′-*O*-sulfate. The overall excretion of flavanone metabolites in urine, which took place mainly 2-10h after ingestion, corresponded to 16.0% of intake. In addition, urinary phenolic acids were excreted in significantly increased quantities after orange juice. These findings show that orange juice flavanones were absorbed and metabolized to some extent in the small intestine but principally in the colon via phase II enzymes to yield the corresponding conjugates, mainly glucuronide and sulfate conjugates. The flavanones are also extensively transformed by colonic microflora yielding phenolic acid catabolites which are absorbed into the circulatory system prior to excretion.

P2.2-04

Absorption, distribution, metabolism and excretion (ADME) of (−)-[2-^{14}C]epicatechin in humans

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While there is increasing interest in the biological properties of flavonoids in the context of nutrition and primary prevention, comprehensive data on thehuman ADME of flavonoids remain uncommon. We report here on the ADME of the flavanol (−)-epicatechin [EC], following the oral intake of isotopically labelled (−)-[2-^{14}C]epicatechin by humans [n=8] observing a flavanol- and procyanidin-controlled background diet. Our findings provide novel insights into the ADME of EC, and demonstrate that the compound is absorbed and subsequently present in circulation in the form of various metabolites that significantly differ in their chemical structure, abundance, and time present in the body. In this context, the gut micriobiome was identified as significant contributor to this diversity, with EC gut microbiome catabolites detected in the body for up to 72h post intake, and metabolites, like γ-valerolactone and hydroxyvaleric acid accounting for 42±5% of the amount of EC ingested. In addition, based on the use of radiolabeled EC, we were able to validate recently developed analytical methods, confirm the chemical structure of previously reported metabolites, resolve some of the ambiguity in the scientific literature around qualitative and quantitative aspects of EC analysis, and provide novel insights into hitherto unknown aspects of EC pharmacokinetics.

P2.2-05

In vitro and in vivo approach of colonic metabolism of olive oil polyphenols

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In vitro colon fermentation has been the model of choice to elucidate the metabolic fate of polyphenols in the large intestine. To acquire knowledge about colon metabolism of olive oil polyphenols we carried out an *in vitro* colon fermentation of hydroxytyrosol (OHT), tyrosol (Tyr), hydroxytyrosol-acetate (OHT-ac) and oleuropein (OL) using human fecal samples as microbial inoculums. The results obtained were then compared with the analysis of feces from 10 healthy adults that consumed a phenol-enriched olive oil (500 mg phenols/kg oil, 25 mL/day) during 3 weeks. Results from *in vitro* colon fermentation showed an important degradation of OHT and Tyr at the first 6 h of incubation; after 12 h were slightly affected by colon bacteria remaining relatively stable until 48 h of incubation. HT-ac colonic fermentation produced free HT as major metabolite. OL was mostly degraded after 12 h of incubation and give as products of it catalysis HT, HT-ac, oleuropein aglycone and the dialdehydic form of elenolic acid linked to hydroxytyrosol. Results from human feces showed an increase of the free OHT after 3 weeks of consumption of phenol-enriched olive oil, which indicate that OHT could play an important role as colon-cancer chemo-preventive.

P2.2-06

Anti-inflammatory effects of peach (*Prunus persica (L.)Batsch*) and derivate products on CCl_4-induced acute general injury in Wistar rats

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Peaches are consumed worldwide and have a high content of carotenoids and phenolic compounds that may exert biological activities. To investigate the anti-inflammatory potential of peaches and its derivate products, such as peel, syrup and peach in syrup, male wistar rats received a daily oral dose of lyophilized extracts (200 and 400 mg/kg) by 30 days through gavage. At the end of this period, animals received one Intraperitoneal injection of carbon tetrachloride (CCl_4) 3 mL/kg to induce an acute toxic response to liver and other organs. Four hours later, tissues were removed for analysis. The presence of pro-inflammatory cytokines (TNF-α and IL-1β) in serum were assessed by ELISA and in tissues by western blot. Liver, kidney and heart were also analyzed by western blot to determine the levels of the receptor for advanced glycation endproducts (RAGE) and NF-kB (p65 subunit). The immunocontent tyrosine hydroxylase at brain cortex and cerebellum were also assessed. Our results show that CCl_4–induced a general acute injury in liver, kidneys, heart and brain. All the products derived from peaches were able to downregulate the increase in pro-inflammatory parameters induced by CCl_4 in all tissues analyzed.

P2.2-07

Utility of the co-culture system of human intestinal Caco-2 cells with hepatic HepG2 cells for investigating flavonoid metabolism

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Flavonoid metabolism mainly occurs in the small intestine and in the liver. Cultural cells are useful models for investigating flavonoid metabolism, and Caco-2 and HepG2 are popular cell lines derived from human tumor cells to estimate intestinal and hepatic metabolism, respectively. In this study, we combined these cells and have made an attempt to simulate the physiological condition of flavonoid metabolism. Caco-2 cells were split on permeable supports and cultured confluently for 3-wk to be fully differentiated. On the day of experiment, the inserts with Caco-2 cells were placed on the wells cultured with confluent HepG2 cells. Quercetin and genistein were administrated on the apical side of Caco-2 cells, and after 2 h, the apical media and the basolateral media, which was co-incubated with HepG2 cells, were analyzed using HPLC-UV and LC-MS/MS for determining their metabolites. In the co-culture system, the apical efflux of metabolites was suppressed and the basolateral aglycones were decreased, together with the increase of methylated quercetin in the basolateral media. Furthermore, LC-MS/MS analysis indicated that the co-culture system, as compared with the single cell line system, produced the metabolite patterns in common with the plasma flavonoid metabolites in humans.

P2.2-08

Bioaccessibility and antioxidant activity of phenolic compounds from Babassu (*Orbignya speciosa*)

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Introduction: The bioaccessibility of phenolic compounds is crucial to knowledge the beneficial effects of their on human health. The babassu palm (*Orbignya speciosa*) tree is one of the most important species of the palms family in Brazil, the eatable parts consists of both the nut and the mesocarp used in human feeding. Objective The aim of this work was to estimate the bioaccessibility and antioxidant capacity of phenolic compounds from mesocarp and nut babassu by an in vitro gastrointestinal digestion. Methods: Samples of mesocarp and nut water extract and the yield of mesocarp and nut in vitro enzymatic digestion (stomach and small intestine) were analyzed for total phenolics by Folin-Ciocalteau and oxygen radical absorbance capacity (ORAC) using fluorescein. Analysis of variance and significance test ($p<0,05$) were realized. Results: The yeld from enzymatic digestion with pepsin, pancreatin and bile salt showed the highest statistically significant values of total phenolis (41,04; 35,27 mgGAE/g) and ORAC (260,33; 83,18 µM Trolox equivalents/g) in mesocarp and nut, respectively, demonstrating that the action of enzymes influences the compound release from food matrix. Conclusions: The results obtained indicate that phenolics compounds from mesocarp and nut Babassu are bioaccessible and have a higher antioxidant capacity after enzymatic digestion.

P2.2-09

Uptake and metabolism of 5-caffeoylquinic and caffeic acids in human colon adenocarcinoma cell

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Studies demonstrate that 5-caffeoylquinic acid (5-CQA) and caffeic acid (CA) have a role in cancer prevention. However, data on absorption of these compounds by human colon adenocarcinoma cells are scarce. Therefore, we determined the uptake of both compounds by human colon adenocarcinoma cells (HT-29). Cells were incubated with 5µM of 5-CQA and CA from 0.5h to 96h and cellular uptake was evaluated by LCMS. After incubation with 5-CQA five phenolic compounds (3-CQA, 4-CQA, 5-CQA, CA and isoferulic acid) were identified. Increase in cellular uptake of 5-CQA began 0.5h after incubation with maximum uptake in 1h. The maximum uptake for 3- and 4-CQA occurred 2h after the incubation, indicating intracellular isomerization of 5-CQA. Caffeic and isoferulic acids were identified as from 6h after incubation with 5-CQA, demonstrating intracellular hydrolysis of 5-CQA. After incubation with CA three phenolic compounds (CA, dihydrocaffeic and 3,4-dihydroxybenxoic acids) were identified. Increase in cellular uptake of CA began 0.5h after incubation with maximum uptake in 2h. Intracellular concentrations of dihydrocaffeic and 3,4-dihydroxybenxoic acids began increased 2h after incubation with CA being the maximum uptake, respectively, in 6h and 24h. Our results demonstrate that in physiological concentrations 5-CQA and CA are absorbed and metabolized by HT-29 cell line. Financial Support: CAPES, CNPq, FAPERJ, UFRJ, UNIRIO

P2.2-10

PhytoHUB: a new online database dedicated to dietary phytochemicals and their human metabolites

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PhytoHUB will contain all the main phytochemicals present in edible plants (polyphenols, terpenes, alkaloids, glucosinolates, …), their major dietary sources and their known metabolitesextracted from the literature. Since the metabolism of many phytochemicals has notbeen studied in humans, a list of predicted metabolites will be generated from expert knowledgeand analysis of functional groups present in the precursor structure. Physico-chemical and mass spectral data will be included from theliterature, other databases and experimental data from our andcollaborative platforms.Built with MySQL and Perl processing chains, an efficient relational design will underpin apowerful and intuitive web interface. For a queried monoisotopic mass or molecular formula, PhytoHUB will return a list of possible metabolites and possible dietary precursors linked to food sources. For a queried food, it will return a list of metabolites likely to be present in biofluids after consumption.
PhytoHUB will be the first publicly accessible database to collate information on phytochemical metabolites from a metabolomics standpoint, and should facilitate identification of unknowns in non-targeted profiling. A first version of the database will be launched by the end of 2013 (www.phytohub.eu). ANR Phenomenep ALIA-2010-007 / Conseil Regional Auvergne-FEDER post-doc grant (YF).

P2.2-11

Application of the Phenol-Explorer database to predict the metabolic profile of polyphenols after wine and wine products intake

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Wine intake has been related to a lower total mortality rate and reduced incidence of cardiovascular diseases. Considering the important role of polyphenols abundant in wine, knowledge of the metabolic profile of polyphenols derived from the consumption of red wine could be key to understand its health benefits.

The goal of this study is to predict the wine polyphenol metabolome in human biofluids as accurately as possible by using Phenol-Explorer. The qualitative prediction is done by using those metabolites described in intervention studies with wine products, and those metabolites derived from the consumption of compounds present in wine.

A total number of 97 metabolites were predicted after wine products consumption in plasma and urine. Metabolites described after wine consumption were 37, while 90 different metabolites were identified in intervention studies with pure compounds known to be present in wine. Thirty metabolites were common to both groups. With all the metabolites retrieved from this analysis, a global pathway has been proposed.

In conclusion, the Phenol-Explorer database has allowed building up a comprehensive pathway for wine polyphenol metabolites. It should help identifying metabolites most promising as biomarkers of wine consumption and could also be key to understand the health effects of wine.

P2.2-12

Changes in microbiota and microbial-derived phenolic metabolites of faeces in mice fed with cranberry and grape seed extracts

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Proanthocyanidin structure features (i.e, interflavanic bond A- or B-type) seems to influence their biological activity. Among others, A-type proanthocyanidins have proven to inhibit the adhesion of pathogenic bacteria to uroepithelial cells of the urinary tract, a property not found for B-type proanthocyanidins. Scientific evidence indicates that the physiological effects of proanthocyanidins could be due to the metabolites formed in the tissues and, mainly, by the colon microbiota during their passage through the gastrointestinal tract. On the other hand, proanthocyanidins and/or their microbial-derived metabolites could exert a selective effect on inhibition or stimulation on the intestinal microbiota that, in turn, would affect microbial metabolizing capacity. The aim of this study was to explore differences in colonic catabolism between A- and B-type proanthocyanidins, by means of experiments in mice fed with cranberry (rich in type-A proanthocyanidins) and grape seed (rich in type-A proanthocyanidins) extracts. After a 3-week adaptation time, JAXc3H/OuJ female mice (n=30) were divided into three groups and maintained in a specific diet (control, 1% cranberry extract and 1% grape seed extract) for 4 weeks. Faecal samples were collected at 5 different times and analysing for phenolic metabolites by UPLC-ESI-MS/MS and for microbial counting of *Lactobacillus*, *Bifidobacterium*, *Enterobacteria*, *Clostridiumgroups and Escherichiacoli* species. Results indicated differences in the metabolism of A-type and B-type proanthocyanidins related to changes in some microbial groups.

P2.2-13

A novel urinary targeted metabolomic approach for discriminatory analysis of food intake biomarkers

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The discovery of biomarkers of intake is essential to associate dietary intake (considering their bioavailability) and diet-related risk factors for diseases in nutritional epidemiological studies. The aim is to establish a new screening method for discover novel potential biomarkers of food intake. High-risk male volunteers (n=36) were included in this randomized, crossover and intervention trial. Subjects received red wine or gin (both 30g alcohol/d) or dealcoholized red wine during 4 weeks. 24h urine was collected at baseline and after each period. Host and microbial wine metabolites (>60) including resveratrol, catechins, valerolactones and phenolic acids were analyzed by targeted UPLC-MS/MS using hydrolyzed and non-hydrolyzed samples followed by multivariate statistics tools (PCA, clustering). A logistic regression analysis using the stepwise variable selection was used for biomarker evaluation for predicting wine-intake. The prediction model included mainly gallic and ethylgallate metabolites whose AUC, sensitivity and specificity were higher than 91% in the training and validations sets. In contrast, lower values were obtained after evaluating individual groups of wine polyphenol metabolites except for resveratrol and its microbial metabolites whose values were similar than those of the prediction model (values higher than 91 and 93%, respectively).

The prediction model established by this targeted metabolomic analysis is a useful tool and has the potential to evaluate biomarkers of intake.

P2.2-14

Uptake and metabolism of the major diterpenoids present in a rosemary extract

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Carnosic acid (CA) and derived diterpenes, abundant in rosemary extracts (RE), exert a wide range of beneficial effects, i.e. anti-obesity, anti-inflammatory, anti-carcinogenic, hepato-protective. The aim of this research was to identify and quantify the main *in vivo* formed metabolites for these compounds using a rat model. A total of 26 compounds were tentatively identified based on accurate mass information and the isotopic pattern provided by TOF-MS analyzer.The main metabolites detected in the gut content, liver and plasma were the glucuronide conjugates of CA, carnosol and rosmanol. Two other metabolites were also identified: CA 12-methyl ether and 5,6,7,10-tetrahydro-7-hydroxyrosmariquinone. All the metabolites were detected as early as 25 min following oral administration. Most of the compounds remained in the intestine, liver and (or) plasma at substantial concentrations for several hours supporting their potential health benefits in these tissues. We also corroborated the presence of small quantities of CA and detected trace quantities of the main CA metabolites in the brain. We report for the first time a comprehensive profile of metabolites in various organs following the oral consumption of a RE enriched in CA and contribute to establish the potential bioactive molecules.

P2.2-15

The interaction of polyphenolic compound curcumin with *Escherichia coli* species

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Curcumin, a polyphenolic compound, is a naturally occurring yellow-orange pigment found in the rhizomes of *Curcuma longa* and other *Curcuma* species. Curcumin is well-known for its broad range of biological activities including its action as an anti-inflammatory and its choleretic and antioxidant properties. Studies on the interaction of curcumin with gut bacteria, however, are scarce. A recent study reported that the enzyme *CurA* isolated from *Escherichia coli* strain *K-12*, substrain DH10B was able to transform curcumin to dihydrocurcumin (DHC) and tetrahydrocurcumin (THC) [Hassaninasab et al., PNAS, 2011]. This project examines the transformation of curcuminby three *E.coli* strains *in vitro*, namely *E.coli fergusonii* (ATCC 35469)*, E.coli* (ATCC 8739) and *E.coli* DH10B. It was found that (i) supplementing the media for bacterial growth with 0.4 mM of curcumin did not have detrimental effects on the growth of all three *E.coli* species and (ii) 30-70% of curcumin was lost over 36 h. The identification and quantification of products from curcumin conversion were also carried out using LC-ESI-MSMS. Both adduct and metabolites of curcumin were formed as a result of bacterial transformation.

P3.1-01

Effect of white grape juice treatment on lipid damage in liver and biochemistry parameters of rats treated with carbon tetrachloride

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The objective of this study was to evaluate the effect of conventional and organic white grape juice on lipid damage in liver and in biochemistry parameters is serum provoked by carbon tetrachloride (CCl_4) in rats.Adult male rats (~300g; n=10/group) were orally treated with 7 µL/g of juices for 14 days. On the 15th day it was administered intraperitonially in half of the rats mineral oil and the other half CCl_4 (3.0 mL/kg). Animals were euthanized and the liver was dissected and used for the analysis of lipid damage (TBARS). The trunk blood was also collected and serum was afterwards separated to performedthe assaysof triglycerides (TG), glycemia (GL), total cholesterol (TC) and its fractions, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltranspeptidase (GGT). We observed that CCl_4increased TBARS and that both juices were able to prevent this increase. In the biochemistry parameters we verified that CCl4 was capable to increased the GL, ALT e GGT and reduced the TC and HDL-cholesterol, the both grape juice was capable, only, to prevent the alters in cholesterol. Therefore, we could propose that white grape juices has hepatoprotective properties reducing the lipid damage and prevent the reduced of cholesterol. Supported by: CNPq and FAPERGS.

P3.1-02

Dimorphism by sex in haemolymphatic distribution of polyphenols and redox response of mice treated with native plant extracts

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Modulation of immune response through the use of diets enriched with polyphenols or development of biodrugs from these compounds, isa promising fieldinthe prevention and treatmentof various pathologies. Nevertheless, have not been fully identified vegetable sources of polyphenols and their actions in the tissues hemolymphatic, therefore were tested Lantana grisebachii (LG), Aspidosperma quebracho blanco (AQB) and Ilex paraguariensis (IP) extracts on a mouse model, and subsequently analyzed tissue levels of polyphenols, nitrite, superoxide anion and the activity of gamma-glutamyl transpeptidase by colorimetric techniques in blood, thymus and spleen, comparing effects of LG, IP and AQB with control group and discriminated by sex for $p<0.05$ (ANOVA). Was found blood levels of polyphenols increased in treated males compared to controls, while females responded with more tissue distribution (spleen and thymus) to intakeof extracts,with the consequentreduction inblood, whichwould indicate adimorphismin thetransport andtissue uptakeofpolyphenolsthatcouldrespond tosexualdifferentiationin the affinity ofthese compoundsfor plasmaand tissue proteins, respectively. Moreover, the response parameters redox-dependent showed equal variation according to sex, showing, in general, the higher antioxidant response in lymphatic organs of females and a potential immunoprotective effect of AQB, similar for both sexes.

P3.1-03

Wistar rats on a diet including peach (*Prunus persica (L.) Batsch*) or derivate products were protected against CCl_4-induced oxidative stress

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The present study was conducted to evaluate the antioxidant potential of a diet including peaches or its derivate products (peach in syrup, peels and syrup) on serum, liver, kidney, heart, lung, brain cortex and cerebellum in male wistar rats. Animals received an oral diet (gavage) of lyophilized extracts (200 or 400 mg/kg) for 30 days. Hepatotoxic oxidative damage was induced by a single injection of CCl_4 (3 mL/kg i.p.). Animals that received the diets consisting on peaches and peels presented varying degrees of protection against toxic effects of CCl_4, as evidenced by assays of aspartate transaminase and alanine transaminase in serum, as well as oxidative damage to lipids (evaluated by a TBARS-based assay) and proteins (assessed by quantification of carbonyl groups) in serum and in all other tissues analyzed. The effect of CCl_4 on catalase and superoxide dismutase was prevented by peaches and peels. Alterations in serum nitrotyrosine and bilirubin levels by CCl_4 were also prevented in animals that received peaches and peach peels in the diet. The present results indicate that diets containing peaches may confer protective properties to different organs, which may be important in the prevention against acute oxidative damage. Financial support: CAPES, FAPERGS, CNPq and PROPESQ-UFRGS.

P3.1-04

Quercetin inhibitory effect on the prooxidant activity of lipoxygenase in reverse-micelle systems

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Quercetin is a natural component of the human diet presenting high antioxidant and metal chelating activities. It has been reported that this flavonoid inhibit the prooxidant activity of certain enzymes as lipoxygenases in direct micelles. Lipoxygenases are non-heme iron metalloenzymes. They catalyze the oxidation of polyunsaturated fatty acids containing a 1,4-*cis*,*cis*-pentadiene system, yielding the lipid conjugated hydroperoxides. Their action affects sensorial and nutritional properties of food. Besides, they have been associated to inflammatory processes. The objectives of this study were to determine the inhibitory effect of quercetin on the prooxidant activity of lipoxygenase using linoleic acid as substrate, to calculate the kinetic parameters and to elucidate the type of inhibition in reverse-micelle systems. The inhibitory action of quercetin was found to increase with its concentration. Furthermore, the flavonoid presence decreases the oxidation reaction rate. The kinetic parameters calculated using the Michaelis-Menten model, for Vmax varied in a range of 267-101 nMs-1 and between 222 and 1034 µM for Km. From Lineweaver-Burk graphic, it is concluded that quercetin behaves as a total mixed noncompetitive inhibitor against lipoxygenase. The dissociation constant of the enzyme-inhibitor complex was K_i = 1.4 µM. These results were analyzed comparatively with those obtained for other polyphenols studied.

P3.1-05

Biochemical and inflammatory parameters of human neutrophils after green tea catechins exposure

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This study aims to evaluate the potential of a mix of catechins in modulating in vitro biochemical and inflammatory parameters of human neutrophils. Neutrophils from healthy subjects were cultivated and treated with a mix of catechins (30 µM of EGCG, 3 µM of EGC, 2 µM of ECG and 1.4 µM of EC) or with the catechins alone. The following assays were performed: intracellular calcium release, chemotactic and phagocytic capacities, mieloperoxidase and G6PDH activities, release of hypochlorous acid (HOCl) and pro-inflammatory cytokines (IL-6 and IL-1β). Production of ROS/RNS was evaluated by measuring $O_2^{\cdot-}$ (dihydroetidium and lucigenin assay) and H_2O_2 (DCFH-DA probe), $NO^{\cdot}$(Griess reagent). The mix of catechins increased the intracellular calcium release. EGCG, ECG and the mix reduced chemotaxis capacity, although, an increase in phagocytic capacity was observed in the same groups. A decrease in myeloperoxidase activity was observed in all experimental groups as well as release of HOCl. IL-6 decreased in all groups, although, IL1β only decreased by EC and the mix. The mix of catechins and each catechins alone, decreased the production of $O_2^{\cdot-}$, H_2O_2 and $NO^{\cdot}$. G6PDH activity decreased only in ECG group. The catechins have an important role as an immunomodulator agent against inflammatory process in neutrophils. Financial Support: Fapesp (2011/19216-8); CNPq (139307/2012-5).

P3.1-06

Relation between in vitro antioxidant capacity and plasmatic antioxidant capacity of hidroalcoholic extracts of *Buddleja globossa* Hope and *Plantago major* L

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The use of medicinal plants today has taken great importance at a national and global level, with Latin America accounting for 60% of the biodiversity of these herbal species. But the development of herbal medicines (formulations containing polyvalent herbal extract) provides stages different than those involved in the formulations containing one single active ingredient or purified synthetic. Because of this we highlight the importance of standardizing natural products. This requires the development of new techniques and/or methodological approaches that allow us to simultaneously evaluate several active compounds and also demonstrate the relationship of their plasma concentrations with some pharmacologic effect. Through different methodologies, we characterized the *in vitro* antioxidant capacity of two widely used medicinal plants in Chile *Buddleja globosa* Hope, known as "Matico" and *Plantago major* L, known as "Llantén", and correlate them with the *in vivo* plasmatic antioxidant capacity. Matico is a perennial native shrub whose leaves present antioxidant, anti-inflammatory, healing and analgesic activity. Moreover, Llantén is an herbaceous perennial, with a worldwide distribution, whose leaves has hepato-protective characteristics, analgesic, anti-inflammatory and wound healing properties. We believe this study represents the first step to develop herbal medicines with recognized efficacy and safety properties.

P3.1-07

Relationship between the molecular structure of natural flavones and some mechanisms implicated in neuronal protection

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The study of the chemical substitutions of neuroprotective flavonoids will be meaningful for the design of new molecules for the treatment of neuropathology. To this aim a structure-activity relationship study was previously performed, evaluating the protective capacity of 13 flavones in primary culture of cerebellar granule cells against oxidative stress. Results showed that only 4 flavones exhibited neuroprotection, indicating that specific structural features would control this activity.In the present study we explored putative mechanisms of action of flavones underlying such protective effect (*in vitro* antioxidant capacity, intracellular levels of reactive oxygen species, lipid peroxidation, intracellular calcium,and cellular labil iron pool). In addition, the bioavailability of molecules in cultures was evaluated by HPLC.
The results showed that all flavones presented good antioxidant capacity *in vitro* although there was no direct relationship with the neuronal protection. Besides, both neuroprotective and non-protective flavones are able to prevent the formation of intracellular ROS and MDA after the oxidative insult. However, while the labile iron pool levels are not affected in this experimental paradigm, preliminary results showed that calcium homeostasis appear to be involved. In addition, cellular bioavailability results showed that neuroprotective flavones without the catechol group in B-ring were more stable.

P3.1-08

Bioavailability of phytochemicals in murine metabolic organs, after consumption of infusions of Argentinean native plants

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The antioxidant properties of plants are of considerable research interest given the intrinsic relationship between oxidative stress and etiophysiopathogeny in many diseases. With the aim of knowing their potential, we sought to define the kinetic behavior of phytochemical constituents due to its conditioning factor in their role as chemopreventive agents. Thus, aqueous extracts of L. grisebachii (LG), A. quebracho-blanco (AQB) and I. paraguariensis (IP) were obtained. A phytochemical characterization was performed, establishing total phenols (TP) and flavonoids (F)[1,2]. Later, was evaluated in-vitro their motion through isolated intestine segments[3], together with organic distribution and redox effect after being consumed by BALB/C mice, estimating TP and superoxide anion (SO)[4] in removed organs (liver and kidney) (TestT, $p<0,05$). The phytochemical analysis indicated that AQB shows 2.6±0.14 mgEAG/g dry extract, corresponding to 37.7% flavonoids; LG 10.2±0.43/58.5% and IP 21.8±1.37/24.5%. Low absorbed-TP concentration was found, AQB resulting in a motion of six times more than LG, and 2.4 times more than IP. These differences did not have effects on TP tissue concentration, therefore males treated with IP showed more TP content and significant increase of SO in liver. The results that indicate AQB with less TP, more absorption and which do not denote oxidizing effects unlike IP, require further research about phenolic bioavailability whose differential kinetics would be determinant of redox-dependant chemopreventive responses. References: [1]Dewanto V et al. J Agric Food Chem. 2002; 50:3010-3014. [2]Salamanca Grossso G et al. Zootecnia Tropical. 2007; 25:95-102. [3]Breda SA et al. Int J Pharm. 2009; 371:106-113. [4]Becerra MC et al. Lumin. 2003; 18:334-340.

P3.1-09

Thermodynamic modeling of the aqueous solubility of some fundamental polyphenols as an approach to understand their bioactivity

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Polyphenols are secondary metabolites widely distributed in the higher plant kingdom that have the ability to complex strongly with carbohydrates and proteins. This ability is strictly related to the antioxidant, antibacterial, antiviral and anti-inflammatory properties of polyphenols, which make them useful bioactive compounds for the food, pharmaceutical and cosmetic industries. The complexation of polyphenols with other macromolecules normally occurs in aqueous media. Therefore, the aqueous solubility of polyphenols is very important to understand how they interact with other macromolecules and how these interactions affect their bioactive properties. Experimental determination of the aqueous solubility of polyphenols is expensive and time consuming since it is extremely difficult to isolate them at the levels of required purity. An alternative is to generate a thermodynamic model to predict the data as a function of temperature, pressure and molecular nature of polyphenols. We have developed a model with those features to describe the aqueous solubility of some fundamental polyphenols such as (+)-catechin, (-)-epicatechin, quercetin and gallic acid using a SAFT-type equation of state. Preliminary results show that the model successfully correlates the experimental aqueous solubility data of the selected polyphenols, which is a fundamental contribution for further understanding their bioactivity.

P3.1-10

Sesamin ameliorates ethanol-induced liver injury via inhibition of NF kappa B activation in rats

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Chronic alcohol consumption is a cause of hepatic injury and inflammation induced by LPS invasion from the intestine. Sesamin is one of major lignans in sesame seeds. We have previously reported that sesamin has protective effects against liver injury and oxidative stress induced by chronic alcohol consumption. To clarify the underlying mechanisms, we investigated the effects of sesamin on liver inflammation.

Wistar rats were divided in four groups and fed on experimental diets for 8 weeks; basal diet (control), ethanol diet containing 20% w/w ethanol powder, ethanol diet containing 0.1% or 0.2% sesamin. After 8 weeks administration, liver gene expressions related with oxidative stress and inflammation were evaluated. Gene expressions encoding NADPH oxidase components and COX-2 in ethanol diet group were significantly increased than the control group. Sesamin suppressed these gene expressions, especially COX-2. Furthermore, in vitro study, we evaluated the influence of sesamin and its metabolites on LPS-induced NO production in HL-60 cells. The metabolites (SC-1) of sesamin was strongly suppressed NO production, suggesting that SC-1 could inhibit NFkB activation by LPS.

These results suggest that the ingestion of sesamin could be beneficial in ameliorating hepatic inflammation resulting from chronic alcohol consumption by inhibiting NFkB signaling pathway.

P3.1-11

Polyphenols from cagaita (*Eugenia dysenterica* DC.) fruit and leaves and their in vitro antioxidant / antidiabetic / antiobesity functionality

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Brazil has a natural abundance of native fruits due to its area, geographic location, climate and soil conditions; however, few species are being exploited commercial and industrially and limited information on their chemistry, biochemistry and nutritional properties is available. This study was conducted to evaluate the concentrations of polyphenols, mainly flavonoids, in cagaita fruit and leaves and their antioxidant / antidiabetic / antiobesity potential. Leaves and fruit showed are a potential source of bioactive compounds, including total phenolics, proanthocyanidins, carotenoids; however, leaves reported values highest in these compounds (146 mg gallic acid equivalents, 136 mg quebracho tannin equivalents and 130 µg/g dw respectively) and antioxidant capacities when compared with the fruit (13, 6 and 84, respectively). Quercetin, kaempferol derivatives, and catequin were the main identified flavonoids in both leaves and fruit. The phenolic-rich extracts showed high inhibitory activity against α-glucosidase (IC_{50} 8.5 and 20.0 ml for leaves and fruit extracts respectively) and pancreatic lipase (IC_{50} 5.7 ml and 10.0 ml); indicating that these phytochemicals may be useful for food-based strategies for complementing effective antidiabetes and antiobesity treating. The results of this study give scientific support on the potential health benefits and to incentive for further research into bioactive compounds of cagaita.

P3.1-12

The biflavonid fukugise from *Garcina madruno* inhibits ROS production in macrophages

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Garcinia madruno (Kunth) Hammel, commonly known as madroño, is a tree endemic to Central and South America. In previous investigations, we reported that a biflavonoid fraction (FB) from *G. madruno* was effective in stabilizing free radicals and inhibit the formation of oxidized low-density lipoprotein (oxLDL). We also isolated and characterized a new biflavonoid (7''-*O*-(6''''-acetyl)-glucoside of morelloflavone), on the basis of 1D, 2D NMR (HMQC and HMBC) spectroscopic methods and chemical evidence, along with five known biflavonoids. Consequently, we became interested in carrying out a comprehensive investigation of the antioxidant activity of these compounds. Thus, the ability of the biflavonoids to inhibit the production of reactive oxygen species (ROS) in macrophages stimulated with oxLDL was determined by using fluorometric techniques and the fluorescent probe DCFH-DA. Fukugiside, a glucoside flavanone-(3→8'')-flavone biflavonoid with a doubling of signals in the 1H and ^{13}C-NMR suggesting the existence of two conformers due its rotational behaviour (atropisomerism), decreased ROS production more efficiently than other bioflavonoids in oxLDL-stimulated macrophages. The flow cytometric analysis also evidenced that Fukugiside (40µM) reduced both the percentage of macrophages producing ROS and the amount of ROS produced per cell. These results suggest that Fukugiside is a potent inhibitor of ROS production in macrophages.

P3.1-13

Luteolin modulates expression of phase II drug-metabolizing enzymes through Nrf2 pathway in HepG2 cells

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Luteolin, a plant-derived flavone, exerts antioxidant and anti-cancer activities, which are involved in theinduction of phase II drug-metabolizing enzymes. Nuclear factor erythroid 2-related factor 2 (Nrf2), a redox-sensitive transcription factor, interacts with antioxidant response element (ARE) and regulates the expression of phase II enzymes. In this study, we evaluated the effect of luteolin on the expression of phase II enzymes and clarified its underlying molecular mechanisms. The results showed that luteolin increased protein expression level of GSTs, NQO1 and HO-1 in a dose-dependent manner, and the significant increases were observed at 1 nM luteolin. At the same concentration, luteolin also significantly increased Nrf2 expression and specific binding between Nrf2 and ARE in nucleus. It was noteworthy that luteolin decreased the degradation of Nrf2 by suppressing its ubiquitination. Moreover, a modified keap1 was observed in luteolin-treated cells accompanying by the reduction of normal Keap1 protein. From these results, luteolin increased Nrf2/ARE-driven phase II drug-metabolizing enzymes by stabilizing Nrf2 that accomplished by modifying Keap1 and inhibiting the ubiquitination of Nrf2.

P3.1-14

Protective effects of pressurized hot water extracts from grape pomace on HL-60 cell culture under oxidative conditions

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Pressurized hot water extraction (PHWE) is a highly promising energy-efficient and environmentally benign technique for recovering polyphenolsfrom natural materials. However in the PHWE of polyphenols from plants the relationship between antioxidant activity and the polyphenol content is disputeddue to diverse phenomena highly dependent on temperature, such as thermal degradationand the formation of neo-antioxidant substances.Therefore, depending on the PHWE temperature used, it is possible to obtain extracts with different compositions and antioxidant activities and, consequently, different bioactive properties. In this work we assessedtwo extracts from grape pomace (a winery byproduct)obtained at 100°C (GPE100) and 200°C (GPE200) by PHWE in terms of antioxidant activity (FRAP assay), total antioxidants (Folin assay), anthocyanins and tannin content (Harbertson−Adams assay),as well as protective effect on cell growth and mitochondrial membrane potential in a HL-60cellculture under oxidative conditions. GPE100 showed a much more elevated anthocyanin and tannin content than GPE200 but less antioxidant activity and total antioxidants content. Only GPE200 showed a protective effect on the growth of HL-60 cells (at the highest concentration tested, 1 mg/mL). Both GPE100 and GPE200 decreased the mitochondrial membrane potential lossbut the effect of GPE100 was threetimes higher than GPE200.

P3.1-15

Peruvian propolis inhibits oxidative damage of liver mitochondria induced by Fe(II)citrate

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Propolis is a mixture of substances derived from vegetable sources, the mixture is made by bees and have high content of polyphenols responsible for its antioxidant capacity in vitro and in vivo.

We assessed the hepatoprotective ability of three sample Peruvian propolis as ethanolic extract (EEP) on mitochondria isolated from rat liver. The isolated mitochondria were induced to oxidative damage by Fe(II)citrate.

The three samples of propolis have between 211-283 µgEAG/mg EEP and between 15-24 µg EQ/mg EEP of phenols and total flavonoids, respectively. Their DPPH radical scavenging activity with a IC50 between 3,7 to 5,2 µg EEP versus 1,8 µg ascorbic acid.

Inhibition of lipid peroxidation in mitochondria induced by Fe(II)citrate is performed in the presence of propolis samples or quercertin and assayed by determination of thiobarbituric acid reactive substances (TBARS). Inhibition of lipid peroxidation induced by mitochondria (1 mg protein) is between 45-73% in presence of 680 µg of EEP or 18% with 4 µM quercetin.

It is suggested that the high content of polyphenols in propolis scavenge generated free radicals or form a complex with Fe(II) to prevent oxidative damage in mitochondria.

P3.1-16

Electrochemical assessment of the antioxidant capacity of 7,8-dihydroxy coumarins towards DDPH and superoxide anion

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Coumarins are widely distributed in the nature and hydroxyl substituted ones have a demonstrated antioxidant capacity. This work reports the assessment of the antioxidant capacity of a new synthesized hybrid coumarin. Measurements were performed in a CH Instrument 760-C workstation. Dry DMSO + 0.1M TBAHFP at 20°C was the electrolytic medium. Cyclic voltammograms were recorded at 0.1 V/s on a glassy carbon electrode, Ag/AgCl was the reference electrode separated from the solution by a salt bridge and a Pt wire was the counter electrode. Tested compounds were 4-chloromethyl-7,8-dihydroxy-coumarin, caffeic acid, 4-methyl-(3,4-dihydroxycinnamate)-7,8-dihydroxy-coumarin, Trolox. Coumarins were synthetized in our laboratory. Superoxide anion was electrochemically generated by the reduction of pure O_2 from a saturated solution according to a previous reported method[1]. 0.5 mM DPPH solutions purged with nitrogen were used for the assay of this radical. A 0.1M stock solution of coumarins was used as work solution. Antioxidant activity (IC_{30} & antioxidant capacity) was obtained from the plot of dimensionless parameter[1] $(Ipa^0\text{-}Ipa^S)/Ipa^0$ *v/s* antioxidant concentration. Synthesized hybrid coumarin exhibited the lowest IC_{30} towards both tested radicals compared with halogen-substituted coumarin and caffeic acid, indicating a synergic contribution of the substituent in 4-position. These results are compared with the obtained by UV-Visible spectroscopy. Reference: [1]Le Bourvellec C. et al. *Talanta* 75**,** 1098–1103 (2008). Acknowledgements: The financial support of Conicyt Doctoral Fellowships 21100054-24121109 and Proyect Fondecyt 1110039 is acknowledged.

P3.1-17

Characterization of antioxidant activity of aqueous extract from *Rosmarinus officinalis*

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Growing interest in natural antioxidants has developed due to a need for more effective, less toxic and cost effective antioxidants, and medicinal plants appear to have these desired advantages. The aims of this work were to evaluate the antioxidant capacity and the effect on lipid-peroxidation of aqueous extracts from *Rosmarinus officinalis*. Infusion and decoction of 5 % w/v were prepared and brain homogenates were used to determine the effect on lipid-peroxidation. Both aqueous extracts presented antioxidant activity measured as DPPH, ABTS, TRAP and reducing power assay, and decoction displayed significant higher values than infusion in terms of DPPH (*p<0.05*) and ABTS (*p<0.01*) assays. In addition, infusion and decoction showed a strong inhibition of lipid-peroxidation of brain homogenates measured as TBARS (IC_{50}=63±1 µg/mL and 56±1 µg/mL, respectively, *p<0.05*). The total polyphenol (TP) and total flavonoids (TF) contents were also evaluated (TP (mg gallic acid/g)= 150.0±4.2 for infusion and 179.9±3.0 for decoction, *p<0.05*; TF (mg gallic acid/g)= 4.9±0.6 for infusion and 8.0±1.6 for decoction). The results obtained in the present study indicate that aqueous extracts of *Rosmarinus officinalis* exhibit antioxidant properties and a protective effect on lipid-peroxidation process. Therefore, they could be use as a source of natural antioxidants in the treatment of several diseases associated with oxidative stress damage.

P3.1-18

Potent anti-inflammatory activity of cyanidin-3-glucoside as compared to 5-aminosalicylic acid in activated RAW 264.7 macrophages

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The release of reactive oxygen species (ROS) and expression of pro-inflammatory mediators are critical events in the inflammatory response underlying several common diseases, such as inflammatory bowel diseases (IBD). 5-Aminosalicylic acid (5-ASA) is anantioxidant and anti-inflammatory drug currently used in these diseases, but polyphenols have been gaining popularity as a complementary strategy for IBD prevention or treatment. Among the common dietary polyphenols, anthocyanins are known to possess several health-promoting effects, including anti-inflammatory activity. Thus, the aim of this work was to study the ability of cyanidin-3-glucoside (Cy3glc), a major dietary anthocyanin, to prevent inflammation, as compared with 5-ASA, using a lipopolysaccharide (LPS)-activated macrophage cell line (RAW 264.7) as a model. Pre-incubation of cells with 25 µM Cy3glc protected them from LPS-mediated NO and ROS formation, much more efficiently than with 25 µM 5-ASA. Moreover, the protection afforded by Cy3glc against the increases in PGE2 and TNF-α productions or in COX-2 and iNOS expressions, was higher than that observed for 5-ASA. Our data suggest that Cy3glc has a much stronger anti-inflammatory activity than 5-ASA in macrophage cells. Considering that inflamed intestinal tissue contains an increased number of phagocytic cells, able to produce reactive and pro-inflammatory species, these results suggest an important role for Cy3glc in modulating intestinal inflammation. Supported by PTDC/SAU-OSM/102907/2008 and PEst-C/SAU/LA0001/2013-2014; Pereira S. is a fellowship recipient from FCT (SFRH/BD/89758/2012).

P3.1-19

Protection against lipid peroxidation in HT-29 cells using a functional powder ingredient obtained from wine-making residues. Changes after in vitro gastrointestinal digestion and colonic microbial fermentation

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Lipid peroxidation in cells has been implicated in the pathogenesis of various diseases, including cancer. Using HT-29 cell cultures, the aim of this study was to evaluate the protection against lipid peroxidation of a functional powder ingredient obtained from grape pomace, a well-known source of polyphenols and dietary fiber, comparing the different effect, the bioavailability and the antioxidant environment generated by the product (Gp) and the fractions obtained after a simulated gastrointestinal digestion (dGp) and microbial colonic fermentation (d+fGp). The identification and quantification of grape main polyphenols and derived metabolites were analyzed by UPLC-MS/MS. Cells were incubated 24 h with a non-cytotoxic treatment of 200 µg/mL of each sample, following an oxidative stress induction with t-BOOH in the absence (-t) or presence (+t) of the treatments. Cell viability was determined by the MTT assay, and the protection against lipid peroxidation was evaluated quantifying malondialdehyde (MDA) levels using HPLC-DAD. The results showed that the phenolic compounds derived from the original product retain or even increase their antioxidant capacity during the processes that take place in the gastrointestinal tract, exerting a protective effect against lipid peroxidation in HT-29 cells (-t % protection: Gp=36.7, dGp=44.6, d+fGp=32.5; +t protection: Gp=56.4%, dGp=65.6, d+fGp=50.3).

P3.1-20

Hesperetin production from Brazilian orange pomace by biotransformation

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Flavanones in citrus are molecules that play an important role in antioxidant activities in nutraceutical products, with potential for therapy on carcinoids. The aim of this study was to evaluate hesperetin production by solid-state fermentation using Brazilian orange pomace. The production kinetic studies were performed to define the time profile of hesperetin/tannase production. The results showed higher concentration of hesperetin after 48h, increasing 40 times. Tannase is supposed to be, partially, responsible for phenolic biotransformation, which presents greaternutraceutical potential. To verify this hypothesis, hesperidin wasbioconverted into hesperetin in reaction catalyzed by the tannase from *Paecilomyces variotii* and the product was assayed for its biological tests.The results for antioxidant, by ORAC method, showed an increase of 180%.The antiproliferative activity of hesperidin after the biotransformation with tannase increased significantly, with the effect observed in concentrations much lower than that of the original compound. In the highest concentrations tested, the biotransformed compound completely inhibited the proliferation of some celllines tested (U251, MCF7, 786-0, OVCAR-3, HT29, HaCat), and in one of them (NCI-ADR/RES), the compound had a cytocidal effect. These results suggest that bioprocess may be a viable alternative for the hesperetin production, a promising flavonoid for health improvement.

P3.1-21

Encapsulation of natural antioxidants of beetroot in capsules of alginate or alginate/espina corona gum

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Beetrrotis an interesting sourceof pigments andantioxidants.These componentsare often unstable, so encapsulating themusingnatural polyelectrolytes and gums emerges as an alternativefor their preservation.The “espina corona” gumis obtained fromseedsof*Gleditsia Amorphoides* which grows in the northern of Argentina,andits properties havebeenpoorly explored.

In thiswork we analyzedthe influence of the confinement in polyelectrolyte capsules and of the incorporation of espina corona gumon physical stabilityandantioxidant capacity ofan aqueous beetroot extract.Capsules were preparedbydroppingalginate (2% w/v)or alginate -gum (0.5% w/v) solutions containing the extract,over $CaCl_2$. Capsules were dried (freeze/vacuum-drying) and exposed todifferent types of light (visible/UV). Characteristicsofcolor/morphological, antioxidant capacity, polyphenols contentand interactions, were evaluated by opticalmicroscopy/SEM, DPPH, Folin-Ciocalteu andFTIR, respectively.

Thedriedcapsulesallowed to mainainedthe antioxidant capacityof the extractprovidig a system easier to storage and handling.The frreze-dried capsulesshowed better structural characteristics and better preserved the color than vacuum-dried capsules. The best resultswere obtained usingalginate/gum capsules; interactions in these systems became evident by shifts in the FTIR spectra.

P3.1-22

Wine polyphenols and their impact on antioxidant capacity *in vivo*

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The moderate red wine consumption has been associated with a lower incidence of chronic diseases due to its polyphenols content. In this work, we evaluated the antioxidant capacity (AC) of three Argentinean red wine varietals(Cabernet Sauvignon, Merlot and Syrah) and studied their polyphenolic profile, in order to identify the active components responsible for their biological activity. *In vitro* AC was determined by ABTS, DPPH and FRAP methods. *In vivo* AC was evaluated using a *Saccharomyces cerevisiae* modelexposed to oxidative stress with H_2O_2. The phenolic profile was performed by HPLC-MS/MS. Three wine varieties showed significant AC, both *In vitro* and *In vivo,* being Syrah the variety with higher AC.We observed that protective wine effect in cells exposed to H_2O_2 was positively correlated with Glutathione Reductase and Glutathione Peroxidase activities as well as DPPH assay. Multiple regression analyses showed that this AC is highly correlated with the content of epicatequin, isorhamnetin and delphinidin glucoside. Our results suggest that the AC of wine depends upon the grape variety used, since it largely determines the qualitative and quantitative composition in polyphenolic compounds. Also, the results highlight the importance of analyzing the polyphenolic profile, in addition to using *In vivo* assays, to understand the differences in the AC of wines.

P3.1-23

Vegetables phenolic content and their role in retarding oxidative processes in avocado purée

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Extracts from *Allium* or *Brassicaceae* families have shown inhibitory capacity of browning and lipid oxidation reactions. The aim of this work was to establish if polyphenolic compounds of those vegetable extracts are involved in the retardation of deteriorative reactions during refrigeration of avocado purée. Aqueous and lipid fraction of purée were separated by centrifugation. Polyphenol content was determined and its correlation with color changes, polyphenol oxidase (POA) and antioxidant (AA) activities was analyzed in the aqueous fraction. Fourier transform infrared spectroscopy (ATR-FTIR) analysis of the lipid fraction was studied by multivariate analysis. Classification strategies investigated were cluster (CA) and principal components (PCA) analysis and Person correlation coefficients. An antibrowning index was defined for the avocado purée with addition of *Allium* or *Brassica* extracts (10%p/p). Polyphenol content was related to the antibrowning index at the first days of storage. PCA showed that polyphenol content and POA allowed differentiation of the inhibitory extracts sources. PCA applied to FTIR and AA allowed discrimination among fresh and oxidized oil, being polyphenols closely related to the second component. A better understanding of relationships that exist between vegetables polyphenols and browning progress or lipid oxidation could be applied for the development of natural ingredients avoiding the questioned synthetic ones.

P3.1-24

Evaluation of the antioxidant, antiproliferative and antimutagenic potential of araçá-boi fruit (*Eugenia stipitata* Mc Vaugh — Myrtaceae) of the Brazilian Amazon Forest

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Eugenia stipitata is a fruit from Amazonia rich in terpene, volatile compounds, fiber, and vitamin C. The fruit is recognized for its high antioxidant activity and has attracted much attention due to their potential health benefits to humans. The total polyphenols, antioxidant, antiproliferative, antimutagenic and antigenotoxic activities of*E. stipitata*ethanolic extract were investigated. Total polyphenols were determined by the Folin–Ciocalteu method and showed 184.05 ± 8.25 mg GAE/100 g. The radical scavenging activity was $DPPH_{IC50}$ 0.69 ± 0.23 µg/mL and $TAC\text{-}ORAC_{FL}$371.98 µmol. TE/100 g. The extract was evaluated for its ability to inhibit the growth of tumor cell lines and had not complete cystostatic effect against any of the tested cell lines. Antimutagenic and antigenotoxic activities were investigated by micronucleus test and comet assay in mice, respectively. Ethanolic extract of*E. stipitata*showed higher antimutagenic and antigenotoxic properties at the highest concentration tested (300 mg/kg of body weight). In conclusion, these results suggest that this fruit could be used as a preventive agent against cancer.

P3.1-25

Antioxidant effect of quercetin on the response SOD induced by ciprofloxacin

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Some side effects of certain antibiotics, would relate with the ability to increase oxidative stress in human cells. In order to find natural compounds that neutralize the toxicity of reactive oxygen species (ROS) generated, we evaluated the effect of Quercetin (Q), a flavonoid with antioxidant and free radical scavenging properties isolated from *Flaveria bidentis*leaves, as potential protective agent. Previous studies carried out by our group demonstrated a marked protective effect of Q against ROS production induced by Ciprofloxacin (CIP) in human leukocytes. For these reason, the aim of this study was evaluate the effect of Q on endogenous antioxidant defenses like Superoxide Dismutase (SOD, endogenous metalloenzyme able to reduce oxidative stress by dismutation of superoxide anion) in presence of CIP. SOD activity in human leukocytes was evaluated by Riboflavin and NBT assay under three conditions: pre-incubation with Q, pre-incubation with CIP and simultaneous incubation with Q and CIP. Ciprofloxacin increased SOD's activity at 0.015 and 0.5 mg/ml. Q at 10, 50 and 250 µM decreased SOD´s activity showing the greatest protective effect at 10 µM. The greatest decrease in SOD activity by Q was observed after pre-incubation with CIP. These results are in agreement with those obtained previously in our group, and demonstrate a marked protective activity against oxidative stress Q generated by CIP in human leukocytes.

P3.1-26

Parameters chemicals, phenolic composition and antioxidant activity of different Brazilian grape juices

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The aim was evaluated 9 different types of grape juice commercial, as the quantification of phenolic compounds, antioxidant activity in vitro and physico-chemical parameters. We used purple grape juices (whole, reprocessed, sweetened and nectar). We evaluated the physical-chemical parameters (total acidity, volatile acidity, density, alcoholic degree and pH), also, the value of phenolic compounds total. The antioxidant activity of grape juice, was measured in vitro, using the test of DPPH radical'. We verified that all physico-chemical parameters of the juices were within the standards established by the legislation, however, some samples showed statistical differences. On the phenolic composition, the nectar, showed lower levels of phenolic compounds than other juices and the whole grape juice showed the highest levels. Also, the whole grape juice showed the highest antioxidant activity, and the reprocessed. The results obtained allow check expressive difference between the juices, and whole grape juice the more rich in phenolic compounds, as well as with greater antioxidant activity, and these then an important choice between the existing on the market.

P3.1-27

Effect of the channelizing agent on the flavonoids release from microparticles in hydrophobic systems

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The lipid oxidation is one of the major deleterious reactions that occur in oils, leading to the formation of primary and secondary oxidation products and therefore to a loss of chemical and nutritional qualities.Natural antioxidants, such as flavonoids, could be used against lipid oxidation. However, its use can be a drawback, because of its unpleasant flavors, low solubility and it can be degraded,being necessary toadd high levels to obtain the desired effects.In this context, the microencapsulation of flavonoids is a potential tool to protect and control the release of the antioxidant in a lipid matrix, extending the lipid shelf-life.

The objective of this study was to evaluate the effect of channelizing agent on the kinetic release of Quercetin (Q) from microparticles in anhydrophobic medium.

Q was encapsulated by spray drying using inulin as encapsulating agent with or without Capsul (channelizing agent), Q-In and Q-(In-C) systems.The release profile was evaluated in hexane and methyl linoleate.

The encapsulation efficiency in Q-In and Q-(In-C) reached values around 60%. The data release profile was fit to mathematical models for determined rate constant and mechanism release. Q release rate constantin hexane for Q-(In-C) system was higher than Q-In. Acknowledgements: Proyecto FONDECYT-Chile N° 1120308; Proyecto FONDECYT-ACT 1105

P3.1-28

Flavonoids microparticles for formulation of functional oils

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Polyphenols act as antioxidant, which prevent diseases associated with oxidative stress, such as cardiovascular diseases and cancer. Polyphenols have limited solubility and stability and some of them have an unpleasant flavor. The use of encapsulated polyphenols as additives for healthy foods, should allow the protection of the polyphenols (preservation of the nutritional properties) until they are consumed within the food vehicles. In this context, fats and oils could be used as a vehicle of microencapsulated flavonoids, being the design of controlled release microparticles a challenge. Inulin (In) and hydroxypropylcellullose (HPC) were selected as encapsulating agent of flavonoids because could behave like inert matrix.

The objective of this study was to evaluate the In and HPC as encapsulant agents on encapsulation efficiency and kinetic release of Naringenin (N) in an hydrophobic vehicle.

N was encapsulated by spray drying(N-In and N-HPC).The release profile was evaluated in hexane and data were fit to mathematical models.

The encapsulation efficiency was determined. HPC was better encapsulating agent of N than inulin, reaching values of60 and 40%, respectively. On the other hand the N release rate constant was significantly lower in HPC than In, showing its potential applicability in formulation of functional oils. Acknowledgments: Proyecto FONDECYT-Chile N° 1120308; Proyecto Anillo-Chile ACT 1105.

P3.1-29

Increase of bioactive isoflavones from soymilk by biotransformation

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Soybean has high isoflavones content and their consumption is commonly associated with beneficial properties for health. Compared to glycoside forms, the aglycone isoflavones have higher bioactive potential. Thereby, in order to increase the content of those compounds, crude extract of tannase obtained from *Paecilomyces variotti* was evaluated for its ability of biotransform soymilk isoflavones. Additionally, it was also investigated the application of the starter and probiotic cultures of lactic acid bacteria and bifidobacteria in soymilk fermentation, to obtain the bioactive isoflavones (daidzein, genistein).The concentration and distribution of isoflavones were evaluated before and after biotransformations using HPLC-DAD. Results demonstrated that the crude extract of tannase and the fermentantion process were able to convert glycoside isoflavones into the aglycone forms. The concentration of daidzein and genistein after enzymatic treatment of soymilk increased about 35 and 31 times, respectively, whereas fermented soymilk, the increase was nearly 3.5 and 5 times. These results indicate that the tannase showed higher hydrolytic catalysis of isoflavone glycosides in relation to the fermentation process, and confirm that enzymatic biotransformation is a efficient strategy to improve bioactive potential of soymilk.

P3.1-30

Evaluation of protective effect of an oil-in-water emulsion containing *Rosmarinus officinalis* in an UVA skin damage experimental model

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The aim of this work was to evaluate the protective effect of emulsions containing *Rosmarinus officinalis* on an UVA skin damage experimental model.

Three different oil-in-water emulsions (E_1, E_2, E_3) were prepared and their antioxidant properties were evaluated by measuring the total reactive antioxidant potential (TRAP), scavenging of ABTS and DPPH. E_3 had the highest values of ABTS and TRAP ($p<0.001$), meanwhile no significant difference was observed in terms of DPPH. Protection against lipid peroxidation was determined by the concentration providing 50 % inhibition (IC50) of brain spontaneous chemiluminescence. E_3 had the smallest IC50 ($p<0.001$) of all emulsions, being the best performing emulsion in terms of ability to neutralize free radicals. Six groups (n=5) of rats were used to determine the in vivo protective effect of E_3 by measuring spontaneous skin chemiluminescence (CL): non irradiated (NIG), irradiated (IG), irradiated after topical application of either, propilengycol (IPGG), glycolic extract (IGEG), E_3 base (IB_3G) or E_3 (IE_3G). The lowest values of CL was observed in IE_3G ($p<0.001$).

These results indicate *Rosmarinus officinalis* emulsions have in vivo and in vitro antioxidant properties. Therefore they could be an alternative form for reducing UVA radiation oxidative damage of skin.

P3.1-31

Obese rats chronically supplemented with green tea show changes in oxidative parameters in the cerebral cortex

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The aim of this study was to evaluate oxidative parameters in the cerebral cortex of obese Wistar rats (male) chronically supplemented with green tea extract (GT) (500 mg/Kg of body weight/12 wk) and treated with a cafeteria diet (8 wk). The rats were separated in: (I) control (standard diet); (II) GT (GT supplemented); (III) obese (cafeteria diet) and (IV) obese+GT (cafeteria diet+GT). After euthanasia the following experiments were performed: plasma leptin levels, FRAP, thiol and carbonyl assays, antioxidant enzymes (total and Mn SOD, glutathione peroxidase and glutathione reductase) and GSH/GSSG ratio. The plasma leptin levels were increased in obese rats and GT did not restore it. Obese and obese+GT groups demonstrated a reduction in FRAP assay. Thiol and carbonyl assays were increased in obese and reduced in obese+GT groups. The obese group showed a reduction in total and Mn SOD and an increase in gluthatione reductase. Supplementation of GT to obese rats caused a reduction in total SOD and an increment in GSH/GSSG ratio and gluthatione reductase. Obesity induced by a cafeteria diet modulated the oxidative parameters in the cerebral cortex as the total antioxidant capacity, protein damage and antioxidant enzymes and the supplementation with GT was not able to restore the effects caused by obesity. Financial Support: Fapesp 2012/19681-5.

P3.1-32

Evaluation of nutraceutical and antioxidant properties of andean landraces tomatoes

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Tomato is one of the most important sources of nutraceutical compounds to the western diet. In this work, we assessed the antioxidant metabolites composition in mature tomatoes from Argentine Andean Valleys and their biological activity, in order to identify the active components responsible of their nutraceutical properties. Antioxidant metabolites from tomato hydrophilic extracts were determined by HPLC-DAD-MS/MS. *In vitro* antioxidant capacity (AC) was determined by TEAC and FRAP methods. Biological activity was evaluated using a *Caenorhabditis elegans* modelexposed to thermal stress.

Two out of seventeen cultivars showed the significant highest AC measured by *in vitro* methods, however, five out of seventeen cultivars showed the significant highest biological activity. The contribution of each phenolic compound to the antioxidant activity and biological properties was evaluated statistically by a multiple regression model. Chlorogenic acid was the compound with the highest positive contribution to the biological activity, which was after confirmed by *C. elegans* assay, using the pure compound. Likewise, chlorogenic acid showed a positive significant correlation with TEAC method. Based on these results it could be found differences in nutraceuticals properties between tomatoes cultivars, and also identify bioactive compound which contribute to these properties.

P3.1-33

Polyphenol-rich blackcurrant juice induces NO-mediated relaxation in porcine coronary artery rings via a copper- and iron-dependent redox-sensitive activation of the Src/PI3-kinase/Akt/eNOS pathway

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The aim of the present study was to determine whether a polyphenol-rich blackcurrant juice (2.7 g/l) improves the vasoprotective endothelial function, and, if so, to characterize the underlying mechanism.
The reactivity of porcine coronary artery rings was assessed in organ chambers, and the expression and phosphorylation levels of proteins in cultured porcine coronary endothelial cells by Western blot analysis.
Polyphenol-rich blackcurrant juice caused potent endothelium-dependent relaxations in coronary artery rings through NO- and endothelium-dependent hyperpolarization-mediated component. Blackcurrant juice-induced NO-mediated relaxations were significantly reduced by membrane permeant analogues of superoxide dismutase and catalase, inhibitors of either Src or PI3-kinase, a calmodulin inhibitor, and chelators of either copper or iron.
In cultured porcine coronary artery endothelial cells, blackcurrant juice increased the formation of NO as assessed by electron paramagnetic resonance spectroscopy. Moreover, blackcurrant juice induced the phosphorylation of Akt and eNOS on activator sites and these phosphorylation were inhibited by membrane permeant analogues of superoxide dismutase and catalase, and inhibitors of Src and PI3-kinase.
Blackcurrant juice is a potent inducer of endothelium-dependent NO-mediated relaxations in porcine coronary artery rings. The NO-mediated relaxation involves an intracellular copper- and iron-dependent redox-sensitive activation the Src/PI3-kinase/Akt pathway leading to activation of eNOS and subsequent NO formation.

P3.1-34

Evaluation of epicatechin and its protective effects against oxidative damage in *Caenorhabditis elegans*

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Catechins are major polyphenols in many plant foods that have been related to have antioxidative properties and possess a remarkable spectrum of biological actions. *Caenorhabditis elegans* has been used as a model organism to develop *in vivo* studies. In previous studies, we had found that these compounds were incorporated by the nematode. It was observed that epicatechin (EC) increased the mean life of the nematode and increased the percentage of survival in conditions of stress, when the worm was within the stages of 1-5 days of adulthood.
The aim was to determine if some markers of oxidative stress in *c. elegans* were modified when they were treated with EC. Also, to deepen our understanding of the mechanisms of action responsible for the biological effects observed previously. We have determined the concentration of the reactive oxygen species, and oxidised macromolecules *i.e*. carbonylated proteins and lipid peroxidation.
The study shows that the concentration of ROS in worms treated with EC, was lower than in control worms until the ninth day of adulthood, from this age this relationship was reversed and the concentration of ROS was higher in those treated with EC. It was determined that from the tenth day of adulthood, worms treated with EC continued having a greater percentage of survival, in conditions of stress. In relation to the lipid oxidation, it was observed that this was lower in worms treated with EC, and there was no significant difference in the concentration of carbonylated proteins.

P3.1-35

Bioactive compounds and antioxidant activity of mixed fruit-vegetable juices: possible synergistic effect?

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Consumption of fruits and vegetables is associated with a lower risk of chronic diseases due to its composition rich in micronutrients, fiber, and bioactive compounds. The objective of this study was to evaluate a possible synergistic effect of fruit-vegetable juices. Two fruits and three vegetables were selected as common samples of mixed juices in Brazil. Individual foods, and mixed juices, were extracted with 80% acetone, the solvent evaporated and residues resuspended with 70% methanol. Total phenolics were analyzed by the Folin-Ciocalteau method and total flavonoids by the $NaNO_2$/ $AlCl_3$ method. Antioxidant capacities were evaluated by DPPH and FRAP assays. To estimate possible synergies, analyzed and calculated values were compared. Mint showed the highest polyphenol and flavonoid contents followed by kale, watercress, orange, and pineapple. For the mixed juices, orange-kale was the juice with the highest polyphenol and flavonoid contents. The antioxidant analysis showed a similar pattern to that of the bioactive compounds. Comparing observed and theoretical values of juices, a possible synergistic effect was observed for polyphenols but not for flavonoids. The combination of fruits and vegetables in a mixed juice can be a simple way to increase the consumption of these foods and bioactive compounds with beneficial health effects.

P3.1-36

Quercetin enhances anti-inflammatory activity of Bifidobacterium adolescentis

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The functional interactions between probiotics and flavonoids are still unclear. Here, we investigated the changes in anti-inflammatory activity of probiotics and flavonoids co-culture supernatants .*Bifidobacterium* and *Lactobacillus* strains (108 cfu/mL) were incubated with quercetin (25 □M) in DMEM under anaerobic conditions, and the supernatants were estimated their effects on nitric oxide (NO) production in lipopolysaccharide-stimulated RAW264 macrophages. After 3 h incubation, the supernatants from the bacteria or quercetin mono-culture as well as almost all of the tested co-culture failed to inhibit the NO production. Interestingly, however, the supernatant from *Bifidobacterium adolescentis* (BA) and quercetin co-culture significantly suppressed it by 50%. This activity increased in a culture period (1-6 h)-dependent manner and was not observed in heat-inactivated BA. The majority (80-90%) of quercetin decomposed during 1 h incubation in both the mono- and the co-culture. The mono-culture with an increasing number of BA (2-4 x 10^8 cfu/mL) showed strong suppression of the NO production which was enhanced by quercetin. The supernatant filtrate (3kDa cut-off) also decreased the NO production. Thus, these results suggest that BA may produce NO suppressant(s) that have a molecular mass <3 kDa, and quercetin may perform a promoting action to its secretion, production, or both.

P3.1-37

Flavonoid microparticles: release in gastro-intestinal simulated conditions

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The intake of phenolic compounds has been associated with the prevention of diseases such as cardiovascular diseases and cancer, due to its antioxidant properties. Thus, the phenolic compounds have been used as additives for healthy foods. However, they haveunpleasant tastes, low solubility and it can be degraded during its passage through the gastro-intestinal tract. In this context, the microencapsulation of flavonoids could be a potential tool toprotect and control the release of phenolic compound in a target site, preserving its functionality and bioavailability.

The objective of this study was to evaluate the effect of flavonoid structure and encapsulating agent on encapsulation efficiency and release kinetic at pH gastric or intestinal simulated. Flavonoids Quercetin (Q), Naringenin (N) and Epicatechin (E) were encapsulated by spray drying with inulin (In) or hydroxypropilcellulose.

The release profile was dependent on flavonoid, encapsulanting agent and pH. The kinetic data were fit to mathematical models.

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P3.1-38

Does encapsulation modulate the biological acitivity and bioavailability of anthocyanins?

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Anthocyanins are attributed with beneficial effects.Especially bilberries have a broad spectrum of anthocyanins. For the described biological mechanisms, the bioavailability of anthocyanins at the site of absorption has a crucial importance. However, anthocyanins are sensitive to environmental conditions, thus their bioavailability in the gastrointestinal tract is an important determinant of their activity. In the studies reported here, the potential benefits of encapsulating an anthocyanin rich bilberry extract (BE) were investigated. In our *in vitro* experiments we have investigated the anti-inflammatory effects of BE on modulation of pro-inflammatory cytokines and anti-oxidative effects of BE (encapsulated and unencapsulated). Additionally, in a human intervention study, we have determined oxidative DNA damage in blood cells after BE consumption (encapsulated and unencapsulated) and analyzed in urine, plasma and ileostomy fluids if the encapsulation has an influence on the availability of anthocyanins.

Our studies reveal the anti-inflammatory and anti-oxidative potential of BE. Moreover the encapsulation of BE could stabilize anthocyanins without loss of their anti-oxidative capacity and could modulate the bioavailability in human in contrast to unencapsulated BE.

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Microarray and pathway analysis of Nrf2/ARE-mediated expression profiling and underlying molecular mechanism by polyphenolic myricetin

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Abstract: Myricetin is a dietary flavonol and widely distributed in many edible plants. It has been reported to have many bioactivities and considered as a promising chemopreventive compound. The present study aimed to investigate the influences of myricetin on gene expressions in genome-wide and underlying mechanisms. Among total 44K gene probes, myricetin treatment upregulated the signals of 143 gene probes (0.33% of total probes) and downregulated signals of 476 gene probes (1.08% of total probes) by ≥2-fold in HepG2 cells. The network pathway analysis revealed that Nrf2-mediated antioxidant response element (ARE) activation is involved in myricetin-induced genes expressions. Molecular data revealed that myricetin activated Nrf2-ARE pathway by: (1) inhibiting Nrf2 ubiquitination and protein turnover, stimulating Nrf2 expression and Keap1 modification in post-transcriptional level; (2) increasing phosphorylation of protein kinases which directly target Nrf2 ; (3) increasing mRNA of Nrf2 in transcriptional level. All of these events finally increased nuclear Nrf2 accumulation and ARE binding activity to enhance ARE-mediated genes expressions. Additionally, treatment with Nrf2 siRNA attenuated the myricetin-induced ARE activity and gene expression. In conclusion, an Nrf2-mediated ARE activation is involved in myricetin-induced expression profiling in hepatic cells.

P3.2-01

Antinociceptive activity of the vanillic acid

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Vanillic acid is found at high concentrations in many plants used in traditional medicine. It has been associated with a variety of pharmacologic activities such as inhibiting carcinogenesis, apoptosis and inflammation but it has become most popular for its pleasant creamy odour. Since there are few reports about the antinociceptive activity of this phenolic compound, the aim of this work was to study this activity on *in vivo* models. Vanillic acid administered by intraperitoneal route (i.p.), produced a dose-related inhibition of acetic acid-induced writhing response (ED50: 18.4 mg/kg). The antinociceptive activity was inhibited for the pretreatment with ondansetron (0.2 mg/kg i.p.) and yohimbine (1 mg/kg i.p.), indicating that serotoninergic and adrenergic system participate in the mechanism underlying the analgesic activity of this compound. In a suitable *in vivo* model, this phenol has shown interact with ASIC channels (Acid-sensing Ion Channels) at dose of 100 mg/kg i.p. Furthermore, it did not produce any interference on locomotor function or motor coordination. Plasmatic vanillic acid, analysed by HPLC method, showed that its $T_{1/2}$ and ABC were 0.123h and 1.38 µg. h/ml, respectively. In conclusion, our results suggested that the vanillic acid might represent potential therapeutic options for the treatment of pain.

P3.2-02

Anthocyanin from purple maize inhibit protein expression of COX-2 and MYD88 in LPS-induced peritonitis in mice

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Anthocyanins display a wide range of biologicalactivities. Here the anti-inflammatory properties of an anthocyanin-enriched fraction (AF) extracted from purple maize (*Zea mays* L.) were studied in peritonitis, induced by lipopolysaccharide (LPS) in mice. In each trial, AF (2 and 4 mg cyanidin 3-glucoside eq/100 g body weight) was orally administered in two different times: 30 min before and 1 h after LPS inflammatory stimulus. The peritoneal exudates was withdraw three hour after cg injection and use to determine total cell counts, COX-2, TLR4 and MYD88 expression analysis by western blotting. AF reduced the polymorphonuclear leukocytes number (PMN) in the exudates when administered 30 min and 1h after LPS injection (70-80% decrease), in both doses, similar to dexamethasone. Also, AF was found to significantly ($p<0.05$) suppress protein levels of COX-2 and presented inhibitory effect on PGE_2 production in the peritoneal exudates up-regulated by LPS, at both administration times with the higher dose. Also, AF suppressed protein level of MYD88, but not TLR4, when administered before LPS induction, at higher dose. Our findings suggest that AF could minimize acute inflammation induced by LPS by attenuating inflammatory factors and possibly regulating the TLR4 signaling pathways. Financial support: FAPESP.

P3.2-03

Quercetin-3-*O*-glucuronide inhibits the binding of noradrenaline to α2-adrenergic receptor, consequently suppresses γ-H2AX induction by co-treatment with 4-OHE2 and noradrenaline in MCF-10A cells

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4-Hydroxyestradiol (4-OHE_2), a metabolite of 17β-estradiol formed preferentially by cytochrome P450 1B1, reacts with DNA to form depurinating adducts thereby exerting genotoxicity and carcinogenicity. Daily stress is considered to promote the development of breast cancer. Concentration of catecholamines such as adrenaline (A) and noradrenaline (NA) are increased under exposure to stress. We found that co-treatment with 4-OHE2 (3 µM) and NA (3 nM) significantly induced phosphorylation of histone H2AX (γ-H2AX), one of the earliest indicators of DNA damage, and apurinic (AP) sites throughα2-adrenergic receptor (α2-AR) in human mammary epithelialMCF-10A cells. As epidemiological studies suggested an inverse association between a higher intake of flavonoids and breast cancer risk, we investigated effects of quercetin-3-*O*-glucuronide (Q3G), a circulating metabolite of quercetin in the blood, on 4-OHE2 and NA induced γ-H2AX and AP sites. Q3G (0.1 µM) suppressed them. HPLC analysis showed that Q3G was not absorbed into the cells. Moreover, Q3G inhibited the binding of [^{3}H]-noradrenaline to α2-AR. These results suggest that Q3G acts as a α2-AR antagonist and can be a chemopreventive agent for daily stress promoted breast cancer.

P3.2-04

Effect of tellimagrandinsin *Rosa rugosa* petals on chemical mediators release from mast cells

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We have reported that petals of Rugosa rose, a rose species native to eastern Asia, contain large amount of hydrolysable tannins such as tellimagrandins. In the present study, we investigated effect of tellimagrandins on release of chemical mediators, histamine and leukotriene B4 (LTB4)that are responsible for Type I allergy symptoms, from mast cells *in vitro*.
Rat basophilic leukemia cell line (RBL-2H3) and mouse mast cell line (PB-3c) were used for histamine and LTB4 release assay, respectively. RBL-2H3 and PB-3c were pre-incubated with tellimagrandin I or II in Tyrode buffer. Stimulations of mast cells were induced by calcium ionophore or cross-linking of high-affinity IgE receptors (FcεRI) *via* IgE-antigen complexes. The secreted histamine and LTB4 were determined by HPLC. Calcium influx into the mast cells cytoplasm was monitored by spectrofluorometry. The phosphorylation of intracellular signaling molecules was analyzed by western blotting.
Histamine and LTB4 releasesfrom mast cells were suppressed by tellimagrandin I and II in a dose-dependent manner.Calcium influx was suppressed by Tellimagrandin I in IgE-antigen stimulation, whereas there was no effectin calcium ionophore stimulation. The phosphorylation of protein kinaseswas inhibited by tellimagrandin I. These results suggest that tellimagrandins may have anti-allergic function.

P3.2-05

Plasma flavanol metabolites modulate expression of miRNA in endothelial cells and affect post-transcriptional regulation of genes regulating adhesion and transendothelial migration

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MicroRNAs (miRNAs) are endogenous, noncoding, single-stranded RNAs and constitute a class of post-transcriptional regulators. We showed that flavanol metabolites decrease adhesion of monocytes to endothelial cells through modulation of expression of genes. The aim of this work was to identify the impact of flavanol metabolites at physiologically-relevant concentrations on the expression of miRNAs in endothelial cells.
The use of microarrays revealed that 4'MEC, 4'MEC7G and EC4'S can modulate expression of miRNAs involved in the regulation of inflammation, cell adhesion or cell invasion, such as miR-221 or miR-181. These metabolites regulate the expression of different miRNAs that could exert post-transcriptional regulation of different target genes thatare however involved in the same cellular pathways. These pathways mainly concern those involved in regulation of cell adhesion and transendothelial migration. Analyses of gene and protein expression of target genes revealed that miRNA could affect mRNA levels of certain genes, such as WASP1 and consequently the level of the protein, but also only decrease the translation and the protein quantity without affecting level of mRNA, as observed for BIRC2.
In conclusion, these data provide an insight into new molecular mechanisms by which plasma flavanol metabolites at physiologically relevant concentrations may preserve vascular endothelium integrity.

P3.2-06

Flavanol metabolites reduce monocyte adhesion and modulate geneexpression involved in atherosclerosis development through MAPK-p38 orNfkB-p65 signaling pathways

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Consumption of flavanol-rich foods is associated with a reduced risk of cardiovascular diseases, which was linked to improvements in endothelial function. The specific flavanolsinvolved in these beneficial effects and underlying molecular mechanisms have not been identified.
The aim of our work was to examine the effect of flavanol circulating metabolites on adhesion of monocytes to TNFα-activated endothelial cells and underlying mechanisms.
4′-O-methyl(−)-epicatechin, 4′-O-methyl(−)-epicatechin-7-β-D-glucuronide and (−)-epicatechin-4′-sulfate decreased the adhesion of monocytes to the endothelial monolayers, particularly at nutritionally-relevant concentrations. Nutrigenomics indicated that metabolites modulate expression of genes involved in the regulation of adhesion and transendothelial migration of monocytes, mainly cell adhesion/junctions, focal adhesion or cytoskeleton remodelling. The gene expression profiles are in agreement with the observed decrease in monocyte adhesion to endothelial cells. The nutrigenomic effect of flavanol metabolites was found to be mediated by their capacity to modulate phosphorylation of p65 and p38 of NF-κB and MAPK cell signalling pathways respectively. Our data suggest that cell adhesion and migration events represent relevant molecular targets of flavanol metabolites with respect to improving the integrity of vascular endothelium. In conclusion, these data provide an insight into the mechanisms by which plasma flavanol metabolites preserve vascular endothelium integrity at the transcriptional level.

P3.3-01

Effect of cinnamon beverage formulations on the bioaccessibility of polyphenols and cinnamaldehyde during in vitro gastro-pancreatic digestion

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This study was undertaken to investigate the in vitro bioaccessibility of phenolic compounds and cinnamaldehyde in cinnamon beverage and formulations during simulated gastro-pancreatic digestion. Cinnamon polyphenol bioaccessibility was 79.8% at the end of the digestion. This decline in total polyphenols found during the gastric step of digestion and was caused by the precipitation of cinnamon proanthocyanidins by pepsin. The addition of sweeteners increased the polyphenol bioaccessibility to value near 90% decreasing the interaction between pepsin and tannins. The addition of bovine milk gave rise to immediate decrease in cinnamon polyphenol concentration and negatively affects their post-pancreatic bioaccessibility. Quercetin-3-rhamnoside, syringic and coumaric acids showed poor bioaccessible whereas kaempferol and cinnamaldehyde were stable during digestion. The addition of sweeteners did not affect the bioaccessibility of these compounds. Milk addition caused an immediate decrease in the concentration of monomeric phenolic compounds partially due to the interaction with milk protein as probed by fluorescence spectroscopy. The final bioaccessibility was affected by milk only for cinnamaldehyde. this study demonstrates that cinnamon beverage provide a significant source of dietary bioaccessible polyphenols and that the modality of consuming cinnamon could represent a crucial factor since the addition of sweeteners improve their bioaccessibility whereas milk negatively affect it.

P3.3-02

Nanocomplexing properties of dairy proteins over green tea polyphenols: Studying the optimum ratio to use in functional foods

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Green tea is a worldwide spread beverage due to their typical flavor and health benefits, which are associated to the presence of polyphenols. Several studies demonstrated the antioxidant and anti-inflammatory capacity of green tea polyphenols (GTPPs), and also, their protective effects over different types of tumors. Among the different aspects to be optimized to use GTPPs into food products, one of the most important is their typical strong bitterness and astringency. In order to mend this negative aspect, several strategies could be used and one of the strategies is to incorporate the polyphenols nanocomplexed with proteins avoiding the interaction with another food ingredient and masking the bitterness and astringency.
The aim of this work was to study the interaction between dairy proteins and GTPPs, analyzing the optimum protein/polyphenol ratio to be used in each case. To this end, several nanocomplexes have been produced using different proteins (α-lactalbumin, β-lactoglobulin, caseinomacropeptide, whey protein concentrate, bovine serum albumin and casein) and commercial GTPPs. The nanocomplexes produced were analyzed by means of dynamic light scattering and quantifying the non-complexed GTPPs using UV-Vis absorption spectroscopy.

P3.3-03

In vitro antiangiogenic activity of a polyphenol-rich extract of Chilean propolis

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Antiangiogenic effect of propolis has been showed; however, the mechanisms implicated in this effect are poorly understood. Thus, the aim of the present study was to investigate the effect of an ethanolic-extract of Chilean propolis (EEP) on *in vitro* angiogenesis and expression of angiogenic microRNAs. *In vitro* "scratch wound assay", "matrigel angiogenesis assay" and the *ex vivo* "rat aortic ring model" were used for assessed the antiangiogenic properties of EEP. HUVECs stimulated with VEGF (20 ng/mL) were treated with EEP (5-25 µg/mL) for 4 h for RNA extraction. MicroRNAs and mRNA expression were studied by real time PCR. ERK1/2 phosphorylation and HIF1-α stabilization was assessed by western blot. At 10 or 15 µg/mL of EEP the *in vitro* and *ex vivo* angiogenic assays were attenuated. In addition, the activation of HIF1α was significantly inhibited in a dose-dependent manner. Moreover, the ERK1/2 activation and the VEGF mRNA expression were slightly inhibited. Finally, miR-19b was overexpressed. *In silico* analysis suggesting that MAPK1 (ERK2) is target for miR-19b. In conclusion, these results suggesting that miR-19b, HIF1α and ERK1/2 phosphorylation could be related with the antiangiogenic effect of Chilean propolis, but more studies are needed to corroborate these findings. Financial support: CONICYT-Fellowship (Chile), FAPESP and CAPES-Brazil.

P3.3-04

Solubility of benzoic acid in water and water-ethanol binary mixtures

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Solubility is a key factor for polyphenol bioactivity (i.e. low aqueous solubility of proanthocyanidins promotes their interaction with other macromolecules and hence their complexation). Thus, knowing the water solubility of polyphenols and proanthocyanidins is very important to understand how they interact with other molecules and how these interactions affect their biochemical and bioactive properties. Furthermore, solubility data are very useful from an industrial point of view, since they provide the necessary information to design and optimize extraction processes.Solubility data of polyphenols and in particular flavan-3-ols and proanthocyanidins are scarce in scientific literature. In this work, we describe the development of a system for measuring experimental solubility of polyphenols, using a Carousel 12 Plus™ Reaction Station (Radleys, Saffron Walden, UK) and benzoic acid as the studied molecule. Solubility experiments between 283.15 and 363.15 Kin water and water-ethanol binary mixtures were performed. Benzoic acid was chosen as the studied molecule due to the availability of experimental water solubility data butalmost no solubility data for water-ethanol binary mixtures,and due to its thermal stability (benzoic acid remains stable in subcritical water up to 573 K).

P3.3-05

Embauba (*Cecropia pachystachya*) extract reduces the renal lesion in rats submitted to 5/6 nephrectomy

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Cecropia pachystachya (CP)is a plant of South America popularly known as embauba in Brazil. CP extract is a rich source of polyphenols as orientin which can inhibit the angiotensin II (AII) converting enzyme (ACE) *in vitro*. This study evaluated the effect of CP extract on the systolic blood pressure (SBP) and renal lesions provoked by 5/6 nefrectomy (NE). Male Wistar rats submitted to 5/6 NE were untreated (NE) or treated (NE+CP) with CP extract (0.6 g/kg/day). The NE rats developed progressive albuminuria and increase of SBP that were less intense in the NE+CP than in the NE group [114 (31; 226) vs 194 (104; 265) mg/24 h], and 181±11 vs 217±13 mmHg, respectively, $p<0.05$]. The NE animals also showed a reduction in glomerular filtration rate, which was attenuated by CP treatment (0.273±0.04 vs 0.125±0.02 ml/min/100 g, $p<0.05$). The treatment with CP extract also reduced the histological changes in the renal cortex of the nephrectomized rats, as well the urinary levels of the monocyte chemoattractant protein and transforming growth factor-β. The reduction of AII production and inflammation was associated with the inhibition of the 60% of the ACE activity in the renal cortex of the NE+CP rats.

P3.3-06

Effect of (+)-catechin on rat heart mitochondria

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Flavonoids are low molecular weight phenolic compounds with a quinonoid-like chemical structure. They have been shown to attenuate the progression of diseases associated with oxidative stress and mitochondrial dysfunction. Mitochondria are the main sources of intracellular reactive oxygen and nitrogen species. The aim of this work was to study the effect of (+)-catechin, a flavan-3-ol, on mitochondrial bioenergetics. Isolated mitochondria from rat heart were incubated in the absence or in the presence of (+)-catechin (3 nM to 100 μM). (+)-Catechin, in μM range (10 μM; ~50 nmol catechin/mg protein), reduced (25%) state 3 O_2 consumption sustained by malate and glutamate, but not the state 4 respiration rate. (+)-Catechin concentrations lower than 1 μM did not modify mitochondrial O_2 uptake. However, (+)-catechin in nM range (10 nM; ~150 pmol catechin/mg protein) inhibited NO (50%) and H_2O_2 (45%) production rates. Moreover, the flavonoid diminished the state 4 mitochondrial membrane potential by about 10 mV, when malate-glutamate were used as substrates. These results show that nM concentration of (+)-catechin reduces mitochondrial membrane potential leading to a decline in mtNOS activity and H_2O_2 production rate, according to the inhibitory effect of flavonoids on complex I activity (Lagoa et al., 2011).

P3.3-07

Structural investigations of the binding of polyphenols to multiple protein targets involved in neurodegenerative diseases

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Progressive neurodegenerative conditions such as Alzheimer's disease (AD), Parkinson's disease (PD) and Creutzfeldt Jakob disease (CJD) are incurable and often fatal conditions. Each of these diseases is caused by the misfolding of one or more proteins leading to the formation of fibrils and localized neural cell death. In AD, the likely culprit is the Abeta protein, in PD it is alpha synuclein and in CJD it is the prion protein. A number of animal and cell culture studies have shown the polyphenolic compounds reduce symptoms or arrest progression of these diseases by inhibiting the misfolding of these three proteins. However, the molecular details of these ligand-protein interactions are lacking. To address these issues we have expressed all three proteins and have undertaken a series of biophysical studies employing NMR spectroscopy, mass spectrometry, chemical modification and gel electrophoresis to investigate how several polyphenolic compounds (curcumin, EGCG, rosmarinic acid, gallic acid, etc.) bind to both the monomeric and oligomeric forms of these proteins. Our findings indicate that these compounds appear to have specific binding sites not only for the monomeric proteins but also for the oligomeric proteins. Additional details regarding the ligand binding position, orientation and affinity are presented in this poster.

P3.3-08

Structure-activity relationship of natural and semi-synthetic proanthocyanidins as anti-*Helicobacter pylori* molecules through the inhibition of its urease activity

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Helicobacter pylori (*Hp*) infect the gastric mucosa of half of the world's population and it is the only microorganism known to successfully inhabit the human stomach. Many studies have established *Hp* as an etiologic agent of duodenal ulcer, chronic gastritis and gastric cancer. In terms of its capacity to colonize the gastric mucosa, one of the most important features of *Hp* is its extremely high capacity to produce urease, whose main function is to generate a neutral microenvironment surrounding the bacterium. In this work we report that procyanidins (PAC) extracted from fruits peels or plants exert part of their antimicrobial action through the inhibition *Hp* urease. PACs were purified by various chromatographic techniques (HILIC/RP-HPLC, CPC) and their structures were confirmed by LC-MS. Inhibition was linked to the procyanidin size and structure. Hence, PACs with higher degree of polymerization were the most effective against urease. Also, catechin-derived PACs were most effective than the epicatechin-derived. Moreover, in order to duly clarify main structure-activity relationships of such compounds, through nucleophilic attack with phloroglucinol, toluene-α-thiol and cysteamine we prepare semi-synthetic derivates from highly polymerized PACs fractions. So, our results also provide evidence that substitution in C-4 can be useful to obtain new urease inhibitors.

P3.3-09

Inhibition of *Leishmania* (*Leishmania*) *amazonensis* and rat arginases by green tea EGCG, (+)-catechin and (-)-epicatechin: a comparative structural analysis of enzyme-inhibitor interactions

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Epigallocatechin-3-gallate (EGCG), a dietary polyphenol (flavanol) from green tea, possesses leishmanicidal and antitrypanosomal activity. Mitochondrial damage was observed in *Leishmania* treated with EGCG, and it contributed to the lethal effect. However, the molecular target has not been defined. In this study, EGCG, (+)-catechin and (-)-epicatechin were tested against recombinant arginase from *Leishmania amazonensis* (ARG-L) and rat liver arginase (ARG-1). The compounds inhibit ARG-L and ARG-1 but are more active against the parasite enzyme. Enzyme kinetics reveal that EGCG is a noncompetitive inhibitor of the ARG-L while (+)-catechin and (-)-epicatechin are competitive inhibitors. The most potent arginase inhibitor is (+)-catechin (IC_{50}=0.8 μM) followed by (-)-epicatechin (IC_{50}=1.8 μM), gallic acid (IC_{50}=2.2 μM) and EGCG (IC_{50}=3.8 μM). Docking analyses showed different modes of interaction of the compounds with the active sites of ARG-L and ARG-1. Due to the low IC_{50} values obtained for ARG-L, flavanols can be used as a supplement for leishmaniasis treatment.

P3.3-10

Effects of prunin- and hesperetin glucoside-alkyl (C_4-C_{18}) esters interaction with Jurkat cells plasma membrane on membrane physical properties and antioxidant capacity

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The effects of prunin (P)- and hesperetin glucoside (HG)-alkyl (C_4-C_{18}) esters (0.1-100 μM) on human leukemia T (Jurkat) cells viability and plasma membrane fluidity were evaluated. After 1 h of exposure, these compounds did not affect cell viability in the range 0.1-10 μM. At 100 μM, a decrease of cell viability was found that depended on the length of the alkyl chain and that reached a maximum with C_6-C_{12} derivatives. Cell hyperpolarization and shrinkage were also observed at this concentration. The fluidity of cell plasma membrane was not affected, regardless the depths of the membrane level evaluated, although mild changes in plasma membrane hydration were found. The antioxidant capacity of P and HG (0.1-10 μM) against 1 mM H_2O_2 was not affected by esterification. When exposed to 1 mM AAPH, P-alkyl esters retained P antioxidant capacity, but HG-derivatives acted as pro-oxidants. Together, experimental evidences suggest that short term exposures to 0.1-10 μM concentrations of P- and HG-alkyl (C_4-C_{18}) esters can be considered safe for cultured human cells, and further studies are required to investigate their long term effects, as well their safety for human consumption. Supported by grants of UBA (B086), UNSa and ANPCyT (PI 2009 and PICTO 36683), Argentina.

P3.3-11

Epigallocatechingallate increases glucose uptake activity and GLUT4 translocation through PI3K-dependent pathway in L6 myotubes

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Purpose. We previously demonstrated that the intake of green teaand its major catechin, (-)-epigallocatechin 3-gallate (EGCg), increased glucose uptake accompanied by a translocation of glucose transporter 4 (GLUT4) in rat skeletal muscle and L6 myotubes. In this study, we investigate the underlying molecular mechanisms by which EGCg promotes GLUT4 translocation in L6 myotubes.
Method. L6 myotubes were treated with various concentrations of EGCg in the presence or absence of the PI3K and PKC inhibitors. Glucose uptake was determined by measuring the incorporated amounts of [^{3}H]-3-O-methyl-D-glucose into the cells. GLUT4 translocation and phosphorylation of proteins in insulin signaling pathway were estimated by Western blot analysis. Glycogen content was measured using a commercial kit.
Result. EGCg increased glucose uptake and GLUT4translocationin a dose-dependent manner. EGCg promoted phosphorylation of PI3K and PKCλ/ζ without affecting phosphorylation of IR-β and Akt. Consistently, a PI3K inhibitor LY294002 and a PKC inhibitor staurosporine suppressed EGCg-induced GLUT4 translocation. EGCg enhanced glycogen accumulation by inhibiting glycogen phosphorylase activity in L6 myotubes.
Conclusion. EGCg promoted GLUT4 translocation with a different action from the insulin's action. This beneficial function of EGCg will contribute to prevent hyperglycemia in diabetes mellitus.

P3.3-12

FKBP proteins as new targets of pholyphenol-mediated NF-kappaB inhibition

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NF-κB (RelA/p50) activation depends on its nuclear translocation. Because FK506-binding proteins (FKBPs) FKBP51 and FKBP52 regulate the subcellular localization of steroid receptors, we studied whether these factors also affect the nuclear translocation of RelA/p50. FKBP51 forms complexes with RelA/p50 delaying its nuclear translocation. Cell stimulation with phorbol-ester (PMA) promotes FKBP51 exchange with FKBP52, favoring RelA/p50 nuclear accumulation. While NF-κB transcriptional response was significantly abrogated by FKBP51 in a concentration-dependent manner, FKBP52 showed a positive effect in a competitive fashion. PPIase-activity is not required for FKBP51 inhibitory action, but punctual mutations in essential amino-acids of the PPIase domain of FKBP52 (FKBP52F67Y and FKBP52F130Y) abolished NF-κB activity, an effect reverted by overexpression of *wt*FKBP52. Epigallocatechin-gallate (EGCG) is a polyphenol previously described as PPIase inhibitor. Accordingly, cells pre-incubated with EGCG (25μM-1h) blocked FKBP52 stimulatory action. Non-transfected cells under the same condition did not show significant NF-κB activation. We postulate that FKBP52 is a novel stimulator factor of NF-κB, whose effect depends on its intrinsic PPIase-activity. These observations raise the possibility that NFκB function may be regulated by the expression balance of both FKBPs, and suggest that FKBP52 could be a critical biological target for the pharmacological mechanism of action of polyphenols. Financial support.PICT2010-2215/2010-1170, UBACyT20020100100237/20020120200335

P3.3-13

Green tea polyphenols-β-lactoglobulin nanocomplexes with anticancer activity as antioxidants in emulsions with fish oil

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There is a strong demand of natural bioactive ingredients with health benefits such as long chain ω-3 polyunsaturated fatty acids (LCω-3 PUFA), which are abundant in fish oil. However, PUFA degradation through auto-oxidation during processing and storage, easily takes to rancidity volatiles formation. Green tea polyphenols have a great antioxidant capacity and are receiving more and more attention because of their beneficial properties to human health. Thus, it is becoming popular to design food products containing polyphenols as functional foods. β-lactoglobulin (β-lg) was used as an emulsifier agent and also as a carrier molecule by spontaneous nanocomplexes formation with green tea polyphenols (size determined by dynamic light scatteing). These nanocomplexes were proved to have antiproliferative activity against different cancer cell lines and were used at pH 6 to formulate oil-in-water emulsions containing fish oil rich in ω-3 fatty acids. The interfacial behaviour of these complexes showed that both surface pressure and dilatational properties decreased as compared with pure β-lg. However, the initial oil droplet size and stability of emulsions were improved in the presence of the nanocomplexes. Moreover, the oxidative stability of liver fish oil was improved by the presence of polyphenols.

P3.3-14

Quercetin modulates insulin secretion and protects pancreatic β-cells against oxidative damage through intracellular calcium increase and ERK1/2 activation

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Pharmacological interventions aiming at protecting or restoring insulin-secreting β-cell function may prevent or treat type 2 diabetes (T2D). As some polyphenols (e.g. quercetin, resveratrol) or other non-polyphenolic antioxidants (e.g. N-acetyl-L-cysteine, NAC) were shown to possess antidiabetic activities *in vivo*, we studied their potential to modulate insulin secretion and protect β-cells against oxidative stress, using insulin-secreting INS-1 β-cells and rat isolated pancreatic islets.
Quercetin, but not resveratrol or NAC, potentiated insulin secretion induced by a stimulating concentration of glucose, the depolarizing agent KCl or the sulfonylurea glibenclamide and protected β-cell function and viability against oxidative damages. Intracellular Ca^{2+} and ERK1/2 signaling pathway played a major role in those effects. Further studies of quercetin mechanism of action allowed us to show that quercetin increases insulin secretion through an extracellular Ca^{2+} influx by a direct activation of L-type voltage-dependent Ca^{2+} channels (patch-clamp technique).
In summary, our study suggests that the flavonoid quercetin modulates insulin secretion and protects the viability of insulin-secreting β-cells through mechanisms not directly linked to its free radical-scavenging activity, interacting with molecular targets within the β-cell. Its potential in the prevention or treatment of T2D deserves to be further studied.

P3.3-15

Polyphenol-rich cagaita (Eugenia dysenterica C) induces pancreatic beta cell proliferation and protects against oxidative and inflammatory damages of diabetes

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Pancreatic beta cell failure and apoptosis, macrophage activation and insulin resistance are key events in the development and progression of diabetes mellitus. We observed that streptozotocin-induced diabetic rats treated with polyphenol-rich Cagaita (Eugenia dysenterica DC) extract (PCE), an exotic Brazilian fruit, showed improvement on insulin tolerante test (ipITT) and triglyceride tolerance test (oTTT) when compare to non-diabetic rats. Besides, PCE also reduced fasting hyperglycemia and hyperlipidemia. Isolated islet from treated animals did not improved insulin secretion, but they had higher levels of insulin gene expression and content, and increased markers of cell proliferation, such as pERK1/2, pAkt and Cyclin D1. The treatment of pancreatic INS1e cells with PCE augmented [3H]-thymidine incorporation, which was inhibited by pre-incubation with inhibitors of MEK and PI3K. LPS-stimulated macrophages J774 treated with PCE showed decreased nitric oxide production, iNOS expression and NFκB binding to iNOS gene promoter. PCE augmented glucose uptake in muscle cells isolated from wild type but not from AMPK knockout mice. Finally, we identified metabolites of quercetin, ellagic acid and coumaric acid in the plasma of PCE treated animals. Thus, PCE is a putative nutraceutical to prevent or treat diabetes due to its capacity of directly modulate tissue-specific signaling pathways. Funding support: FAPESP, AUF, INAF, MDEIE, CMDO and FRQS.

P3.3-16

Effect of phenolic compounds to the structure of model lipid membranes

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Many phenolic compounds of plant origin have an antimicrobial effect, are very effective chelators of metal ions and powerful antioxidants. In addition to specific effects, the antioxidant activity of phenolics, results in reduced oxidation of biologically important molecules and consequently has a beneficial effect on health. The focus of our research has recently been turning primarily to the study of the interactions of phenolic acids and flavonoids (catechin, epicatechin, epigallocatechin, epigallocatechin-3-gallate, quercetin), which are known constituents of fruits, vegetables, wine and green tea extracts and synthetic antioxidant butylated hydroxytoluene with membranes. Large unilamellar vesicles (ULV) were used to follow changes in membrane fluidity induced by selected phenolics by electron paramagnetic resonance (EPR), fluorescence anisotropy and differential scanning calorimetry (DSC). From the line-shape of the EPR spectra ordering and dynamics of phospholipids alkyl chains were estimated. With both methods we obtained comparable results. Addition of tested phenolic compounds to the ULV increased the ordering and decreased dynamics of phospholipids alkyl chains. This means that the membranes became less fluid. In contrast, the addition of the antioxidant BHT to the ULV decreased the ordering and increased dynamics of phospholipids alkyl chains. Membrane became more fluid. The largest effect was obtained by quercetin and epigallocatechin-3-gallate, although relatively hydrophilic compounds. We believe that in both cases, conversion occurred just below the surface of the liposome membrane. Additionally, DSC results show that epigallocatechin-3-gallate, in addition to BHT, have the highest impact on the thermodynamic profile of gel-to-liquid phase transition of DPPC liposome. Both compounds can form a number of hydrogen bonds with the hydrophilic parts of phospholipids and therefore the dynamics of movement of the alkyl chain decreased. We discuss the implications of our results for understanding the mechanism of interaction of phenolic compounds with biological membranes.

P4.1-01

Polyphenol in the pomace of the red wine fermentation as a functional food

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So much are known about thevarious healthy effects by the antioxidant action of the polyphenol contained in wine (especially red wine). By increased production of wine, abundant by-product (pomace) is produced. Pomace are used for raw materials of brandy such as grappa and the marl, but most are used as compost. Therefore, there is a need to re-utilize pomace. It is known well that a lot of polyphenol is contained in its seeds. I examined its functionality. I divided the grape pomace into rinds and seeds, freeze dried and transformed them into powder. Samples were extracted from the powder using acetone, etc. (GSE). The functional food potential was examined by checking for the presence of antimutagenicity through Ames Mutagenicity Assays performed using *Salmonella typhimurium* TA98 and TA100. Antibacterial study of GSEis performed usingTA98 and TA100andconcentration was measured without antibacterial. Antimutagenicity and Desmutagenicity assay of GSP were performed. It is Trp-P-2 (3-Amino-1-methyl-5-H-pyrido[4 and 3-b] indol) to a mutagen, S-9mix was used for enzyme activity. Both assay of GSE decreased significantly the revertant counts of TA98 and TA100.Manufacture of a supplement or functional food is expectable from now on using the seed of primary red wine fermentation pomace.

P4.1-02

Resveratrol increases the cytotoxic effect of Melphalan in breast cancer cells by cell cycle arrest

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Melphalan (MEL) is a chemotherapeutic agent used in breast cancer therapy; however, MEL's side effects limit its clinical applications. Among the potential sensitizers cancer cells to chemotherapy are phytochemicals such as resveratrol (RSV), a polyphenol found in grape skins. The aim of this study was to investigate the antitumor effects of RSV in combination with MEL in MCF-7 and MDA-MB-231 breast cancer cells. RSV increased the cytotoxic effect of MEL in breast cancer cells. The proposed mechanism for the chemosensitization effect of MCF-7 cells to MEL by RSV was the cell cycle arrest in the S phase by RSV. Combinations of specific cell cycle inhibitors of G1, S, or G2/M phase with MEL also potentiated the ability of MEL to decrease the viability of MCF-7 cells, suggesting an important role of cell cycle progression in increasing MEL's cytotoxicity. While CDK2 expression remained unchanged by the treatments, its active form (Thr160-phosphorylated CDK2) was decreased by treatment with RSV and its association with MEL. The activity of CDK7, responsible to phosphorylate CDK2 at Thr160, was inhibited by RSV and by its combination with MEL. These results indicate that RSV could be used as an adjuvant agent during breast cancer therapy with MEL. Supported by: FAPERJ, CAPES and FAF.

P4.1-03

Antineoplastic and antioxidant effects of camu-camu (*Myrciaria dubia*) fruit fractions: pulp, seeds and peel

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Phenolic compounds such as ellagic acid derivatives, which are abundant in camu-camu (*Myrciaria dubia* Mc. Vaugh), have been shown to display antioxidant and antineoplastic activities. Since lung cancer has been the leading cause of cancer-related death in human, the aim of this study was to evaluate different parts of camu-camu fruit cultivated in São Paulo State (Brazil), and also of the depulping residue in relation to total phenolics, ascorbic acid and proanthocyanidins contents, *in vitro* antioxidant capacity (Folin-Ciocalteu reducing capacity, FRAP ferric reducing power and DPPH radical scavenging activity), and antiproliferative activity (human lung cancer cells line A549). Ascorbic acid contents were higher in pulp and peel. However, total phenolics were higher in the order seed> residue>peel>pulp, varying from 59 to 96 mg GAE/g DW. Proanthocyanidins contents followed the order peel>residue>seed>pulp, varying from 36 to 117 mg QTE/g DW). There was no significant differences between antioxidant capacities of pulp and peel, and also, between residue and seed ($p<0.05$). The first ones were higher than the last ones. These results were related to antiproliferative inhibition, with IC50 of 0.08, 0.16, 0.70 and 0.87 mg/mL, for pulp, peel, seed and residue, respectively. In conclusion, this work showed a good relationship between antioxidant and antineoplastic activities of bioactive compounds in camu-camu fruit fractions. Acknowledgements:CNPq.

P4.1-04

Apoptosis induction activity of green tea polyphenols in human tumor xenograft model

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Green tea (GT) is chemically characterized by the presence of large amounts of polyphenolic compounds. The beneficial effects of green tea are well known, including the ability to inhibit the different stages of cancer development. Acute myeloid leukemia (AML) is an aggressive hematologic malignancy. The development and progression of the leukemic disease involve deregulation in the apoptotic response. Accordingly, the goal of this work was to identify the GT effects using leukemia cells in xenografted mice. HL-60 cells were inoculated subcutaneously in NOD/SCID mice and GT (100 mg/kg) was administered daily by gavage. After 21 days, the mice were sacrificed and the tumors were removed for western blotting and immunohistochemistry analyzes. GT treatment reduced 42% of tumor volume compared to control. The main proteins related to the apoptosis process were analyzed and we found decrease in Bcl2 expression, increase in Bax and any modulation of BcL-xL. The Bcl-2 family proteins are the central regulator of cytochrome *c* release and caspase activation. We found pronounced increase in the cytochrome *c* expression, active caspase 3 staining and in phosphorylation of ERK1/2 and JNK in GT mice treatment. The GT treatment in xenotransplant model reduces the tumor development, with pronounced activation of apoptotic process.

P4.1-05

Soy isoflavones genistein and daidzein reduce human colon adenocarcinoma cell viability and induce arrest in G2/M cycle phase

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Colon cancer is the third most prevalent cancer worldwide, with complex interactions between genetic and environmental aspects. Currently, great attention has been paid to preventive strategies that use functional foods with bioactive compounds. Among these, soy isoflavones stand out since their intake is often associated with a decreased incidence of chronic diseases, including cancer. In this context, we investigated the influence of genistein and daidzein on cell viability and cell cycle of human colon adenocarcinoma cell line (HT-29). Cells were incubated with both isoflavones at 2 µM to1000µM for 6h, 12h, 24h and 48h. MTT assay demonstrated that daidzein and genistein reduced cell viability at concentrations starting from 250µM and 8µM, respectively, when compared to control. The reduction in viability ranged from 19% to 49% and from 3% to 62% when cells were incubated with daidzein and genistein, respectively. Both reductions were time-dependent dose. After incubation with daidzein and genistein a decrease in the percentage of cells at G0/G1 phase and an increase in G2/M phase was observed. Our results suggest that both isoflavones may module cell cycle by arrest in G2/M phase in a time-dependent dose in human colon adenocarcinoma. Financial Support: CAPES, CNPq, FAPERJ, UFRJ, UNIRIO.

P4.1-06

***Rhodiola rosea* extract reduces autophagy in human tumor xenograft model**

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Myelodysplastic syndromes treatment usually focuses on reducing or preventing complications, and single-target drugs are ineffective. The autophagy process has been implicated for new anti-cancer therapies. In this concern, natural compounds such as *Rhodiola rosea* are considered interesting for the development of drugs against various molecular targets. Phytochemicals analyses of *R. rosea* extracts (RRE) revealed the presence of several components including polyphenols. The aim of this study was to study RRE effects using human tumor xenograft model. P39 myeloid cell line ($2x10^6$) were subcutaneously injected in dorsal region of NOD.CB17-$Prkdc^{scid}$/J mice (n=6). Tumor volume was measured once every 7 days and RRE treatment initiated when tumors reached 100-200 mm^3. The dose of 250mg/Kg body was given once every day by oral route (gavage). Control group received vehicle only. After 14 days, mice were sacrificed; tumors were removed and homogenized in protein extraction buffer. Detection of autophagy process was then performed. After 14 days treatment, there was reduction of 29.6% in tumor volume compared to controls. The proteins related to the autophagy process were analyzed and we found increased expression of beclin-1 and STSQM1/p62, and also Bcl-2 expression. RRE treatment in xenograft model reduces tumor development, with pronounced inhibition of autophagy process.

P4.1-07

Antiproliferative activity of fruits in A549 cell line (human lung adenocarcinoma)

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The consumption of fruits and vegetables has been associated with a reduced risk of developing diseases related to oxidative stress, including certain types of cancer. The main protective effect of fruits has been particularly attributed to compounds with antioxidant activity, such as phenolic. This study evaluated the antiproliferative activity of water extracts of the fruits camu-camu (*Myrciaria dúbia* H. B. K. McVough), cupuaçu (*Theobroma grandiflorum*Willd. ExSpreng), graviola (*Anona muricata* Linn.) and jabuticaba (*Myrciaria jaboticaba* (Vell.) O. Berg) in A549 cell line (human lung adenocarcinoma). Cells were treated at different extracts concentrations (50; 5; 0.5 and 0.05 mg/ml). The antiproliferative activity was measured by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-difeniltetrazole) assay. Camu-camu showed the highest inhibitory effect with an IC50 of 0.27mg/ml followed by graviola (2.4 mg/ml), cupuaçu (5.4 mg/ml) and jabuticaba (5.9 mg/ml). In addition, the results of IC50 were correlated with phenolic compounds amounts and showed a good correlation (r = 0,824).These results suggests that bioactive compounds of fruits may provide a new alternative for future cancer prevention and health promotion.

P4.1-08

Integration of new data on food processing and cooking from the Phenol-Explorer database improves estimation of polyphenol intake in the EPIC cohort

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Although it is well established that polyphenols can be degraded during cooking or food processing, food transformation has not usually been considered in the estimation of polyphenol intake in epidemiological studies.The Phenol-Explorer database has recently been updated to incorporate data on the effects of food processing on polyphenol contents in foods. The database now contains4,600 retention factor values, collected from peer-reviewed literature, which encompassaround 100 foods and 150 polyphenols.These new data, in combination with the existing Phenol-Explorer polyphenol composition data, were used to build new composition tables for EPIC foods and thus estimate intake from37,000 single standardized 24-hour dietary recallscollected inthe 23 centers participating in the European Prospective Investigation on Cancer and nutrition (EPIC) cohort.The application of retention factors substantially changed the estimation of polyphenol intake for some foods, particularly for commonly consumed vegetables that are often boiled, fried or baked. The improvement of dietary polyphenol measurements will aid correlation with their related biological markers and investigation of their associations with different disease outcomes, including cancer.

P4.1-09

Cytotoxic activity of an Andean potato variety from Argentinean northwest on human hepatocarcinoma cells

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Hepatocellular carcinoma is one of the most frequent cancers and is associated to high mortality.The research for new anti-cancer drugs is focused on compounds from plant origin. Polyphenols are associated to beneficial effects for human health, including antimicrobial, antineurodegerative and antiproliferative activities. Potato is one of the major sources of polyphenols in the human diet. The aim of this work was to study the cytotoxic activity of Andean potato extracts on human Hepatocarcinoma cells. Methanolic extracts of Andean potato variety (CL658) were obtained. Phenolic acids, anthocyanins and flavan-3-ols were quantified. The cytotoxic activity was studied on HepG2, Hep3B, Huh7 cells. They were incubated with different concentrations of the extracts for 24 h, and cell viability was analyzed by the MTS assay. To analyze if the cytotoxic activity is exerted by apoptosis, it was determined by ethidium bromide/acridine orange staining, DNA laddering and flow cytometer. The concentrations in the extract were: phenolic acids, 9.65 mg chlorogenic acid equiv/mL; anthocyanins, 2.13 mg cyd-3-glu equiv/mL; flavan-3-ols, 0.05 mg catechin equiv/mL. The cytotoxic concentrations 50% and 90% were: HepG2 93µg/mL and 351µg/mL; Hep3B: 140µg/mL and 364µg/mL; Huh7 137µg/mL and 442µg/mL. Andean potato extracts present cytotoxic activity on human hepatocarcinoma cells.

P4.1-10

Association of piperine and curcumin with viability of breast cancer cells lines

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Breast cancer is the second most frequent type in the world and spices have bioactive compounds (BCs), such as piperine and curcumin which are responsible for their chemopreventive and chemotherapeutic activities. The aim of this study was to investigate the effect of piperine and curcumin, either alone or associated with the viability of the breast cancer cells lines (MCF-7 and MDA-MB-231). Cells were treated with concentrations ranging from 1 to 300 µM of BCs and MTT reduction method was used for cell viability (CV) analysis. In the treatment with BCs isolated, curcumin presents better cytotoxic effect in both cells lines, mainly in 48h, when it was compared with piperine. IC_{50} values was 11,21 and 66,91 µM on MCF-7 and 18,62 and 246,6 µM on MDA-MB-231 using piperine and curcumin, respectively.MDA-MB-231 cell line was more resistant than MCF-7 cell line by BCs effect except in the association of curcumin and piperine during 48h of treatment. The treatment with the low concentration on MDA-MB-231 was able to reduce the CV in 100%. In general, results suggest that combination of piperine and curcumin can potentiate the cytotoxic effects on MCF-7 and MDA-MB-231 breast cancer cells lines rather than are used alone. Supported by: FAPERJ, FAF, CAPES,CNPq.

P4.1-11

Quercetin induces cell cycle arrest in human tumor xenograft model

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Quercetin is ubiquitously found in plants and plant food sources, several beneficial health effects have been associated with the dietary uptake of this polyphenolic compound. A variety of studies indicate a possible use of quercetin for cancer treatment through its interaction with multiple cancer-related pathways. The underlying mechanisms of myelodysplastic syndromes involve signal proteins and transcription factors which gives the cell a growth advantage over its normal counterpart. Accordingly, the goal of this work was to identify the quercetin effects using P39 cells in xenografted mice. The myeloid cell line P39, derived from a patient with MDS-chronic myelomonocytic leukemia, was inoculated subcutaneously in NOD/SCID mice and quercetin (120mg/Kg) was administrated once every four days by intraperitoneal. After 21 days, the mice were sacrificed and the tumors were removed for western blotting and immunohistochemistry analyzes. Quercetin treatment reduced 31.6% of tumor volume compared to control. Quercetin induces cell cycle arrest in G1 phase, with reduction of cyclin D and E expression and phosphorylation of Rb. The p21 staining was increased compared to control. We did not observe any significant difference in cyclin A, Cdk2, 4 and 6. The quercetin treatment in xenotransplant model reduces the tumor development, with pronounced induction of cell cycle arrest.

P4.1-12

Effects of resveratrol, curcumin and piperine on MCF-7 breast cancer cells viability and expression of glyoxalase-1

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The increase of the glycolytic pathway in tumors justifies the intense formation of glyoxal (GO) and methylglyoxal (MGO). These substances are formed during glycolysis and promote oxidative stress becoming aggressive towards cells suggesting a potential chemotherapeutics agents. The glyoxalase system detoxifies GO and MGO, physiologically, being dependent of the enzyme glyoxlase 1 (GLO1), which is overexpressed in tumors. Bioactive compounds (BCs) from functional foods have chemopreventive and chemotherapeutic activities. The aim of this study was to evaluate the action of BCs in viability of breast cancer cells (MCF-7) and expression of GLO1. Cells were cultivated in D-MEMmedium and treated with different concentrations of GO, MGO, resveratrol, cursumin and piperine. Cell viability was determined by MTT assay. The expression of GLO1 was determined by Western Blotting. The IC50 values for MGO, GO, resveratrol, curcumin and piperine during 24 hours of treatment were 2800µM, 2800 µM, 131 µM, 24,5 µM and 94,5 µM, respectively. In the cells treated with BCs, it was observed that curcumin and resveratrol, but not piperine, diminished GLO1 expression. This inhibitory effect is related to increased intracellular concentrations of GO and MGO, due to the low activity of the enzyme that detoxifies it, explaining a possible mechanism of action for apoptosis. Financial support: FAPERJ and FAF.

P4.1-13

Resveratrol as a pro oxidant: a new strategy for breast cancer treatment

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The breast cancer is the second most deadly cancer among women. Resveratrol (RSV), a polyphenol, has been reported to exhibit a wide range of pharmacological properties and is believed to play a role as anti- cancer agent. In the present study, the anticancer activity of RSV towards MCF-7 cells was investigated. Cell viability was determined by MTT assay. The cells were treated with RSV for 24h and a decrease of the cell viability towards the production of reactive oxygen species occurred. The generation of ROS was measured by H2DCF-DA. N-Acetyl-L-cysteine, L-reduced glutathione, Catalase, Peg-catalase, Superoxide dismutase were used in different concentrations to evaluate the capability of reverse the pro-oxidant effect of RSV. This polyphenol inhibited cellular proliferation in time and dose-dependent manner. ROS production was increased when MCF-7 cells were treated with RSV in a high concentration (200μM) for 24h. NAC, GSH and Peg-catalase completely reverted the ROS generation, demonstrating a pro oxidant effect of RSV which caused the decrease of the MCF-7 cell viability. However, Catalase and Superoxide dismutase hadn't show similar responses. Our results demonstrate that RSV may be applied as a new strategy of breast cancer treatments. Key words: Breast cancer, Resveratrol, reactive species of oxygen, MCF-7 cells. Support: FAPERJ, CAPES, CNPq, and FAF.

P4.1-14

Procyanidins inhibit Akt and induce colorectal cancer cell apoptosis via the mitochondrial pathway

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Colorectal cancer (CRC) constitutes the third highest cancer incidence worldwide. Epidemiological studies indicate that consumption of fruit and vegetables containing procyanidins (PCAs), polymers of flavan-3-ols, is associated with CRC lower risk. This study investigated the capacity of PCAs with different degrees of polymerization to decrease CRC cell survival, characterizing the underlying mechanisms. The largest PCA studied (hexamer (Hex)) was the most active decreasing CRC cell viability. Hex caused a dose (2.5-50 μM)- and time (24-72 h)- dependent decrease in the viability and cell colony formation of 6 human CRC cell lines. Hex induced increased CRC apoptotic cell death within 24 h, as evidenced by caspase 3 and caspase 9 activation, DNA fragmentation, changes in nuclear morphology, and Annexin V (+) staining. Hex-induced apoptosis occurs through the mitochondrial pathway, as evidenced by an increased translocation of Bad to the mitochondria, and the release of mitochondrial cytochrome c to the cytosol. The mitochondrial translocation of Bad is driven by a decrease in Bad phosphorylation attributed, in part, to the capacity of Hex to inhibit the upstream kinase Akt. In conclusion, results show that large PCAs can induce CRC apoptotic cell death, which supports epidemiological evidence of a role of PCAs-rich diets in the prevention of CRC.

P4.1-15

Antiproliferative activity of the flavonoid quercetin-3-methyl-ether on a lymphoma cell line: an approach to its mechanism of action

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Leukemia and lymphoma are a group of heterogeneous neoplastic disorder of white blood cells. Nitric oxide (NO) is involved in modulation of proliferation/death balance in cells. Conventional medicine can be inefficient or also results in side effects. Previously it was reported the antiproliferative effect of a fraction named AE (ethyl acetate), obtained from an aqueous extract of *Larrea divaricata Cav* which was rich in polyphenols. One of the compounds identified was quercetin-3-methy-ether (Q-3ME).

The aim of this work was to determine the effect of Q-3ME on the proliferation of the lymphoma cell line EL-4, to analyze the mechanism of action in relation to nitric oxide production and to determine the effect on cell cycle and apoptosis by flow cytometry.

Q-3ME exerted antiproliferative activity (EC_{50}: 46.7± 4 μg/ml) in relation to an arrest of cell cycle and the induction of apoptosis mediated by NO production.

It conclusion, Q3-ME could be a potential therapy for lymphoma and leukemia treatments.

P4.1-16

The effect of pomegranate active component (punicalagin) on Caco-2 human colon cancer cells

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Background: Recent evidence indicates that some fruit species have antioxidant and antimutagenic properties. In particular, punicalagin extracted from pomegranate is reported to have anti-proliferative activities and can induce toxicity in colon cancer cells; however, the mechanism of action at a molecular level is still poorly understood. In this study, in vitro apoptotic and anti-proliferative activities of punicalagin were investigated in human colon cancer caco2 cells.

Methods: The cell viability of Caco-2 cell line was identified and morphological changes in the presence of punicalagin were investigated. In addition, reactive oxygen species in response to punicalagin injury were evaluated by flow cytometry. The effect of different concentrations of punicalagin on caco-2 cell cycle was also studied by flow cytometry. Further, the effects of punicalagin on caco-2 cells were compared to those on immortalized human colonic epithelial cells (HCEC).

Results: Punicalagin (50 and 75 μM) showed significant anti-proliferative activity against caco-2 cells by arresting the cells at G1/S phase of the cell cycle (30% compared to 12% in untreated cells)

Conclusion: Punicalagin has the potential for use as an antioxidant and anticancer compound.

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P4.1-17

Cytotoxic activity of the organic extract and isolation of an active flavonoid from *Mikania periplocifolia* (Asteraceae)

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According to the World Health Organization, lymphoma stands at the fifth place of cancer type related to death over the world. It isnecessary to search newandmore selective drugswith fewer adverse effects to treat this disease.

Natural productsplay an important rolein the process ofdiscovering and developingnew drugs. In this sense, the aim of the present study was to isolate cytotoxic compounds from *Mikania periplocifolia.*

The aerial parts of *M. periplocifolia* were extracted with dichloromethane and this extract was fractionated by column chromatography. After purification process, a yellow powder (compound A) was obtained. Cytotoxic activity of the extract and the isolated compound was investigated in a murine lymphocitic leukaemia cell line (EL-4) by MTT assay.

The organic extract of *M. periplocifolia* inhibited by more than 75% cell proliferation at 100 µg/ml. The chromatographic behaviour and UV analysis of compound A allowed us to identify it as a methoxy tetrahydroxylated flavone. This compound showed a 50% effective concentration of 20.02 µg/ml when tested for its *in vitro* cytotoxic activity.

This is the first time that the cytotoxic activity and the isolation of a bioactive flavone from *M. periplocifolia* are reported.

P4.1-18

Epigenetic regulation of breast cancer metastasis by the natural steroid withaferin A

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Because a vast majority of cancer patients succumb metastatic disease, interfering with metastatic cascade remains one of the main challenges in cancer therapy. This complex proces includes several discrete steps: local invasion, intravasation (or dissemination in lymph nodes or body cavities), circulation and survival, extravasation, growth at distinct sites and angiogenesis, all of which occur in a context of tumour promoting microenvironment. It is now becoming apparent that these cell-microenvironment interactions are highly susceptible to epigenetic regulation, both by internal and external cues.

Here we show that several essential components of metastasis, including urokinase plasminogen activator (PLAU), ADAM8 metallopeptidase, and tumour promoting cytokine TNFSF12 are regulated epigenetically by DNA methylation in breast cancer as revealed by 450K Illumina BeadChip Array and EpiTyper Mass Array. Moreover, Withaferin A, a natural compound derived from *Withania somnifera* decreases breast cancer invasion by increasing methylation of these genes leading to lowered gene expression as revealed by qPCR.

P4.1-19

Bioactivity of by-products extract from Brazilian wine industry in cultured human hepatocarcinoma cells

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Background: Wine industry produces large quantities of by-products rich in bioactive compounds from grapes, which could be obtained for nutraceutical purposes. Aim: To evaluate antioxidant capacity (AC) and bioactivity of an extract made from by-products of Brazilian wine industry in human hepatocarcinoma cells (HepG2). Methods: Hydro-alcoholic extracts were obtained from Brazilian wine by-products and concentrated by reverse osmosis. Bioactive compounds and antioxidant capacity were determined by ORAC, TEAC ($ABTS^{\bullet-}$) and TRAP assays. *In vitro* bioactivity was assessed on HepG2 and normal human fibroblasts (BEAS). Cellular viability and AC were investigated, respectively, by MTT reduction and dichlorofluoresce in oxidation, under short-medium-long-term incubation. Results/Extract: Total phenolics, flavonoids and anthocyanins where, respectively, (mean±SD) 473.8±52.4 mgGAE/100g, 147.5±2.77 mgCE/100g and 105.5±7.27 mg-cyanidin3-glycoside/100g. AC was (mean±SD; µmol TE/g): 27.1±0.33, 9.33±6.74, 122.2±2.40, by TEAC, ORAC and TRAP assays, respectively. Results/Cellular: HepG2 viability reduced in a time- and dose-dependent manner. Short-term incubation had no effect whereas medium- and long-term incubation induced, respectively, a maximum of 37% and of 75% reduction in viability. BEAS cells viability was slightly affected by the extract. Interestingly, short-term incubation promoted a dose-response increase in AC on HepG2. Conclusion: Wine-industry by-products present high AC and potential selective anticancer and antiproliferative effects on HepG2 cells.

P4.2-01

The effect of red wine polyphenols on endothelial function and oxidative stress in borderline hypertensive rats

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This study evaluated the effects of red wine polyphenols (Provinols™) on blood pressure (BP), oxidative status and the femoral artery (FA) reactivity in the borderline hypertensive rats (BHR).

Male 12-week-old BHR and Wistar-Kyoto (WKY) rats were treated by Provinols™ (20 mg/kg/day) for 8 weeks. Provinols™ had no effect on BP in any group investigated, however, it reduced conjugated dienes and TBARS (measured in the left ventricle and kidney for assessment of oxidative load) in BHR. No differences were seen in lipid profile.

Endothelium-dependent vasorelaxation induced by acetylcholine (ACh) was measured in the FA. Endothelial dysfunction was more pronounced in untreated BHR vs. Provinols™–treated BHR. ACh (at concentrations higher than 10^{-6} M) caused the release of endothelium-dependent vasoconstrictors (EDCFs) only in BHRs and this effect was partially prevented by Provinols™. Nitric oxide (NO)-dependent relaxation was similar in both control BHR and WKY. Results showed that reduced vasorelaxation in the BHR was associated with increased release of EDCFs rather than with impaired release of NO. Red wine polyphenols partially reduced the release of EDCFs in BHR, suggesting favourable effect of Provinols™ on endothelial function in rats with mild elevation of BP. Supported by the VEGA grant No. 2 /0084/10 and APVV-0523-10.

P4.2-02

Chronic consumption of blackcurrant juice prevents endothelial dysfunction in the mesenteric artery of cirrhotic rats with portal hypertension

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Portal hypertension (PH) characterized by generalized vasodilatation associated with endothelial dysfunction affecting nitric oxide (NO) and endothelium-dependent hyperpolarization (EDH). The aim of the present study was to evaluate the effect of a polyphenol-rich blackcurrant juice (PRBJ) on the endothelial function and, if so, to determine the underlying mechanism.

Male Wistar rats received either control drinking water or PRBJ (60 mg total phenols/kg) for 7 weeks. After 3 weeks, the rats underwent surgery either with the ligation and resection of the common bile duct (CBDL rats) or sham surgery (sham rats).

Both the NO- and the EDH-mediated relaxations to acetylcholine were significantly reduced in CBDL rats compared to sham rats. The CBDL rats exhibited a reduced vascular expression of Cx37 and $SK_{Ca,}$ increased expression of eNOS and NADPH oxidase subunits, and increased vascular formation of ROS and peroxynitrite. The PRBJ treatment improved blunted EDH-mediated relaxation, and this effect was associated with an improved vascular expression of Cx37, $SK_{Ca,}$and eNOS, reduced vascular oxidative stress, and improved plasma levels of pro-inflammatory cytokines.

Altogether, these findings indicate that chronic ingestion of PRBJ improved the CBDL-induced blunted EDH-mediated relaxation, most likely by normalizing the vascular oxidative stress and the inflammatory response.

P4.2-03

Nutrigenomic and vascular protective effects of a long-term grapefruit juice consumption: a controlled randomized cross-over study in post-menopausal women

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Flavanones are highly and exclusively present in citrus; however clinical evidences on the role of flavanones on health effects are scarce.

We aimed at 1) characterizing the effect of a long-term consumption of grapefruit juice on vascular function in humans and assessing the specific role of naringenin in the observed effects, 2) analyzing change in PBMCs transcriptome profile induced by naringin.

52 healthy post-menopausal women were enrolled in randomized, controlled, cross-over trial. For two periods of 6 months, subjects consumed 340ml/d of grapefruit juice (212mg naringenin-glycosides) or control beverage without naringenin. The effect on conventional risk factors for CVD, endothelium-dependent vasoreactivity in both macro- and microcirculation (FMD and PAT signal), arterial stiffness and blood pressure were analyzed. Furthermore, a global gene expression profiles were determined using microarrays.

We showed that grapefruit juice could protect from age-related arterial stiffening, the effect specifically related to the presence of naringenin in juice. Modulation of arterial stiffness could be an interesting target by which grapefruit juice exerts beneficial effect on vascular health. The PBMCs nutrigenomic analyses showed that naringenin regulates the expression of genes involved in inflammatory processes. Further analyses may shed light on the molecular mechanisms involved in vascular effects of grapefruit flavanones.

P4.2-04

Yerba mate (*Ilex paraguariensis*) enhances the gene modulation and activity of paraoxonase-2: *In vitro* and *in vivo* studies

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Objective: Paraoxonase-2 (PON2) is an intracellular antioxidan enzyme that can be modulated by polyphenols. The aim of thi study was to verify whether yerba mate (*Ilex paraguariensis* modulates the gene expression and activity of PON2 i macrophages *in vitro* and *in vivo*. Methods: THP-1 macrophage were incubated with increasing amounts of yerba mate extract or chlorogenic and caffeic acids (1-10 µM). The *in vivo* effects o yerba mate or water (control) intakes were evaluated acutely (2 h after ingestion) and in the short-term (after daily ingestion for 7 days) in 20 healthy women. Results: Yerba mate extracts o chlorogenic acid at 1 and 3 µM increased PON2 relative gene expression in THP-1 macrophages (P< 0.05). Caffeic aci induced PON-2 activity only. The acute ingestion of yerba mat infusions increased relative gene expression and PON2 activity i monocytes (P< 0.05), while consumption of yerba mate for 7 days increased PON2 relative gene expression (P< 0.05) an had a tendency to enhance PON2 activity in monocytes and i monocyte-derived macrophages. Conclusion: It is suggested tha yerba mate modulated positively mRNA expression and the activity of the PON2 enzyme in monocytes and macrophages which may prevent cellular oxidative stress.

P4.2-05

Effect of grape pomace (GP) on weight gain and adipose inflammation in high-fat-fructose diet-induce metabolic syndrome

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Western diet high in fat and sugars has been related with high incidence of cardiac arrhythmias. Food rich in polyphenols has been proposed to prevent cardiovascular disease. Our aim was evaluate if a high-fat-fructose diet (HFFD) increased arrhythmia in a model of injury by ischemia-reperfusion and the cardioprotective effect of dietary supplementation with wine grape pomace (WGP). Male Wistar rats were fed for 6 w: control diet (C), HFFD (20 % fat and 20% fructose w/w), and HFFD supplemented with WGP in a dose of 1 g/Kg/d (n=5 each group). The heart was extracted, reperfused with modified Krebs-Henseleit solution and performed in 5 m from preisquemia, 10 m of regional ischemia (by ligation of the anterior descending coronary artery) and 5 min of reperfusion. Incidence and severity of arrhythmias were analyzed with the scale of Curtis-Walker. HFFD showed high incidence and duration of severe arrhythmias group (4/5) compared with the C group that showed low incidence of severe arrhythmias (1/5). HFFD supplemented with WGP showed high incidence but low duration (4/5) restoring the normal electric activity. Our results suggest that HFFD predispose the occurrence of arrhythmias in the ischemia-reperfusion model. Interestingly, supplementation with WGP could attenuate this effect.

P4.2-06

Combinations of flavonoid metabolites reduce tumour necrosis factor-α to a greater extent than the constituent compounds alone

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Epidemiological studies suggest flavonoid-rich dietsare associated with reduced cardiovascular disease incidence. Flavonoids are consumed in combination, and are present in the circulation as complex mixtures of metabolites. This study investigated the potential additive/synergistic effects of flavonoid metabolites. 28 combinations of 6 parent flavonoids and 14 metabolites were screened for their ability to reduce lipopolysaccharide-induced tumour necrosis factor-α (TNF-α) secretion in THP-1 monocytes. A combined 10 μM treatment of protocatechuic acid (PCA) and 4-hydroxybenzoic acid (4HBA) resulted insignificant (p=0.008) TNF-α reduction (42.7% ± 13.5), compared to vehicle control (VC). This was greater than the response from 10 μM of either constituent alone, wherePCA reduced TNF-αby 20.5% ± 36.3 (p=0.666) and4HBA reduced TNF-α 8.4% ± 11.6 (p=0.970). Treatment witha 1 μM combination of PCA, 4HBA and vanillic acid (VA) resulted in significant (p=0.011) TNF-αreduction47.4% ± 12.3. This reduction was greater even thanthat elicited even by 10 μM of the constituents: VA 12.6% ± 29.9 (p=0.846) compared to VC. These data suggest the complex mixtures of flavonoid metabolites found after consumption of a diet of mixed flavonoids, may be more beneficial than consuming flavonoids as single purified compounds.

P4.2-07

Quercetin induces acute vasodilator effects in healthy volunteers: correlation with beta-glucuronidase activity

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Quercetin exerts vasodilator, antiaggregant and antiproliferative effects and reduces blood pressure, oxidative status and end-organ damage in hypertensive humans and animal models.We hypothesized that oral quercetin might induce vasodilator effects in humans and that they might be related to the deconjugation of quercetin-3-glucuronide (Q3GA). Design: double blind, randomized, placebo-controlled trial. Sixteen healthy volunteers (26 ± 5 years, 6 female) were given a capsule containing placebo, 200 or 400 mg of quercetin in random order in three consecutive weeks. At 2 h a dose-dependent increase in Q3GA was observed in plasma with minor levels of quercetin and isorhamnetin. No changes were observed in blood pressure. An increase in brachial artery diameter was observed 2 h after 400 mg quercetin which correlated with the plasma flavonoid levels. At 5 h the change in diameter increased 5 fold (over the effect at 2 h) and correlated with the product of the levels of Q3GA at 2 h by the plasma glucuronidase activity. There was no increase in urinary nitrites plus nitrates. In conclusion, quercetin exerts acute vasodilator effects in vivo in normotensive, normocholesterolemic human subjects. These results are consistent with the effects being due to the deconjugation of the metabolite Q3GA.

P4.2-08

Effects of polymeric proanthocyanidins and their gut metabolites on endothelial dysfunction in deoxycorticosterone acetate (DOCA)-salt hypertensive rats

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Proanthocyanidins are a class of polyphenols which are mainly contained in plants such as grapes. It is well-known that Proanthocyanidins improve endothelial function in some animal models and human studies. However the contribution of Proanthocyanidins themselves on improvement of endothelial function is unclear, because of its poor absorption. On the other hand, some studies have reported that Proanthocyanidins metabolites produced by intestinal bacteria were detected in urine.

We first evaluate the effects of grape extract and its high-polymer fraction on endothelial function in deoxycorticosterone acetate (DOCA)-salt hypertensive rats. The decreased vasorelaxant responses to acetylcholine in endothelium-intact aortas were significantly improved by administration of grape extract and its high-polymer fraction, respectively. For the purpose of predicting influence of Proanthocyanidins metabolites, we examined the effects of six Proanthocyanidins metabolites on angiotensin 2-induced superoxide (O_2^-) production in HUVEC cells. Three metabolites reduced gene expression of NADPH oxidase components, IL6, ICAM1 and MMP-1. Furthermore, 3,4-Dihydroxyphenyl acetic acid, a main Proanthocyanidins metabolite, could significantly ameliorate the impairment of endothelium-dependent relaxation.

These results suggest that Proanthocyanidins metabolites produced by intestinal bacteria may in part account for the effectiveness of Proanthocyanidins.

P4.2-09

Polyphenols from propolis regulates the gene expression in advanced atherosclerosis

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Polyphenols from diverse sources have been demonstrated its capacity of modulates many biological processes, includinginflammation. Atherosclerosis is defined asa chronic inflammatory process which progress from initial to advanced lesion by expression of genes involved in monocyte transmigration, foam cell formation and extracellular matrix formation and degradation.The modulation of these inflammatory factors by polyphenols might result in a lesion with lesser progression. Thus, the aim of the present study was to evaluate the effect of a propolis polyphenols extract and Pinocembrin on the progression of atherosclerosis lesion. Atherosclerosis was induced in homozygous LDL receptor-deficient mice using a protocol to achieve an advanced lesion in whichafter 12 weeks of cholesterol-enriched diet the mice divided in 4 groups that received polyphenols extract (250 mg/kg body weight), pinocembrin (250 mg/kg body weight), vehicle or saline solution respectively. The size of lesion was determined by oil red O stain. Total RNA was extracted from atherosclerotic lesion area to evaluate gene expression. The size of lesion was similar between studied groups. However, polyphenols from propolis showed an inhibitory effect on PCAM, PDGF, TIMP2, VEGF gene expression. These data suggest a beneficial role of polyphenols on processes related to advanced atherosclerotic plaques as angiogenesis and matrix degradation, being interesting the study of cellular composition of these plaques to evaluate a possible effect on its stability. Financial support: CONICYT-Fellowship (Chile), FAPESP and CAPES-Brazil.

P4.2-10

Effect of tomato industrial processing on phenolic profile and antiplatelet activity

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Regular consumption of fruits and vegetables (e.g. tomatoes) has been shown to be beneficial in terms of reducing the incidence of cardiovascular diseases. The industrial processing of tomatoes into tomato-based products includes several thermal treatments. Consequently, very little is known on the effect of tomato industrial processing on antiaggregatory activity. We assessed the effect of tomato and by-products extracts on platelet aggregation challenged by ADP, collagen, TRAP-6 and arachidonic acid. Under standardized conditions. A set of antiplatelet compounds were selected for HPLC analysis in the different extracts. Interestingly, some natural compounds (chlorogenic acid, caffeic acid, ferulic acid and p-coumaric acid) consumed regularly in the diet may inhibit platelet activation. Of this form tomatoes, and its products (salsa, ketchup, juice) and by-products extracts inhibited platelet aggregation induced by ADP, collagen, TRAP-6 and arachidonic acid, but to a different extent. In conclusion, processed tomatoes may have a higher content of health-benefiting compounds than fresh ones. Even, pomace present the best antiplatelet activity. Finally, tomatoesmay be used as a functional ingredient adding antiplatelet activities to processed foods.Acknowledgements: To CONICYT REGIONAL/GORE MAULE/CEAP / R09I2001, Programa de Investigación de Excelencia Interdisciplinaria en Envejecimiento Saludable (PIEI-ES), and supported by grant no. 1130216 (I.P., M.G., R.M., M.A., J.C.) from Fondecyt, Chile.

P4.2-11

Effect of Alibernet red wine extract on endothelial function and lipoperoxidation in young normotensive and hypertensive rats

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As wine polyphenols were shown to possess many positive effects, including improvement of endothelial function, this study investigated the effect of the Slovak Alibernet red wine extract (AWE) on blood pressure (BP) and vascular function in young Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats. Six-weeks-old, male, WKY and SHR were treated with AWE for three weeks at the dose of 24.2 mg/kg/day. BP (tail-cuff) was significantly elevated in SHR vs. WKY and AWE failed to affect it. Lipid peroxidation was evaluated by determination of thiobarbituric acid-reactive substances. AWE reduced lipid peroxidation in the left ventricle of both genotypes. Endothelium-dependent relaxation was evaluated in the femoral arteries (FA) as the responses to acetylcholine (ACh). Relaxation of the FA was reduced in control SHR vs. control WKY, which was associated with a significant decrease of its nitric oxide (NO)-independent component. AWE failed to affect ACh-induced relaxation, both its NO-dependent and independent components, compared to controls of the same genotype. In conclusion, three-week administration of AWE failed to reduce BP and to improve endothelial function in the FA of both genotypes investigated, however significantly reduced oxidative damage in the left ventricle. Supported by the grants VEGA No. 2 /0084/10 and APVV-0523-10.

P4.2-12

Cardiovascular benefits of combined flavan-3-ol and isoflavone intake in statin treated postmenopausal women with type 2 diabetes: potential impact ofequol producer status

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Introduction: Prospective studies observe an association between high flavonoid intake and a reduced risk of type 2 diabetes (T2DM), with supportive findings of glycaemic and vascular benefits *in vitro.* However, to date few randomised controlled trials (RCTs) have examined the chronic effects of flavonoids in patients with T2DM. Objective and methods: In medicated postmenopausal women with T2DM, the effect of 1-year intake offlavonoid-enriched chocolate (850mg flavan-3-ols (90mg epicatechin), 100mg isoflavones (aglycone equivalents)/d) on vascular endpoints (common-carotid artery intima-media thickness (CCA-IMT), pulse wave velocity (PWV), augmentation Index (AIx), blood pressure (BP)) and insulin resistance was assessed. Results:In 93 patients, no significant change in CCA-IMT, AIx or BP was observed, however intervention significantly reduced total:HDL cholesterol ratio (-0.2±0.1, p=0.01), LDL(-0.1±0.1mmol/L, p=0.04), insulin and insulin resistance (-0.8±0.5mU/L, p=0.02; HOMA-IR -0.3±0.2, p=0.004) and PWV was improved(flavonoid (n=18), -0.07 ± 0.38 *m/s*, placebo (n=17) 0.68 ± 0.25 *m/s*; p=0.01). Notably, equol-producers (EPs; n=17), had significantly reduced diastolic BP, mean arterial pressure and PWV (-2.24 ± 1.31 *mmHg,* -1.24 ± 1.30 *mmHg,* -0.68 ± 0.40 *m/s*; p<0.01) compared to non-EPs (n=30). Conclusions: Chronic flavonoid intake resulted in improvements in CVD risk markers and augmented therapeutic strategies to reduce CVD risk in T2DM.

P4.2-13

Intake and time dependence of blueberry (poly)phenol-induced improvements in vascular function: mechanistic insights into biological activity

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Limited data exists regarding the effects of berryintake on vascular function.This work aimedto investigate the impact of blueberry (poly)phenolintake on endothelial function in healthy men and to assess potential mechanisms of action by assessment of circulating metabolites and neutrophil NADPH oxidase activity.Two randomized, controlled, double-blind, cross-over intervention trials were conducted in 21 healthy male subjects. The impact of blueberry (poly)phenol intake on flow-mediated dilation (FMD) was assessed following the consumption of different amounts of blueberry containing 319to 1791 mg of total blueberry (poly)phenols (TP). We observed a time-dependent increase in FMD at 1, 2, and 6 hours post-consumption and a dose-dependent increase in FMD up to 766 mg TP intake, after which FMD plateaued.Increases in FMD at 1-2 hours were closely linked to increases in circulating blueberry-derived phenolicmetabolites, whereas increases in FMD at 6 hours were linked to increases in blueberry-derived phenolic metabolites of the colonic microflora. Decreases in neutrophil NADPH oxidase activity were linked to circulating metabolites and FMD. In conclusion, blueberry intake acutely improves vascular function in healthy men in a time and intake level-dependent manner. These benefits may be mechanistically linked to the actions of circulating phenolic metabolites on neutrophil NADPH oxidase activity.

P4.2-14

Considering the cardiovascular benefits of cocoa flavanols in the context of the general population: Do we meet a prerequisite for dietary guidelines?

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Accumulating evidence from dietary intervention studies suggest that diets rich in flavanols are causally related to cardiovascular health benefits in humans.In o rder to consider these findings in the context of potential futuredietary recommendations, it is necessary to assess their relevance and applicability to the general public. In this context, the pan-european research consortium FLAVIOLA aimed at investigatingthe cardiovascular effects of cocoa flavanol(CF) intake in various cohorts representing a broader healthy population. In particular, we investigated the efficacy of CF as well as their absorption, distribution, metabolism, excretion (ADME) from a gender-and age perspective in healthy people. Taken together, our data demonstrate that CF intake mediates beneficial effects on various cardiovascular endpoints in healthy men and women across all the ages and groups investigated. Furthermore, the intra- and inter-individual variability of flavanol ADME is relatively low, and while small differences in the metabolism and catabolism of flavanols were identified as a function of age, in general, flavanols were absorbed, metabolised and excreted in a similar fashion across study populations. Taken together, we provide direct evidence for population-based health benefits, thus supporting a potential role for cocoa flavanols in primary cardiovascular disease prevention and future dietary guidelines.

P4.2-15

Transcriptomic and epigenomic changes in human leukocytes upon 8 weeks supplementation with monomeric and oligomeric flavanols

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A recent randomized, double-blind, placebo controlled clinical trial unveiled pleiotropic health benefits in the vasculature of healthy male smokers upon 8 weeks supplementation with daily 200 mg flavanols. In order to unravel the flavanols' underlying molecular mechanisms, we investigated the transcriptomic and epigenomic changes in leukocytes. Gene expression analysis (microarrays) revealed significant changes in various cellular processes like chemotaxis, cell adhesion, or cytoskeleton organization. Transcription factor analysis identified NF-κB as a main affected inflammatory regulator. In-vitro studies have confirmed inhibition of NF-κB-mediated gene transcription in reporter-gene assays and attenuation of monocytes' adhesion to endothelial cells. Genome-wide DNA methylation changes occurred in gene clusters involved in detoxification, metabolism and cell adhesion. Although individually the flavanol intervention triggered significant changes in DNA methylation levels (>10%) of 0.2-1% of the methylome, no common flavanol-specific DNA methylation response was seen. Inter-individual variability in genes' DNA methylation levels could be linked to long-term smoking history, overruling diet specific effects of an 8-weeks intervention. Altogether, flavanols may elicit protective effects in the vasculature by decreasing inflammatory and cell adhesion pathways at the transcriptional level. Moreover, smoking history may be a confounding factor in epigenetic profiling studies of leukocytes from subjects involved in a flavanol-rich diet intervention.

P4.2-16

Daidzein metabolizing phenotypes influence metabolomic responses in individuals with cardiometabolic risk factors

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Consumption of polyphenol-rich diets, including soy, is associated with beneficial health effects and chronic disease prevention. However, large variability exists in individual responses to soy. Equol, a daidzein metabolite produced by some colonic microbiota, contributes to this variability. The metabolomic profile associated with Equol producing individuals compared to those who do not, is unknown. Therefore, this pilot study utilized untargeted metabolomics to assess effects of a soy intervention on metabolites in participants at cardiometabolic risk. 17 individuals completed a randomized, controlled, crossover study receiving 70 g soy nuts (100 mg aglycone equivalents) or control food daily for four weeks, separated by two week washout. Serum and urine were used for clinical biomarkers and NMR analyses. Statistical analyses included multivariate PCA and PLS-DA. Metabolomic responses related directly to phenotypes based on gut microbial products of daidzein and were not distinguished by pre/post-soy changes. Three metabolomic phenotypes were identified: Equol+ODMA, ODMA only, and non-producers. The Equol+ODMA phenotype had significantly lower levels of multiple metabolites including TMA, creatinine, and aromatic and branched-chain amino acids, which typically increase in obesity and diabetes. Paradoxically, this phenotype also had significantly higher pro-inflammatory cytokines (TNF-α, IL-6, IL-18). Conclusion: Metabolic profiling identified phenotypic responses in at-risk individuals.

P4.2-17

The beneficial vascular effects of coffee polyphenols from high and low roasted coffee

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There is strong evidence suggesting that polyphenols reduce the risk of cardiovascular disease; the biggest contributor to morbidity and mortality internationally. Coffee is a rich source of a class of polyphenols called chlorogenic acids (CGA), however, during roasting, CGA levels are decreased.
A randomised, three-armed, crossover intervention study in healthy individuals was used to assess acute vascular responses to coffees subjected to different degrees of roasting. Subjects (n=14) consumed 236mg CGA (low roast), 108 mg CGA (high roast) and a caffeine control (110mg). All interventions were matched for caffeine, macronutrients and micronutrients. Vascular responses were assessed by flow mediated dilatation (FMD) and blood was collected for plasma CGA metabolite and nitric oxide (NO) analysis at baseline, 1, 3 and 5h.
A biphasic increase in FMD was observed at 1 and 5h post CGA intake (1.6% and 1.5% at 1h and 1.7% and 1% at 5h for low and high roasted coffee respectively) and a significant increase from baseline was seen at 5h with the low roasted coffee ($p<0.05$). Increases in FMD were correlated with total plasma CGA metabolites (54 were identified) ($r=0.21$, $p<0.005$), with, ferulic and isoferulic acids and native CGA correlating with the FMD peak at 1h ($r=0.25$-0.33, $p<0.001$) and methylferulic and dihydrocaffeic acid correlating with the peak at 5h ($r=0.25$-0.30, $p<0.001$). We hypothesise that increases in FMD following CGA intake are mediated by specific circulating CGA metabolites and their ability to maintain NO levels by inhibiting NADPH oxidase.

P4.2-18

Acute consumption of curcumin differently affects vascular function in middle-aged healthy male and female smokers: a clinical and molecular analysis

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Curcumim is poorly studied regarding cardiovascular health. The aim of this study was to determine the effect of curcumin on vascular endothelial function in healthy smokers and its nutrigenomic impact in circulating white blood cells.
The effect of an acute intake of curcumin (5g) on endothelial function was evaluated by Flow mediated dilatation (FMD) and Reactive Hyperaemia Index (RHI) in a randomized, double-blind, cross-over study on 9 men and women. Measurements were done before and 2-hours after curcumin or placebo intake. Impact of curcumin on expression of 93 genes involved in inflammation, cell adhesion, cholesterol absorption/efflux was evaluated by RT-PCR using TaqMan-Low-Density-Array.
We failed to show significant difference between curcumin and placebo for FMD and RHI partly due to inter-individual variability. Analysis of data separately for men and women revealed a significant effect of curcumin for FMD in women, but not in men. PCR analysis suggests that curcumin modulated expression of genes and nutrigenomic response to curcumin seems to be different between women and men.
Although results did not account for a beneficial effect of curcumin on vascular function in the whole studied population, gender comparison analysis seems to reveal a difference in response to curcumin between male and female.

P4.3-01

Synergistic effects of sesamin and gamma- or alpha-tocopherol on lipid metabolism in high sucrose-fed rats

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We have found that ingestion of sesamin together with α-tocopherol (α-VE) synergistically reduced the blood cholesterol in rats given high-cholesterol diet. This study was designed to investigate the effect of sesamin and γ-tocopherol (γ-VE) ingestion on blood triglyceride (TG) in rats given high-sucrose diet (HS). Rats were divided into eight groups and fed on experimental diets for 2 weeks as follows; 1) HS diet (control group), 2) HS diets containing 0.1% sesamin, 3)-7) HS diets containing 0.1% sesamin and various doses (0.02, 0.05, 0.1, 0.2, and 0.5%) of γ-VE (sesamin+γ-VE), 8) HS diet containing 0.5% γ-VE. Blood samples were collected after 4 hour fasting. Serum TG levels in sesamin, sesamin+γ-VE (0.2 and 0.5%) groups reduced by 29, 59 and 52% compared to the control group, respectively. Since TG level in γ-VE group did not differ from the control group, these results might be regarded as synergistic hypotriglyceridemic effect. Moreover, we also confirmed that sesamin with α-VE synergistically reduced serum TG in the similar experiment. Red blood cell hemolysis, peroxides in liver and plasma ε-hexanoyl-lysine adduct in S+γ-VE groups were decreased compared to sesamin or γ-VE group. These results suggest that ingestion of sesamin with α- and γ-VE might be beneficial in preventing metabolic syndrome.

P4.3-02

Concord grape pomace polyphenols complexed to soy protein isolate are stable and hypoglycemic in diabetic mice

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Polyphenols were extracted from Concord grape pomace using food-compatible solvents. LC-MS and MALDI-TOF analysis of the extract showed a broad range of phytochemicals. Extracted polyphenols were complexed to soy protein isolate (SPI) to produce grape polyphenol-SPI complex (GP-SPI) containing 5% or 10% grape polyphenols. After 16 weeks of incubation at 37 ºC, anthocyanins and total polyphenols demonstrated marked stability in the GP-SPI complex compared to dried pomace extract. Single dose administration of GP-SPI (300 or 500 mg /kg) containing 5% grape polyphenols formulated in Labrasol demonstrated significant hypoglycemic activity in obese and hyperglycemic C57BL/6 mice compared to before treatment or controls. GP-SPI (containing 10% polyphenols) or SPI alone were incorporated into the high fat diet (HFD) of C57BL/6 mice and feeding studies were performed to investigate the treatment and preventive effects of grape polyphenols on metabolic syndrome endpoints. Compared to mice fed the HFD + SPI, mice fed the HFD + GP-SPI showed improvement in fasting blood glucose, oral glucose tolerance as well as decreased body weight gain and adiposity. GP-SPI allows the capture of polyphenols from grape pomace in a protein-rich food matrix and may be useful as a functional food ingredient for the management of blood glucose levels.

P4.3-03

The impact of coffee consumption on body fat and some effect biomarkers in humans: results of two intervention studies

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Coffee is one of the most popular beverages consumed worldwide. To assess the influence of regular coffee consumption over four weeks on body weight/composition and on food intake in healthy subjects (N=84) a randomized, controlled cross-over intervention study was carried out. As biomarkers, blood platelet cAMP and phosphodiesterase activity (PDE) were investigated as well as plasma adenosine, adenosine deaminase activity and DNA damage. Additionally, body composition, plasma cholesterol, triglycerides, and lipoproteins were monitored. Two different coffee blends were tested differing in caffeoylquinic acids and N-methylpyridinium. Results show a significant reduction of body fat over the whole study period by each coffee, more pronounced after consumption of one coffee. In platelets, inhibition of PDE activity and increased cAMP concentration were observed.High density lipoprotein was found significantly enhanced after the consumption of one coffee. In a two weeks pilot intervention study (N=8) effects of regular and of caffeine reduced coffee on the above mentioned parameters were monitored. This short term intervention revealed a highly significant inhibition of PDE activity by regular coffee; whereas cAMP increased significantly ($p<0.01$) after regular but not after caffeine reduced coffee. These studies were supported by BMBF (grant no. 0315692), the DAAD, and the Tchibo GmbH.

P4.3-04

(-)-Epicatechin and its metabolites inhibit hyperlipidemia-induced oxidative stress through the transcriptional and posttranscriptional modulation of NADPH oxidase

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Oxidative stress is a major contributor to obesity-associated altered hepatic glucose and lipid homeostasis. Consumption of (-)-epicatechin (EC)-rich foods improves obesity-related metabolic diseases. This work investigated whether EC and its metabolites (ECM) inhibit hyperlipidemia-induced hepatic oxidant production regulating NADPH oxidase (NOX) activity and subunit expression (NOX3, p47, p22). This was tested in conditions of hyperlipidemia: i) induced in rats by high fructose (HFr) consumption, ii) HepG2 cells treated with palmitate. EC supplementation mitigated HFr-induced hyperlipidemia in rats and alleviated the increased expression of NOX3, but not that of p22 andp47. In HepG2 cells, palmitate (0.25 mM) treatment caused an increase in cellular oxidants which was prevented by EC and ECM. Palmitateincreased mRNA and protein levels of NOX3, p47 and p22. Treatment with EC or its metabolites (0.25-1 µM) attenuated palmitate-induced NOX3, but not p47 and p22 expression. Palmitate also caused NOX activation, measured as both enzyme activity, and p47 translocation to the cell membrane. EC and ECM (1 µM) inhibited NOX activation, but not p47 translocation. Our results show that EC modulates NADPH oxidase, both at the expression and activity levels *in vivo* and *in vitro*. Thus, treatment with EC could be relevant to the improvement of obesity-associated hepatotoxicity.

P4.3-05

(-)-Epicatechin mitigates metabolic syndrome-associated insulin resistance in rats

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The urgent need for strategies to prevent the emerging global epidemic of metabolic syndrome (MetS) led to the identification of nutritional complementary therapies that can counteract the risk factors for MetS; insulin resistance (IR) in particular. The present study investigated the ability of dietary supplementation with (-)-epicatechin (EC) to prevent IR in a rat model of high fructose (HFr)-induced MetS. Adolescent rats were given regular water or water sweetened with high-fructose (10% (w/v)) syrup in addition to a standard diet of rat chow for 8 w. The chow diet of a subgroup of HFr rats was supplemented with EC (20 mg EC/d/kg BW). Adipose and liver lysates from rats receiving fructose alone exhibited: i) significant alterations in the insulin signaling pathway (insulin receptor, IRS-1, Akt and ERK1/2); ii) activation of endoplasmic reticulum (ER)-stress pathways, as evidenced by increased PERK (Thr980), eIF2α (Ser51) and IRE1α (Ser724) phosphorylation; and iii) increased inflammation as evidenced by increased activation of NF-κB. Importantly, EC supplementation enhanced HFr-induced insulin signaling, attenuated HFr-induced ER stress, and mitigated HFr-induced activation of NF-κB in liver and adipose tissue. In summary, these findings demonstrate that Dietary supplementation with (-)-epicatechin may provide potential benefit against insulin resistance and metabolic syndrome.

P4.3-06

Supplementation with *Linum usitatissimum* L. increases polyphenols and vitamin C levels in patients with metabolic syndrome

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Metabolic syndrome (MS) is a group of metabolic disorders, with many studies demonstrating a reduction in antioxidant defenses of these patients and, consequently, increased oxidative damage to biomolecules. Flaxseed (*Linum usitatissimum* L.), a functional food rich in polyphenols and vitamin C, has been studied to produce an improvement in antioxidant defenses. The aim of this study was evaluate the effect of flaxseed golden on polyphenols and vitamin C levels in patients with MS. The study included 15 volunteers with MS and 15 healthy subjects. The volunteers received aliquots of 40g golden flaxseed for daily use for 28 days. We collected venous blood sample of volunteers to measure the polyphenols and vitamin C levels, using standard techniques, before and after supplementation. After 28 days of supplementation with flaxseed golden, there was a statistically significant increase ($p <0.05$) in the vitamin C levels and a significant reduction in the polyphenols levels in both the control subjects and those with MS. The results show that the intake of flaxseed for 28 days is able to improve the vitamin C levels as well as promoting increased consumption of polyphenols in free radical scavenging, in both healthy subjects and patients with MS.

P4.3-07

Dietary (-)-epicatechin lowers blood pressure in fructose-fed rats modulating nitric oxide bioavailability

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Flavonoids have shown to be efficient decreasing blood pressure (BP) in human and animal models of metabolic syndrome (MS). This study examined the antihypertensive effect of dietary (-)-epicatechin (EC) in fructose-fed rats, a model of MS. Male Sprague-Dawley rats were divided into 3 groups: Control (C), Fructose (F) and Fructose + EC (FEC) groups. Fructose was administered in the drinking water (10% w/v) and EC was supplemented in a solid diet (20 mg/kg BW/d) during 8 w. The FEC group did not show the increase in BP observed in F group. Fructose treatment showed a significant increase in NADPH oxidase (NOX) subunits expression and NOX activity in aorta, however these changes were not present in FEC group. The modifications in NOX subunits were produced essentially in the intima and adventitia layers. The expression of phosphorylated endothelial nitric oxide synthase (NOS) was significantly increased in F and FEC groups. However, EC treatment showed a higher NOS activity than F group. To conclude, EC normalized the increase in BP associated with in vivo fructose overload treatment. This effect could be related to the attenuation in superoxide anion production through the inhibition of NOX expression and activity and the increasein NOS activity and therefore increasing nitric oxide bioavailability. Supported by UBACYT 20020100100659 and 20020100100060 and CONICET (PIP-11220110100612).

P4.3-08

Effect of wine grape pomace extract (WGPE) and wine grape pomace (WGP) on weight gain and adipose tissue inflammation in high-fat-fructose diet-induce metabolic syndrome

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Polyphenols from grape has been found to exert beneficial effect on disorders associated with metabolic syndrome (MS). The aim of this study was to evaluate the effect of wine grape pomace extract (WGPE) and wine grape pomace (WGP) on weight gain, and metabolic alteration related with high fat-fructose diet-induce MS. The total phenolic content of WGPE and WGP were 196,2 and 41.6 mg of gallic acid equiv/g respectively. Catechin and epicatechin in WGPE were 2.14 and 3.29 mg/g, respectively. Other major polyphenols in WGPE included quercetin (1.54 mg/g) and trans-resveratrol (322 µg/g). Thirty male Wistar rats were fed for 6 w: control diet (C), high fat-fructose diet (20 % each w/w) (HFFD), HFFD supplemented with WGPE (100 and 300 mg/kg BW/d respectively), and HFFD plus WGP in a dose of 1 g/Kg BW/d. HFFD increased weight gain, systolic blood pressure, triglycerides and decreased HDL-cholesterol that were ameliorated/prevented by high doses of WGPE and WGP. HFFD also increased the area of adipocytes and parameters of adipose tissue inflammation that were partially reversed by high doses of WGPE and WGP. Consumption of food or food-rich in polyphenols may be useful in the prevention and/or amelioration of MS and MS-associated diseases.

P4.3-09

Effects of consumption of red wine treated with salicylic acid in a rat model of metabolic syndrome

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Anthocyanins are a subclass of flavonoids occurring in fruits, vegetables and beverages such as wines, which are synthesized by plants in response to adverse conditions. Stimulation of grapes with salicylic acid (SA) triggers the production of anthocyanins. We investigated the effectsof red wine Syrah,treated or not with SA, on metabolic alterationsin fructose-fed rats (F), ametabolic syndrome (MS) model. Twenty eight male Wistar rats were fed with or without (F) fructose 10% (w/v) in drinking water for 8 wk. A group of F rats received red wine, and SAtreated red winein a dose of 10% (v/v) in drinking water (n=7 each group). Subgroups of rats in each group wereinjected with insulin 10 min prior euthanasia. SA (8mM) was pulverized during maturation of grapesfor 45 days, from painting of clusters until the enological maturation of the plant. SA increased 64% of the total anthocyanins levels in red wine. F increased systolic blood pressure, triglycerides and decreased HDL-cholesterol and increased parameters of insulin resistance and inflammation in visceral adipose tissue such as PTB1B, pIRS-1, pJNK and reduced pAKT and PPARγ that were ameliorated/prevented by treatment with both wines.The administration of red wine regardless of treatment with SA attenuates F-induced insulin resistance.

P4.3-10

Evaluation of the anti-obesity and the antidiabetic properties of polyphenol-rich berries from northern Canada and Brazil

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Obesity and Type 2 Diabetes (T2D) profoundly affect Western Societies. The use of polyphenol-rich berries represents an interesting approach against these diseases. Wescreened 8 polyphenol-rich berries using obese micein order to identify novelanti-diabetic molecules. Administration of *M. dubia* and *V. trilobum* whole extracts prevented obesity in high fat/high sucrose (HFHS)-fed mice as revealed by a marked reduction in weight gain and adiposity, findings unrelated to changes in energy intake. These effects wereassociated with blunted adipose tissue inflammationevidenced by reduced crown-like structure (CLS) density and decreased MCP1 and VEGF content in fat tissue. *M. dubia* and *V. trilobum* further ameliorated glucose intolerance and hepatic triglyceride accumulation in HFHS-fed mice. Interestingly, *L. caerulea* and *P. virginiana* wholeextracts improved glucose intolerance and hepatic triglyceride content without affecting body weight gain or adiposity. *P. Virginiana* diminished CLS density and MCP1 and VEGF contents in adipose tissue. In conclusion, consumption of polyphenol-rich berries reduces obesity and T2D in HFHS-fed mice. Whereas *M. dubia* and *V. Trilobum* exert beneficial effects through prevention of adiposity, *L. caerulea* and *P. virginiana* appear to have anti-diabetic properties independent from body weight regulation. Identification of the bioactive molecules underlying the observed effects is underway.

P4.3-11

Cupuassu phenolic extract supplementation attenuates the development of glucose intolerance associated with a high fat/ high sucrose diet in C57BL/6J mice

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Obesity is a major health problem worldwide, increasing the risk of type 2 diabetes and other chronic diseases. Cupuassu is a native fruit from the Amazon region with a large number of phytochemicals that can act on body metabolism and influence our overall health. Thus, we aimed to investigate the effects of phenolic extracts of cupuassu in the prevention of insulin resistance and obesity induced by high fat/ high sucrose diet (HF/HS). Male C57BL/6J mice were fed astandard dietora HF/HS for 9 weeks. A phenolic extract of cupuassu was administered to mice at 2 different concentrations (1 or 3 mg of galic acid equivalent/kg body weight). Glucose and insulin tolerance test, measurement of body weight and fasting glucose were performed during the experiment. Cupuassu extracts did not affect body weight gain or mass of several fat depots, indicating that extracts of cupuassu were not effective on preventing obesity. Despite of the absence of effects on adiposity, cupuassu extract at lower, but not higher dose, completely prevented the development of glucose intolerance. In conclusion, phenolic extracts of cupuassu have preventive effects against the glucose intolerance associated with HF/HS diet, but the mechanisms underlying these actions need to be further investigated.

P4.3-12

Fermented tea improves glucose intolerance in mice by enhancing translocation of glucose transporter 4 in skeletal muscle

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The antihyperglycemic effects of tea are well documented. Glucose transporters play an important role in the regulation of blood glucose level. Of these, insulin-sensitive glucose transporter 4 (GLUT4) has been recognized as a novel target for prevention of hyperglycemia. GLUT4 is specifically expressed in skeletal muscle and adipose tissue, where it takes up glucose to reduce postprandial hyperglycemia. The insulin and AMP-activated protein kinase (AMPK) signaling pathways are the major regulators of GLUT4 translocation from intracellular storage vesicles to the plasma membrane. In this study, we investigated the translocation of GLUT4 and its related signaling pathways in skeletal muscle of male ICR mice given fermented tea. Intake of oolong, black, or pu-erh tea for 7 days enhanced GLUT4 translocation to the plasma membrane of skeletal muscle. Each type of fermented tea stimulated the phosphorylation of phosphoinositide 3-kinase (PI3K), Akt/protein kinase B, and AMPK. Fermented tea also increased the protein expression of insulin receptor. These results strongly suggest that fermented tea activates both PI3K/Akt- and AMPK-dependent signaling pathways to induce GLUT4 translocation and increases the expression of insulin receptor to improve glucose intolerance.

P4.3-13

Cardamonin and flavokawain B suppress differentiation of preadiopocytes to adipocytes through ERK activation

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To search for the effective compounds for the inhibition of differentiation of preadipocytes to the adipocytes, we treated with 50 polyphenols to 3T3-L1 preadipocytes for the first 3 days during the differentiation and measured lipid accumulation into the cells. We found seven effective polyphenols including cardamonin and flavokawain B. Cardamonin and flavokawain B are chalcones that exhibit various biological activities such as antitumor, anti-inflammatory and antioxidant activities. However, the anti-obesity effect and its underlying mechanism have not been documented. Both cardamonin and flavokawain B significantly inhibited intracellular lipid accumulation at 5 μM without any cytotoxicity. As the mode of inhibitory action of these chalcones, they inhibited lipid accumulation through down-expression of C/EBPα and PPARγ as the master regulators for adipocyte-differentiation. Furthermore, both cardamonin and flavokawain B increased phosphorylation level of ERK in a dose-dependent manner. ERK inhibitor, PD98059 canceled the down-expression of C/EBPα and PPARγ by these chalcones, and subsequently abolished the inhibition of the lipid accumulation. These results indicate that inhibitory effects of cardamonin and flavokawain B on adipocytes differentiation are involved in the ERK activation, resulting in the down-expression of C/EBPα and PPARγ levels.

P4.3-14

Inhibitory effects of food intake and fat accumulation by resveratrol derivative in mice

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Introduction. In the previous study, it has reported that resveratrol (Res) has suppressive action of fat accumulation. Therefore, we made several kinds of resveratrol derivatives (RDs), and their suppressive activities of fat accumulation screened by *in vitro* culture test using 3T3-L1 adipocytes. As a result, we found that a RD (RS-2) had strong inhibitory action of fat accumulation.

Therefore, in this study, we examined inhibitory effects of food intake and fat accumulation by RS-2 in mice.

Materials and Methods. Nine weeks old female C57BL/6J mouse used for feeding experiment. We administered AIN-76 diet with high-fat (high fat diet) and high fat diet mixed with Res and RS-2 at the concentration of 0.05 to 0.4% to the mice during 6 weeks and the food intake and body weight were measured every week. After the feeding, the various organ weights and the blood and liver lipid levels were measured.

Results and Discussion. Body weight increase of all RS-2 groups significantly suppressed. In addition, the food intake was remarkably reduced by RS-2. Moreover, Weights of intraperitoneal adipose tissues and liver were significantly lowered by it.

These results indicated that RS-2 had suppressive activity of food intake in mice and body weight and fat accumulation might be reduced by the action.

P4.3-15

Evaluation of energy metabolism and mitochondrial complexes in the heart of diabetic rats treated with resveratrol

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Resveratrol, polyphenolic phytoalexin, is a component of grape products, including red wine and grape juice, with antioxidant activity and exhibit beneficial effects on chronic degenerative disease, such as diabetes mellitus (DM1). It is characterized by hyperglycemia, which causes oxidative stress and changes the energy metabolism. This study evaluated the effect of resveratrol (RSV) on key enzymes of the energetic metabolism in myocardial of diabetic rats. Thirty-two male rats were divided into four groups (*n*=8): G1: control; G2: RSV-treated; G3: diabetic; G4: RSV-treated diabetic. DM1 was induced by streptozotocin (60 mg/kg, i.p.). RSV (1mg/weight/day) was administrated by gavage for 30 days. RSV decreased ($p<0.05$) β-hydroxyacyl-CoA-dehydrogenase (β-OH) and citrate synthase (CS) activities in diabetic rats, when compared to the non-treated animal (β-OH: G3=173,44±8,75; G4=140,06±7,85 nmol/mg; CS: G3=164,52±20,44; G4=137,50±16,61 □mol/g tissue). RSV increased of the activity pyruvate dehydrogenase (G4=107,46±14,47 nmol/mg), but had no effect on activity of the lactate dehydrogenase and mitochondrial respiratory complexes (I, II and ATP synthase) in myocardial of the diabetic rats. In conclusion the RSV normalized the activity of key enzymes of β-oxidation and citric acid cycle in animals with DM1, decreasing the oxidation of fat acids and increased utilization of carbohydrates for generation of the ATP in cardiac muscle.

P4.3-16

Phenolic antioxidants in foxtail and little millet varieties and their distinct inhibitory effects on α-glucosidase and α-amylase activities

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Phenolic extracts of four varieties each of foxtail millet (CO5, CO6, CO7 and PS4) and little millet (CO2, CO3, CO4 and TNAU13) were evaluated for phenolic contents, antioxidant capacities and α-glucosidase and α-amylase inhibitory activities. Little millet varieties contained higher total phenolic content (TPC), total flavonoid content (TFC), proanthocyanidin content (PC) and anthocyanin contents compared to foxtail millet varieties. Among foxtail millet varieties, PS4 variety contained highest amounts of TPC, TFC and anthocyanins. However, TNAU-13 variety of little millet contained higher amounts of both TPC and anthocyanins among little millet varieties. CO7 variety of foxtail millet and CO2 and CO4 varieties of little millet presented the highest DPPH scavenging activity. Millet varieties exhibited ferrous reducing antioxidant power in the range of 0.14–1.29 mg/g ascorbic acid equivalents. PS4 variety of foxtail millet (57.6%) and TNAU-13 variety of little millet (52.5%) possessed stronger inhibitory activity against α-glucosidase than other varieties. CO5and TNAU13 varieties showed higher α-amylase inhibitory activity. These studies indicated that PS4 and TNAU-13 varieties have higher content of phenolic compounds, antioxidant activity and α-glucosidase inhibitory activities. These results are helpful in effective utilization of underutilized millets as functional food ingredientsin the prevention of diabetes and its related complications.

P4.3-17

Biochemical and morphologic alterations in copper deficient bovines

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Copper deficiency is an important cattle disease, with several clinical signs due to alterations in copper-dependent enzymes. This deficit is produced by an excess of molybdenum and sulphur in the diet. These elements cause a reduction of copper absorbability. The aim of this work was to determine the effect of copper deficiency on bovine heart. Two groups of five Holstein calves were used: the control group received 9 mg of copper/kg DM in the diet, while the deficient group received 11 mg of molybdenum/kg DM and 3 g of sulphur/kg DM. After 8 months of treatment, copper levels (plasmatic, < 57 µg/dl; hepatic, < 25 ppm), indicative of deficiency,were observed. Animals were sacrificed after 12 months of treatment and myocardial samples were taken. Mitochondrial structure was evaluated using electron microscopy. Copper concentration, lipids oxidation (TBARS), cytochrome *c* oxidase (COX) and Cu,Zn-superoxide dismutase (Cu,Zn-SOD) activities were measured in myocardialsamples. Cardiac copper levels (23%), COX (27%) and Cu,Zn-SOD (38%) activities were reduced in deficient animals. Mitochondrial damage, determined as swelling, cristae and membranes disruption; and lipids oxidation (TBARS, 58%) were increased in deficient animals. Heart COX and Cu,Zn-SOD activities reduction from copper deficient bovines could lead to mitochondrial respiration impairment and damage, withan increase onoxidative damage parameters (TBARS).

P4.3-18

Green tea extract restores obesity-related dysfunction of obese rats

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We examined the effect of daily green tea extract intake on plasma obesity-related and adipocyte parameters of obese rats. Wistar male rats (150±40 g) were treated with green tea extract (500 mg/BW) by gavage, 5 days a week (12 wks). Obesity was induced by cafeteria diet for 8 wks. Experimental animals were: (i) control, (standard chow); (ii) obese (cafeteria diet); (iii) green tea; and (iv) obese + green tea. After 90 days rats were killed and plasma and adipose tissue (epipidimal, subcutaneous and retroperitoneal) were collected. We evaluated: GTT, ITT, glycemia, lipidemia, adiponectin, and lipolytic and lipogenic mRNA expression. Green tea rats reduced BW gain by 22% compared to the control and adiposity index by 23%. Obese + green tea reduced BW gain by 34% and index of adiposity by 30% as compared to the obese. GTT and ITT indicated insulin resistance in obese rats. Increased plasma levels of glucose, NEFA, adiponectin and glycerol in the obese group were reversed by green tea. Adipocytes mRNA levels of lipolytic and lipogenic proteins were decreased in obese and the supplementation with green tea restored mRNA expression. Green tea was effective in increasing mRNA expression of FABP4 and CD36 whereas decreased PPARy. Financial support: FAPESP (2011/19216-8), CNPq.

P4.3-19

Meal replacement, rich in natural antioxidants, improves the diet quality and decreases metabolic risk parameters in overweight women: a crossover study

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"Human Ration" (HR) is a mixture of ingredients rich in phytochemicals, consumed by Brazilian people for management of overweight. We investigated the effect of HR drink, compared to placebo drink, replacing breakfast, on anthropometric and metabolic parameters in women (n=22, overweight and obese), on a crossover study (two periods of five weeks and one week of washout). In both interventions was applied mild caloric restriction (15% in regardingEER). HR product was source of antioxidants compounds such as: zinc (5.5 ± 0.20 mg), manganese (6.4±0.74 mg) and vitamin E (19.84±1.45 mg). These antioxidants and total phenolic content (42.7±0.74 mg), contributed for high potential to eliminate radical DPPH$^{\bullet}$ (86.1%), comparing with white bread (39,95%), usually consumed at breakfast. HR drink decreased waist circumference (-2.54 2.74 cm, p=0.0003), and increased levels of HDL-C (3.09±6.67 mg/dL, p=0.04). Both drinks resulted in decrease in body weight and BMI. No significant changes were observed for CAT in plasma, lipid peroxidation and inflammatory biomarkers during study. Therefore, HR consumption, associated with caloric restriction is favorable, because improves the diet quality and positivelycontributesto modulate metabolic risk factors.

P4.3-20

Prebiotic effect of cranberry phenolic compounds on the prevention of metabolic disorders linked to obesityand influence of a probiotic on phenolics bioavailability

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Scope: This project aimed to study the impact of a cranberry extract, combined or not with a probiotic, on intestinal microbiota and physiological impairments on adiet-induced obesity mouse model.
Experimental design: 48 mice were fed with a high-fat high-sucrose diet for 8 weeks. They received dailya dose of cranberry extract, a probiotic, the combined treatment, or only a vehicle. Feces were collected throughout the study for microbiological analysis while blood and tissues where sampled upon sacrifice for phenolic compounds bioavailability analysisby UPLC-MS/MSand physiologicalexperiments.
Results: Cranberry phenolic compoundsdecreased food intake and body gain weight of treated mice. The two separated treatments showed a beneficial effect on adiposity. The combined treatment improved insulin sensitivity and strongly modulated the intestinal microbiota, with significant increase or decrease of specific bacterial families. The combined treatment leaded to a stimulation of microbial degradation of cranberry phenolic compounds.
Conclusion: Cranberry phenoliccompounds improved metabolic parameters in obese mice, an effect that appearsto result from interactions with gut microbiota.Indeed, we are reporting here that cranberry phenolic compounds display a prebiotic effect and that their bioavailability can be modulated by probiotics.

P4.3-21

Geraniin purified from Nephelium lappaceum (rambutan) rind as a potential insulin sensitizing agent in adipose tissue for the treatment of hyperglycaemia

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Geraniin, an ellagitannin is the major constituent in rind extracts of Nephelium lappaceum (rambutan). The compound has been credited with a range of bioactive properties including antioxidant and free radical scavenging activity, antimicrobial and antihypertensive properties and in vitro antihyperglycaemic activity. In this study, geraniin was purified from crude ethanolic extract of rambutan rind using reverse-phase C18 column chromatography and crystallization to obtain geraniin of approximately 98% purity. 3T3-L1 mouse adipocytes were utilised as a cell-based model to study the effects of geraniin (0.2-20 µM) in adipose tissue in the presence of insulin. Geraniin exhibited low cytotoxicity towards 3T3-L1 cells. The differentiation of 3T3-L1 pre-adipocytes into mature adipocytes was inhibited when the cells were treated with geraniin. Furthermore geraniin also enhanced 2-NBDG (a fluorescent glucose analogue) uptake in 3T3-L1 adipocytes. Free glycerol released from the adipocytes increased upon treatment with geraniin indicating an involvement in lipolysis. Further study on the molecular interactions and mechanism of action of geraniin in 3T3-L1 adipocytes is currently in progress. The ability of geraniin to stimulate glucose uptake in adipocytes while simultaneously inhibiting adipocyte differentiation show potential for geraniin to act as an agent for the treatment of hyperglycaemia associated with diabetes mellitus in obese individuals.

P4.3-22

(-)-Epicatechin ameliorates cardiometabolic and oxidative modifications in fructose-fed rats

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We studied the effects of (-)-epicatechin (EC) dietary supplementation on heart metabolic and oxidative modifications in a model of Metabolic Syndrome (MetS). Male Sprague-Dawley rats were divided in three groups and received for 8 w: i) control diet + tap water (C), ii) control diet + 10%(w/v) fructose in drinking water (F), and iii) control diet supplemented with EC (20 mg/kg BW/d)+ 10%(w/v) fructose in drinking water (FEC). Systolic blood pressure, triglycerides and cholesterol were higher in F ($p<0.05$) compared to C and FEC. Regarding oxidative alterations, GSSG/GHS^2 index was lower in F respect to C and FEC ($p<0.05$). FEC nitric oxide synthase activity was higher ($p<0.01$) than in F and C. Increased superoxide production with higher expression of NOX4 and p47^{phox} proteins were observed in F ($p<0.05$) compared to C and FEC. Superoxide dismutase and glutathione peroxidase activities in F were lower than in C and FEC ($p<0.05$). Mitochondrial Mn-SOD activity and expression were significantly higher in F compared to C and FEC. In conclusion, EC prevented the effects observed in the heart of fructose fed-rats, and these effects could be related to a decrease in the oxidative stress evidenced in MetS.

P4.3-23

Postprandial metabolic changes induced by consumption of barbecue and phenolic compounds from açai

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Barbecuing meat over a direct flame leads the production of polycyclic aromatic hydrocarbons (PAHs), contaminants known to induce oxidative stress. On the other hand, phenolic compounds exhibit *in vivo* antioxidant activity. The objectives of the study were to evaluate metabolic changes after barbecue intake associated or not to phenolic compounds from açai (*Euterpe oleracea*), in comparison with a control meal. Twenty-three healthy subjects were selected and randomly assigned to three different sequences of test-meals (crossover design): barbecue associated with phenolic compound-rich capsules (450 mg gallic acid equivalents/g of powder; 160 mg of anthocyanins/g of powder) from açai; barbecue associated with placebo and oven cooked meat associated with placebo. Blood was collected at baseline, 3 and 5 hours after the meal intake. PAHs in meat samples were analyzed by HPLC/FLD. Barbecue showed significantly higher PAH levels than oven cooked meat. Blood changes of TBARS, hs-CRP, ALP, GPT, GOT were not significantly different between the test-meals. GSH-Px tended to decrease with barbecue consumption (-17.71 ± 27.37%), and to increase with phenolic compound intake (67.82 ± 124.58 %), at 3h post-ingestion. Results suggest that barbecue intake leads to an antioxidant status impairment, which may be attenuated by phenolic compound intake.

P4.3-24

Adipose tissue modifications during endotoxemia: modulation by dietary (-)-epicatechin

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Adipose tissue is a key player in inflammation and its dysregulation is responsible for deleterious effects on several organs. In this work we evaluated the effects of dietary (-)-epicatechin (E) on parameters associated to global and local nitric oxide (NO) production and oxidative modifications in white adipose epididymal tissue (EWAT) in rats subjected to endotoxemia. Male Sprague-Dawley rats were fed on a diet enriched with E (ca. 100 mg/kg bw) during 4 d. After this pretreatment, lipopolysaccharide (LPS, 4 mg/kg) was ip injected to produce acute endotoxemia, and animals were sacrificed 6 h after LPS administration. The 4 experimental groups were: Control (C), Control+E (CE), LPS (L) and LPS + E (LE). The increase in NO level in blood after LPS-challenge, evaluated by electronic paramagnetic resonance, was significantly higher in LE than in L. In EWAT from LE there were significant attenuations in the increases observed in TBARS and NADPH-dependent SOD inhibitable superoxide anion production in L. The same effect of E was found in the expression of inducible-NO synthase (iNOS). These results suggest that dietary E should be able to attenuate the EWAT dysregulation in terms of oxidative metabolism and iNOS induction during the onset of acute endotoxemia.Supported by: UBACYT 20020100100659 and 20020120100177, and ANPCyT PICT-2012-0765.

P4.4-01

Cyanidin-3-glucoside inhibits pro-inflammatory mediators expression in human intestinal cells by down-regulating STAT1 activation: a comparative study with 5-aminosalicylic acid

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Cyanidin-3-glucoside (C3G) is a typical anthocyaninwidespread in fruits and vegetables used in human diet. Anthocyanins exert different biological effects, including anti-inflammatory activity by interfering with cellular signaling pathways and enzymatic reactions, which are up-regulated in inflammatory diseases such as Inflammatory Bowel Disease (IBD). The aim of this study was to assess the protection afforded by C3G against cytokine-induced inflammatory response in the human intestinal HT-29 cell line, in comparison with 5-aminosalicilic acid (5-ASA), a well-known anti-inflammatory drug currently used in IBD. HT-29 cells were pretreated with 25 µM C3G and/or 500 µM 5-ASA and then stimulated with a mixture of inflammatory cytokines for a certain period of time. Some inflammatory mediators and inflammatory enzymes were evaluated. Cyanidin-3-glucoside reduced cytokine-induced inflammation in intestinal cells, in terms of NO, PGE_2 and IL-8 production and of iNOS and COX-2 expressions, at a much lower concentration than 5ASA, suggesting a higher anti-inflammatory efficiency. Interestingly, C3G and 5-ASA neither prevented IkB-α degradation nor the activation of NF-kB, but significantly reduced cytokine-induced levels of activated STAT1 accumulated in the nucleus of HT-29 cells. These data suggest that C3G may be promising at least as an anti-inflammatory nutraceutical in the context of IBD. This work was supported by the grants PTDC/SAU-OSM/102907/2008 and PEst-C/SAU/LA0001/2013-2014 funded by FCT and FEDER/COMPETE. D. Serra is a fellowship recipient from FCT (SFRH/BD/75418/2010).

P4.4-02

Comparative effects of A- and B-type proanthocyanidins in the prevention of urinary tract infection in mice

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Consumption of cranberry (*Vaccinium macrocarpum*) is widely recommended forprophylaxis against urinary tract infections (UTI) in women. Among cranberry components, A-type proanthocyanins would be implicated in these preventive effects against UTI. However, proanthocyanidins are poorly absorbed in the small intestine, but subjected to extensive biotransformation in the colon, although studies are almost restricted to B-type proanthocyanidins. Therefore, the hypothesis of this study is that urinary metabolome from of A-type and B-type proanthocyanidins-mainly derived from their colonic catabolism-differ,and only metabolites from the A-type procyanidins have protective effects against UTI. To test this hypothesis, JAXc3H/OuJ female mice previously fed with specific diet (control, 1% cranberry extract and 1% grape seed extract) for 2 weeks, were inoculated with the uropathogenic *E. coli* (ATCC 53503™) to provoke infection, and maintained 2 weeks more before being sacrificed. Urine samples were collected at different times and subjected to *E.coli* counting, leukocytary esterase and nitrites analyses, and mieloperoxidase task. Samples of kidney and bladder tissues were also collected for *E. coli* counting and histopathologic analysis. Additionally, the capacity of the urine samples to inhibit bacterial adherence was tested in the T24 bladder cell line (ATCC HTB4 ™).

P4.4-03

Preventive effect of grape pomace extracts on dextran sodium sulfate (DSS)-induced colitis in rats

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Several studies have shown that polyphenols may exertbeneficial effects on inflammatory bowel diseases. This study aimed to compare the preventive effects of a dietsupplementation with three polyphenol rich grape pomace extracts (GPEs) on DSS-induced colitis in rats. GPEsare from two grape varieties (Alicante or Pinot) and two extraction processes (classic: C or patented: P). Rats were divided into 5 groups (n=8) and fed for 21 days with a semi-synthetic diet (control and DSS groups) or the same diet enriched with GPE (Alicante-C, Alicante-P and Pinot-C groups). Colonic inflammation was induced by DSS administration in drinking water (4% w/v) during the last 7days of experimentation. Animal weight, colonic tissue damage, inflammatory (myeloperoxidase (MPO) activity and cytokine levels) and antioxidant (superoxide dismutase (SOD) activity) status and gene expression (qRT-PCR) of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) were evaluated. Compared to DSS-treated group, Alicante GPEs limited body weight loss and histological lesions. Alicante-P and Pinot-C decreased MPO activity and Alicante-P increased colonic SOD activity. All GPEs counteracted the colonic increase of pro-inflammatory cytokine levels. GPEs down-regulated COX-2 (Alicante-P) and iNOS (Alicante-C and Alicante-P) gene expression. Thus, preventive GPE diet supplementation offered protection against DSS-induced colitis in rats.

P4.4-04

Influence of consumption of anthocyanin purified extract from açai (*Euterpe oleracea*) in the short- chain fatty acids profile in male wistar rats

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The purpose was to determine whether consumption of anthocyanin rich extract from acai would influence the gastrointestinal short-chain fatty acids bacterial production as a protective factor in colon carcinogenesis model (DMH). Was used a purified extract from açai (16,413mg/100g of anthocyanins). The rats were divided into 8 groups (6 animals/ treatment) during 28 days. The acai extract doses used were 30, 50 and 180mg/kg and combined treatment with DMH (80 mg/Kg)and control groups. For the analysis of SCFA'scaecal content was acidified with 40% m-phosphoric acid for GC/FID analysis using Nukol™ Capillary Column, Split mode (1:50) at 220°C and SCFA analytical standard mixture (46975-U Supelco®).It was detected statistical significance in the increase of production for the acids acetic, propionic and caproic in the all groups that fed with the acai extract ($p < 0.001$). A production of isobutiric was detected only in these groups and an increase was observed ($p=0.003$) in the group with combined treatment DMH and extract high level dose. There was a significantly increase for butyric only for the extract high level dose group ($p < 0.001$).This demonstrate that the consumption of acai extract stimulated the SCFA production, even in colon cancer model groups.

P4.4-05

Dietary polyphenols induce the production of NO and ethyl nitrite in the human gut: ethanol nitrosation as a novel signaling pathway with implications for gastric motility

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Nitric oxide (NO), anubiquitous molecule involved in a plethora of signaling pathways, is produced from dietary nitrate in the gut through the so-called nitrate-nitrite-NO pathway. In the stomach, dietary polyphenols have been shown to induce nitrite reduction to NO that, given its diffusional properties, diffuses towards the gastric muscular layer, inducing smooth muscle relaxation. Herein we show that polyphenols also modulate the network of reactions involving nitrogen oxides in the stomach by promoting nitrosation of ethanol from alcoholic beverages. Hence, we show that ethyl nitrite, a potent vasodilator, is produced in the human stomach upon the consumption of lettuce and red wine. Although polyphenols decrease the yields of nitrosation *in vitro* when compared with beverages without phenolics, we provide the proof of concept that after a meal containing nitrate, polyphenols and ethanol, ethyl nitrite is produced in the stomach. Moreover, we demonstrate that, at physiological pH, ethyl nitrite induces gastric smooth muscle relaxation through a cGMP-dependent pathway. Overall, these results suggest that ethyl nitrite is formed at acidic pH, acquiring the ability to release NO at physiological pH thereby modulating gastric motility. This data highlights new signaling properties of dietary polyphenols in the gut with connection to NO biology. This work was supported by Foundation for Science and Technology (Portugal) through the grant PTDC/AGR-ALI/115744/2009.

P4.5-01

Molecular basis of the effects of flavonoids on neuronal functions

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Flavonoids have been recognized as promising agents capable of influencing different aspects of synaptic plasticity resulting in improvements in memory and learning in both animals and humans. In particular, we have demonstrated that chronic interventions with flavonoid-rich foods are able not only to reverse age-related impairments in memory in aged rats but are also effective at improving learning in young healthy rats. Furthermore, in both young and aged models, the improvements in hippocampal dependent learning are accompanied by activation of ERK/CREB/BDNF and Akt/mTOR/Arc pathways, which are critical in memory formation. We have additionally shown that pure flavanols and anthocyanins are both effective at modulating memory formation and hippocampal BDNF levels in aged animals, strongly suggesting that flavonoids are the active components driving the beneficial effects of flavonoid-rich foods in brain function. Most recently, we have identified new important molecular targets for the action of flavonoids in the brain, in particular the polysialylated form of the neural adhesion molecule (PSA-NCAM), which has an important-role in activity-dependent changes in synapse strength. The additional increase in hippocampal NR2B-containing NMDA receptors suggests an enhancement of glutamate signalling following flavonoid feeding. Through these mechanisms, the consumption of flavonoid-rich foods throughout life holds the potential to limit neurodegeneration and to prevent or reverse age-dependent loses in cognitive performance.

P4.5-02

Methods to alter the natural metabolism of resveratrol and effects on cerebral blood flow, cognitive performance, and subjective health, mood and sleep in healthy, young humans

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The natural metabolism of resveratrol results in quick excretion and poor bioavailability in human plasma. This lab has conducted 2 studies which attempt to alter natural resveratrol metabolism, to increase bioavailability, and to assess the effects of this on cerebral blood flow (CBF) and behavioural outcomes in healthy humans. Study 1 co-supplemented 250mg resveratrol with 20mg of the bioenhancer piperine and demonstrated increased efficacy on CBF but no significant alteration in plasma levels or cognition/mood. Study 2 investigated whether repeated dosing of resveratrol (500mg daily, for 28-days) could inculcate increased plasma levels and improve CBF, health, mood, sleep and cognition/mood. Here resveratrol demonstrated acute CBF effects, attenuated fatigue across the entire 28-days, and suggests that cumulative plasma resveratrol levels can be achieved by chronic consumption. These studies are the first to attempt to modulate resveratrol metabolism *in vivo* and to ascertain the effects in healthy humans.

P4.5-03

The effects of single doses of resveratrol on cognitive function and transient mood in healthy young adults

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Previous research from our own laboratory has demonstrated that single doses of resveratrol can increase cerebral blood-flow in the frontal cortex. However,these hemodynamic effects were not accompanied by any modulation of task performance. This disjunction between physiological and psychological factors may be related to the cognitive tasks utilised, the sample sizes employed, or the physical constraints of concomitantly using near-infrared spectroscopy. The current study therefore assessed the cognitive effects of a single dose of resveratrol in a larger sample, utilising a broader range of cognitive tasks without any NIRS measurements.
In this double-blind, placebo-controlled, cross-over experiment 50 healthy young participants received 500 mg resveratrol or placebo on separate days 7-days apart. Attention, executive function, working memory, secondary memory and transient mood were assessed pre-dose and at 40, 150, 240 and 360 minutes post-dose utilising a comprehensive range of computerised cognitive tasks and the Bond-Lader mood scales.
Following resveratrol participants rated themselves as significantly less 'alert', but more 'content' and 'calm' on the Bond-Lader mood scales. There were no effects on cognitive performance.These results confirm a lack of benefits to cognitive function seen previously, but suggest a modulation of transient mood states following single doses of resveratrol.

P4.5-04

Quercetin liposomes acutely stabilize multisystemic failure on a perinatal asphyxia model in newborn piglets

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Perinatal asphyxia is a main cause of neonatal mortality. Although hypothermia has demonstrated to decrease mortality it did not show long-term neurological change, being the search of new therapies a big concern. Quercetin is a ubiquitous flavonoid that has shown to be neuroprotective in neuronal cultures and in rat´s brain after an ischemic episode. When administered intravenously in a liposomal preparation in a model of asphyxia in newborn piglets, it showed good plasmatic and brain bioavailability. In anesthetized and mechanical ventilated newborn piglets, we induced severe asphyxia demonstrated by a voltage lower than 7µV, measured by the amplitude-integrated electroencephalogram after breathing 8% of oxygen. Another newborn group was treated with 10 mg/kg of quercetin liposomes during the reanimation period. The hypoxia + quercetin group, monitorized during 8 h, significantly improved systemic arterial blood pressure, decreased oxygen requirements and improved brain voltage. The use of quercetin after a severe hypoxic insult can acutely improve functional parameters indicating better lung, circulatory and brain conditions, highlighting key functional aspects in this multisystemic pathological condition. These studies are part of the preclinical assays previous to scaling-up this preparation, towards a future evaluation in human asphyctic pathologies in an effect likely synergistic with hypothermia.

P4.5-05

Effects of polyphenol-rich extracts from plants on age-related cognitive decline

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During aging, cognitive deficits are commonly observed associated with synaptic plasticity impairments. Recent studies have highlighted the beneficial role of nutrition to prevent this decline and polyphenols have recently been identified as potential functional food candidates. They could indeed potentiate the signaling pathways of synaptic plasticity and learning and memory performances.
This study aims at investigating the effects of a polyphenol-rich plant extract supplementation on hippocampal-dependent learning and memory, and the neurobiological mechanisms underlying these effects.
The memory deficits of aged mice have been first highlighted in the Morris water maze task, dependent on the hippocampus, particularly affected with aging. In order to assess the effectiveness of nutritional polyphenols to delay the occurrence of age-related cognitive decline, young and aged mice have been fed a polyphenol-enriched diet for 6 or 12 weeks. Our results show that polyphenols are able to reverse age-induced learning deficits and to improve memory retention. We have further investigated the expression of gene encoding proteins involved either in synaptic plasticity (BDNF, GAP43, RC3) or in oxidative stress (SOD1, Nrf2) by RT-qPCR.
Our results suggest that supplementation with polyphenols could thus be a potential nutritional way to prevent age-induced cognitive decline.

P4.5-06

Guaraná (*Paullinia cupana* Mart.) prevents beta-amyloid aggregation, advanced glycation end products formation, and acrolein-induced cytotoxicity on human neuronal-like SH-SY5Y cells

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A number of polyphenolsfound in plants extracts have been shown toinhibit the aggregationof amyloid-β peptide (Aβ), to act as scavengers of Advanced Glycation End-Products (AGEs), andALEs (lipid peroxidation ends-products)such as Acrolein (ACR). The aims of the present study were to evaluate the potential effects of guarana against AGEs and ACR-induced toxicity on SHSY-5Y neuronal-like cells, Aβ aggregation, and protein glycation. *In vitro* toxicity was evaluated by the sulphorodamine B assay, intracellular ROS production was determined by using the 2,7 dichlorofluorescein (DCFDA), as well as Bovine Serum Albumin (BSA) glycation assay for sugars (glucose/fructose) and AGEs (glyoxal and methylglyoxal). Aβ aggregation experiments were performed by utilizing the Thioflavin T assay. Our results demonstrate that guaraná extract is able to prevent AGEs and sugars-mediated proteinglycation, ACR-induced ROS production and cytotoxicity, as well as Aßpeptide aggregation. Since these are typical Alzheimer's disease hallmarks, we postulate about the future therapeutic potential of guaraná in this pathological context.

P4.5-07

Sex-dependent changes in the level of reactive species in phenols and brain regions of mice treated with plant extracts

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Neurodegenerative processes and oxidative stress underlie the development of chronic diseases that present with dysfunction of central nervous system (CNS). Objective: To determine the neuroprotective effect of consumption of Argentine plants of plant extracts with antioxidant activity. We analyzed tissue levels of total phenols (TP), hydroperoxides (HP) and superoxide anion (SO) by colorimetric techniques in different brain regions (weight), comparing the effects of *L. grisebachii* (LG), *I. paraguariensis* (IP) and A quebracho-blanco (AQB) with $p < 0.05$ (ANOVA). We found a moderate increase telencephalon TP, AQB female and male and IP, while the TP in the cerebellum LG and IP were significantly lower than controls (C) in females. In midbrain only found higher levels of TP with AQB. There were no significant changes in the rest of the brainstem. Found only in the telencephalon increased SO AQB and IP females. In telencephalon AQB only HP levels remained similar to C in both sexes, HP lipid lowering in males, while the other extracts showed antioxidant activity. There were similar trends in cerebellum and brain stem, while AQB increased in diencephalon HP aqueous feminine and reduced in males. AQB and LG were associated with increased hydroperoxidation midbrain. Because the results are encouraging but inconclusive, further research is required in this regard.

P4.5-08

Effects of epicatechin on reference spatial memory in the aged mouse

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Cognitive decline is observed with ageing, which particularly affects the hippocampus. Among the polyphenols proposed as functional food, epicatechin, a protoanthocyanidin, has recently been shown to improve spatial memory of running mice and to reverse the learning and memory deficits in a transgenic model of Alzheimer's disease.

Here, we focused on the effects of epicatechin on the reference spatial memory of aged mice. Indeed, young and aged mice, supplemented or not with epicatechin (2.5 mg/day), were trained in the spatial version of the Morris water maze and submitted to two probe tests: 48h and 2 months later. Subsequently, mice were trained to locate a new position of the platform and submitted to another probe test.

While spatial learning and memory deficits could be observed in aged mice, neither young nor aged supplemented mice were able to perform better than their respective control mice. Besides, young epicatechin-supplemented mice could not adapt to the new location of the platform as fast as young control mice, suggesting a lack of behavioral flexibility as in aged mice.

Thus, our results suggest that epicatechin supplementation may not be sufficient to reverse age-induced spatial learning and memory deficits but may still alter learning and memory processes.

P4.5-09

Protective effect of *Piper aduncum* in an experimental model in vitro of β-amyloid-induced neurodegeneration

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Oxidative stress is considered in the pathogenesis of neurodegenerative process such as Alzheimer's disease, characterized by the development of neuritic plates (amyloid-β peptide, Aβ) in areas of the brain confined to memory and cognition. There is a wide report that Aβ alters the homeostasis of neuronal calcium, oxidative damageand disruption of neuronal homeostasis.

Objetive was evaluating the neuroprotective effects of ethanolic extracts of *Piper aduncum*. In addition, damage was induced with $A\beta_{1-42}$ and NMDA to cultured cells. So hippocampal cells were treated with $A\beta_{1-42}$ 1 µM and expressions of NMDA receptors in synapses were evaluated and intracellular calcium influx (overestimulation with NMDA) was registered in treatments with *P. aduncum*. In the neuroprotector evaluation, the results revealed a cytoprotector effect (concentration of 20 mg/ml of *P. aduncum*); there is one increase of 9.6% of NR1 and recovery of 20.86% of SV2 proteins over control. Besides, there is a reduction of more than 50% of cellular calcium. These results demonstrate neuroprotector effects of *P. aduncum* for the studied models. However, more detailed in *vivo* and *in vitro* studies are needed to establish the specific activities of *Piper aduncum.* Supported by: CONCYTEC, UNMSM (Lima – Perú).

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