

October 15 - 17, 2006  
Karlsruhe, Germany

Ernährungstage



*10<sup>th</sup> Karlsruhe Nutrition Congress*

**Final Programme  
and Abstracts**

# **HEALTH ASPECTS OF VEGETABLES AND FRUITS:**

## **Scientific Evidence for “5-a-day”**



Federal Ministry  
of Food, Agriculture and  
Consumer Protection

**BfEL** 



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**World Health  
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## **Contents**

- I. Programme
- II. Proceedings
- III. List of participants
- IV. Notes

# **10<sup>th</sup> Karlsruhe Nutrition Congress**

15<sup>th</sup> - 17<sup>th</sup> October 2006

## **Health Aspects of Vegetables and Fruits: Scientific Evidence for “5-a-day”**

### **Programme**

Sunday, 15<sup>th</sup> October 2006

16:00 Opening and Welcome

***B. Kühnle (Federal Ministry of Food, Agriculture and Consumer Protection, GER)***

***B. Watzl (Federal Research Centre for Nutrition and Food, GER)***

#### **Opening Lecture:**

**Chair: B. Watzl, GER, and U. Oltersdorf, GER**

16:30 From ‘weak food’ to vital food: The changing attitudes towards fruits and vegetables in 19<sup>th</sup> and 20<sup>th</sup> century Germany

***U. Spiekermann, GER***

#### **Session 1: Epidemiology I**

**Chair: B. Watzl, GER, and U. Oltersdorf, GER**

17:20 Fruit and vegetable consumption and risk of cardiovascular diseases

***L. A. Bazzano, USA***

18:00 Vegetables and fruits: The key to diabetes prevention

***M. B. Schulze, GER***

18:40 *Welcome Reception*

Monday, 16<sup>th</sup> October 2006

**Session 1:        Epidemiology II**

**Chair.            B. Watzl, GER, and U. Oltersdorf, GER**

09:00            Vegetables, fruits and cancer prevention: The EPIC-Study  
**H. Boeing, GER**

09:40            Design, mechanism, and measurement: Improving epidemiologic  
studies of fruits, vegetables and cancer risk  
**A. R. Kristal, USA**

10:20            Vegetables and fruits for cancer prevention: The nutrigenetic  
approach  
**C. M. Ulrich, USA**

11:00 - 11:20        *Coffee Break*

**Session 2:        Human Intervention Studies**

**Chair:            C. Rüfer, GER, and A. Bub, GER**

11:20            Benefit-risk assessment of vegetable and fruit consumption  
**G. Rechkemmer, GER**

12:00            Modulation of disease biomarkers by vegetables and fruits  
**A. Bub, GER**

12:40 - 14:00        *Lunch Break*

14:00            Vegetables and fruits: The colourful way to blood pressure reduction  
**M. A. Martínez-González, ESP**

14:40            The role of fruit and vegetables in maintaining healthy bones  
**H. M. Macdonald, UK**

15:20 - 15:40        *Coffee Break*

15:40 **Poster viewing (guided tour)**

**Poster Session 1: Plant food: Constituents, processing and quality**

S. Kulling, GER

**Poster Session 2: Biological effects in vitro**

D. Marko, GER

**Poster Session 3: Animal and human intervention studies: Biological effects**

C. Gerhäuser, GER

**Poster Session 4: Consumer research and epidemiology**

C. Brombach, GER

17:30 *Departure time Social Event*

18:30 *Social Event*

Tuesday, 17<sup>th</sup> October 2006

**Session 3: Mechanisms of risk reduction**

**Chair: K. Briviba, GER, and G. Rechkemmer, GER**

08:30 Brassica vegetables and cancers: Molecular mechanisms underlying the observed risk reduction

**R. F. Mithen, UK**

09:10 Cancer-preventive mechanisms of apples

**S. W. Barth, GER**

09:50 - 10:10 *Coffee Break*

10:10 Quercetin: Tissue distribution and in vivo bioactivity

**J. Keijer, NL**

10:50 Vegetables and fruits: Implications for vascular protection

**V. B. Schini-Kerth, F**

11:30 - 13:00 *Lunch Break*

**Session 4: Programmes to increase the intake of vegetables and fruits**

**Chair: U. Schlemmer, GER, and J. Brug, NL**

13:00 The Pro Children Study: The development and evaluation of school-based fruit and vegetable promotion across Europe

**J. Brug, NL**

13:40 The '6-a-Day Campaign' in Denmark and the intake of vegetables and fruits in the Danish population

**E. Trolle, DK**

14:20 - 14:40 *Coffee Break*

- 14:40 WHO programmes on fruit and vegetable promotion  
***U. Trübwasser, DK***
- 15:20 National Nutrition Survey II: Vegetables and fruit in German diets  
***C. Brombach, GER***
- 16:00 Concluding Remarks  
***B. Watzl (Federal Research Centre for Nutrition and Food, GER)***

## **Oral presentations**

## **From 'weak food' to vital food: The changing attitudes towards fruits and vegetables in 19th and 20th century Germany**

**U. Spiekermann**

University of Göttingen, Göttingen, Germany

In the early 19<sup>th</sup> century fruits and vegetables only played a minor role both in health advices and food patterns in Germany. Transport of fresh products was complicated and expensive, conservation was homebound and focused mainly on a small range of items, such as dried apples and preserved cabbage. Beside local and seasonal production, fruits and vegetables were expensive luxury goods, well known on the tables of nobility and burghers, but not by ordinary peasants and workers. Doctors were not really interested in these foodstuffs, which had no relevance for the dietetics in early hospital meals. And early nutritional science, dominated by the work of Justus Liebig and his followers, was focused on animal protein primarily – while the nutritional value of fruits and vegetables was reduced to minerals (“ash”).

It was not before the middle of the 19<sup>th</sup> century, when fruits and vegetables got growing relevance for the daily diet. Market-orientated agriculture widened the area under cultivation and provided quickly growing urban markets. Water and railway transport, canning and improved drying technology were decisive for a growing consumption, which included even some tropical fruits. While breeding research was established and institutionalized since the late 19<sup>th</sup> century, for nutritional science fruits and vegetables were only a 'weak' side-dish. Although attacked by the vegetarian movement and many dietetic doctors, the supremacy of animal protein as a superior nutrient remained unchallenged, because it was backed by the everyday believe of protein as the main source of strength, vitality and masculinity. These attitudes were seriously hit by the detection of vitamins in 1911. Although it took more than a decade before German scientists accepted the existence of this new group of nutrients, the effect in public was remarkable. Fruits and vegetables became fashionable foodstuffs, because vitamins and mineral nutrients became symbols of health. Nutritionists followed and gave evidence to this public perception. The result can be seen in the first international food recommendations of the League of Nations in the mid-1930s, which build the background for the high impact of fruits and vegetables in the rationing system of World War II. This development was supported by intensified marketing of German agriculture to consume German products and effective health promotion campaigns in favour of fresh and seasonal diet, including relevant proportions of vegetables and fruits.

After World War II we find a shift back to “modern” eating patterns, including more meat, butter, etc. But this development was accompanied mainly by a reduced consumption of staple foods, such as potatoes and bread, while vegetables and especially fruits became more important. This was not only the result of ongoing scientific advice, but mainly a consequence of price reduction and intensified imports of tropical fruits, caused by the reconstruction of international trade relations. New technologies (freezing) and product innovations (fruit juices) backed this change. On the other side we find new risk debates since the early 1950s, focused on the effects of pesticides, preservatives, radioactivity, wrapping, international transport and monocultures. Chemical and physiological studies were intensified, but nutritional science failed to analyse relevant social and environmental patterns. Contemporary health promotion is dominantly based on the

evidence of natural sciences, while the contribution of economic and social, environmental and cultural sciences is neglected. Instead, the history of fruits and vegetables in 19<sup>th</sup> and 20<sup>th</sup> century Germany shows, that the dominant forces of changing eating patterns were on the one hand economic and social one, on the other hand a kind of common sense belief of “healthiness” in public. If we want to improve public health it is not sufficient – although indispensable – to look for scientific evidence on the biological, medical or epidemiological level, but we have to integrate scientific evidence of environmental, social and cultural studies.

## **Fruit and vegetable consumption and risk of cardiovascular diseases**

**L. A. Bazzano**

Tulane University, New Orleans, Louisiana, USA

Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality world-wide. The health benefits of a diet rich in fruits, vegetables, and legumes, have been recognized for some time, however, epidemiologic investigations have only recently begun to clarify the role of specific foods and food groups in the development and management of CVD. Evidence suggests that persons who consume more fruits and vegetables often have lower prevalence of important risk factors for CVD, including hypertension, obesity, and type 2 diabetes mellitus. In addition, recent large prospective studies have shown a direct association of higher levels of fruit and vegetable consumption with lower risks of stroke and coronary heart disease. Particular types of fruit and vegetable consumption have also been shown to have distinctive benefits. For example, legume consumption has been shown to decrease blood cholesterol levels, and has been associated with a decreased risk of developing coronary heart disease. The mechanisms by which fruits vegetables and legumes exert these effects may involve not only known nutrients and phytochemicals such as fibre, potassium, and folate, but also functional aspects of fruits and vegetables such as their low glycemic load and energy density. Based on available evidence and given the enormous potential for benefit, greater efforts and resources are needed to support dietary changes that encourage increased fruit and vegetable consumption.

## **Vegetables and fruits: The key to diabetes prevention**

**M. B. Schulze**

Department of Epidemiology, German Institute of Human Nutrition, Nuthetal, Germany

Diabetes mellitus is a group of metabolic diseases whose common feature is an elevated blood glucose level resulting from defects in insulin secretion, insulin action, or both. Type 2 diabetes mellitus accounts for 90%–95% of all diabetes cases, and it develops when the production of insulin is insufficient to overcome the underlying abnormality of increased resistance to its action. Diabetes is associated with serious health consequences, particularly coronary heart disease and stroke as well as microvascular complications, putting an enormous economic burden on societies. Because there is no cure for diabetes, primary prevention through diet and lifestyle modification is of paramount importance.

It has convincingly been shown by intervention studies that the development of type 2 diabetes can largely be prevented or postponed by lifestyle modifications (Pan et al. 1997, Tuomilehto et al., 2001; Knowler et al., 2002). Although a higher fruit and vegetable consumption has been a component of some intervention diets, these studies are multifactorial studies making it impossible to separate the effects of fruits and vegetables from those of other dietary characteristics or physical activity patterns. Several prospective observational studies have evaluated whether the intake of fruits and vegetables is associated with the risk of developing type 2 diabetes. A higher consumption of F&V was associated with lower diabetes risk among women in the National Health and Nutrition Examination Survey (Ford & Mokdad 2001), although not among men. Women who consumed  $\geq$  5 portions per day had a RR of 0.61 (95 % CI: 0.42-0.88) compared to women who did not consume F&V. In a Finnish cohort study, a higher intake of fruits and berries (>136 g/d compared to <33 g) was associated with a reduced diabetes risk (RR: 0.69, 95 % CI: 0.51-0.92). However, vegetable intake was not significantly associated with risk in this study (Montonen et al. 2005). In contrast to these studies, other studies did not observe significant associations between the intake of fruits and/or vegetables and the risk of type 2 diabetes (Lundgren et al. 1989, Feskens et al. 1991, Colditz et al. 1992, Meyer et al. 2000, Liu et al. 2004). Studies which have evaluated the association between fruit or vegetable fiber consumption and diabetes risk have generally not found a relation. The majority of studies therefore support that the intake of fruits and vegetables is probably not related to diabetes risk.

However, estimates of relative risk have generally been adjusted for BMI in cohort studies, because it is impossible to separate effects of dietary intake on body weight from potentially confounding effects of body weight in these studies. This might have led to an underestimation of the association between fruit and vegetable consumption and diabetes risk by controlling for a major mediating factor by which consumption could be related to risk.

## **Vegetables, fruits and cancer prevention: The EPIC-Study**

### **H. Boeing**

Department of Epidemiology, German Institute of Human Nutrition, Nuthetal, Germany

In 1981, the impact of nutrition on cancer death in the US was estimated to be around 35%. This estimate based on descriptive epidemiology by comparing the diet in different regions in the world and cancer risk of groups with specific diets. At that time, the prime dietary variable explaining cancer occurrence was fat intake. Ten years later, fruit and vegetables appeared to be the driving dietary factor for cancer risk and prevention, mainly due to results from case-control studies. Nowadays, with the incoming data from prospective cohort studies, neither dietary fat nor fruits and vegetables appeared to be related to cancer risk. The most obvious explanation for the phenomenon that dietary factors appears and disappears overtime to be related to cancer risk relates to the study designs being used in each time period. Within the hierarchy of generating evidence, the design of cohort studies are considered as being superior to the case-control design. However, we also have to acknowledge that about 3 generations were studied with different designs over the last decades. Case-control studies in the 1980ies investigated those born around 1920, whereas the current data from cohort studies refer those born between 1930 and 1950. It is obvious that supply of dietary factors during life differed between these generations and might be partly responsible - aside the study design issue - for divergent results regarding cancer risk.

The largest initiative to study the war and post war generation regarding diet and cancer risk in Europe is the EPIC-Study. This study recruited about 520,000 study participants of both genders and preferentially within the age range 35 to 65 during 1992 and 1998 in 10 European countries, spanning from South to North of Europe, for prospective investigation of the link between diet and chronic diseases, particular cancer. This study is using new approaches in dietary assessment by combining two assessment instruments and includes the use of biomarkers and genetic parameters. The food group fruits and vegetables and their subgroups were among the first variables which were studied for various cancer sites. EPIC could show that risk of hormonal dependent cancer sites as breast and prostate does not depend on intake of fruits and vegetables. Also risk of kidney and ovarian cancer was not changing with intake of fruits and vegetables but associations with particular subgroups were noted (Kidney: root vegetables, ovary: allium vegetables). No specific effect of intake of fruits and vegetables was also seen for stomach cancer. Intake of fruits was associated with decreased risk of lung cancer and intake of fruits and vegetables with decreased risk of cancer of the upper aero-digestive tract. Non-linear relationships were observed for cancer of the upper aero-digestive tract, kidney and ovary in that low intake was associated with risk but not medium and high intake. The analytical strategy in EPIC to study the effect of intake of fruits and vegetables is exemplified for cancers of the aero-digestive tract.

In conclusion, there is limited support from the EPIC study that intake of fruits and vegetables in middle age determines cancer risk in this generation.

## **Design, mechanism, and measurement: Improving epidemiologic studies of fruits, vegetables and cancer risk**

**A. R. Kristal**

Cancer Prevention Program, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

Over the past decade, we have learned a great deal about the optimal methods for research in diet and cancer. We have come to recognize the serious limitations of case-control studies of diet and cancer, and it is becoming clear that randomized clinical trials cannot be used to study effects of long-term diet on cancer risk. Large cohort studies are likely to be the only approach available to study directly the associations of fruits and vegetables with cancer risk in humans.

Our understanding of cancer biology has also grown substantially. We now understand many of the molecular mechanisms that underlie neoplasia. We recognize the phenotypic diversity of many cancers and the likelihood that risk factors may differ by phenotype. We are also beginning to understand how individual genetic characteristics and environmental exposures affect mechanisms such as carcinogen metabolism, protection from oxidative stress and DNA repair, and recognize that the associations of compounds in fruits and vegetables with cancer risk could differ by individuals' genetic characteristics or exposures to environment factors. Finally, there have been changes in cancer detection, which have made studies of diet and some cancers strongly confounded by use of cancer screening. Future cohort studies investigating fruits and vegetables will need to collect extensive data on both genetic and environment characteristics, use of screening, standardized clinical data such as grade and stage, and biomarkers of tumor characteristics such as estrogen receptor status.

Perhaps most controversial are growing concerns about assessments of fruit and vegetable consumption. Of many possible biomarkers of fruit and vegetable consumption, only serum carotenoids are sufficiently specific and reliable to be useful. Food frequency questionnaires (FFQs) and measures similar to FFQs have been used in every cohort study examining fruits and vegetables and cancer risk. Some researchers believe that FFQs are good measures of fruit and vegetable consumption, but there is increasing evidence that this is not the case. Correlations between FFQ-derived fruit and vegetable intake with serum carotenoids are low, ranging from a low of -0.17 to 0.4, with most observations between 0.15 and 0.25. Further, in the few studies that have directly compared the validity of FFQs with 24-hr recalls or diet records, using serum carotenoids as the criterion measure, have found that validity is higher for recalls and records. Several studies report associations of serum carotenoids with reduced cancer risk, but no association when analyses are based on FFQ-based measures of fruit and vegetable intakes. These findings are similar to two recent studies showing strong associations of dietary fat with breast cancer when intake is measured by diet record, but no association when based on FFQ, suggesting that the predictive validity of FFQs is poor. Statisticians have developed methods to adjust for error and bias in FFQs, but these approaches are not likely to be useful. We can inflate the magnitude of an association by adjusting for error, but the confidence intervals are similarly inflated. We can adjust associations for systematic bias, for example the tendency of obese persons to underreport energy intake, but because these methods can actually change statistical inference, they must be subjected to careful scrutiny. Valid

adjustments for bias can only be made using true criterion measures, which are currently limited to recovery biomarkers for energy and protein, and cannot be applied to fruits and vegetables. While further evidence is needed, it is prudent for current and new studies to use measures based on actual foods consumed, such as 24-hr recalls and food records, for dietary assessment.

Calculating fruit and vegetable intake from food recalls and records requires specialized food databases and coding techniques, however when implemented it becomes feasible to calculate not only the amount of fruits and vegetables consumed, but the classification of fruits and vegetables into categories based on their phytonutrient content. Several groups are developing both study designs and data collection methods to make using recalls and records feasible for large cohort studies. One approach is to collect and store dietary records, and subsequently analyze only those records needed to support case-cohort or nested case-control studies. Technologies are under development to support computerized self-administered 24-hour recalls, which will make multiple recalls an inexpensive and feasible approach to dietary assessment.

## **Vegetables and fruits for cancer prevention: The nutrigenetic approach**

### **C. M. Ulrich**

Fred Hutchinson Cancer Research Center, Cancer Prevention Program, and University of Washington, Department of Epidemiology, Seattle, Washington, USA

Vegetables and fruit have been associated with a reduced cancer risk in a number of epidemiologic studies. However, these foods contain numerous bioactive substances, and it is difficult to discern what specific ones may be most critical in the prevention of carcinogenesis. Nutrigenetic studies can help to identify which components of foods are causally linked to carcinogenesis. The B-vitamin folate is essential for one-carbon transfer reactions, including those related to the methylation of DNA or other substrates, and to nucleotide synthesis. Epidemiologic and experimental studies implicate low-folate intakes in elevated risk of colorectal and hematopoietic malignancies and suggest that biologic mechanisms underlying this relationship include disturbances in DNA methylation patterns or adverse effects on DNA synthesis and repair. The nutrigenetics of folate metabolism has been studied extensively and provides evidence for a causal link between this pathway and colorectal carcinogenesis. This research on genetic characteristics can help clarify whether certain individuals may benefit from higher or lower intakes of folate or nutrients relevant to folate metabolism. Studies on genetic polymorphisms can also generate hypotheses regarding possible biologic mechanisms that connect this pathway to carcinogenesis. Epidemiologic studies are now complemented by mathematical modeling approaches, which enhance our understanding of this complex pathway. This presentation will illustrate knowledge that can be gained from molecular epidemiology in the context of nutrigenetics, and the research questions that this approach can answer or raise.

## **Benefit-risk assessment of vegetable and fruit consumption**

### **G. Rechkemmer**

Technische Universitaet Muenchen, Muenchen, Germany

A high consumption of vegetables and fruit, as enforced by the 5-a-day or similar campaigns in different countries, is generally considered to be beneficial for human health and is thought to reduce the risk of several chronic diseases and of obesity, respectively. The evidence supporting these views is primarily provided by large-scale epidemiological studies. Several recent studies, however, concluded that the effects of a high consumption of vegetables and fruits, particularly in relation to the reduction of risk of certain cancers, might not be as high as earlier studies suggested. Only few long-term human dietary intervention studies investigating the relationship between vegetable and fruit consumption and the reduction in disease risks are available.

Animal studies often times have provided conflicting data and mechanistic studies using cell culture systems and molecular biology technologies (Omics) to study the effects of individual bioactive components contained in vegetables and fruits are of the times difficult to interpret. The relevance of these findings for assessing the health benefit for humans has to be discussed critically.

The debate about benefit-risk assessment of foods has recently been sparked by a report about contaminants in farmed and wild salmon and calculations of the tolerable intake of these foods based on toxicological risk assessment. While nutrition societies promote the consumption of two portions of fatty fish a week to increase the dietary intake of n-3 fatty acids, the toxicological data indicated that for some farmed salmon only one portion every other month would be safe to eat.

Furthermore the debate in Europe about a new regulation for health-related claims on foods has also initiated a discussion on the scientific assessment of benefits and risks associated with the consumption of functional foods, including natural foods like vegetables and fruits.

While the risk assessment of bioactive components and contaminants in foods, respectively, is well established and is based on internationally accepted toxicological standards and methods, the assessment of health benefits is much less rigorous and there are no commonly accepted procedures available. It is a great scientific challenge to find common measures for benefit-risk assessment of foods. This task will be tackled in a new European Commission funded activity, the BRAFO-project (acronym stands for: Benefit-Risk Assessment of Food). In this activity also natural foods will be discussed and it is the aim to develop common standards for benefit-risk assessment.

## **Modulation of disease biomarkers by vegetables and fruits**

### **A. Bub**

Federal Reserach Centre for Nutrition and Food, Institute of Nutritional Physiology,  
Haid-und-Neu-Strasse 9, 76131 Karlsruhe, Germany

Diet-related diseases such as obesity, type 2 diabetes, cardiovascular disease (CVD) and cancer are the major causes of death in Germany and other European countries. Human intervention trials are designed to causally link nutrition with disease and to support dietary prevention strategies. To find specific and valid biomarkers indicating the risk for or the stage of a diet-related disease is another goal in nutrition research. However, in most cases dietary intervention studies will not be able to study disease endpoints and surrogate markers are investigated instead. A broad collection of miscellaneous surrogate markers are currently applied to study the pathophysiology of e.g. cardiovascular diseases (CVD), cancers and other possibly diet-related diseases. In the case of CVD such markers cover the range from lipid metabolism and endothelial integrity to oxidative stress, inflammation and e.g. homocysteine. Surrogate markers for cancer risk and development also include markers of oxidative stress, inflammation and homocysteine. In the past, homocysteine has increasingly come to be seen as a CVD risk factor linking nutritional status (folate) with endothelial damage (protein *N*-homocysteinylolation), smooth muscle proliferation (DNA hypomethylation) and subsequently increased CVD risk and mortality. However, DNA hypomethylation as a consequence of low folate intake and hyperhomocysteinaemia has also been linked with increased risk for certain cancers. A further example of a common marker for CVD and cancer is oxidative DNA damage. A great body of evidence exists linking DNA oxidation causally with cancer development and today various methods are available that may be valid for oxidative DNA damage determination (8-oxodG, DNA etheno adducts, comet assay). But recently oxidative DNA damage has been shown to be elevated in the serum and myocardium of heart failure patients and this has again raised the question of surrogate marker specificity. Despite this lack of specificity, such similarities may point to general mechanisms among these diet-related diseases - as has already been shown for chronic inflammatory processes. Although specificity for a single pathophysiological entity may be weak such surrogate markers can be strong enough to demonstrate dietary intervention effects on common disease mechanisms. Future investigations will show whether the new upcoming "OMICS" technologies will add specificity to the biomarker field.

## **Vegetables and fruits: The colourful way to blood pressure reduction**

**M. A. Martínez-González**

University of Navarra, Pamplona, Spain

There is evidence that a diet rich in fruit and vegetables (F&V) reduces blood pressure. The DASH trial, the Oxford trial (John, Lancet 2002. 359, 1969-74) and, more recently, the PREDIMED trial (Estruch, Ann Intern Med 2006. 145, 1-11) have shown reductions in blood pressure with a randomised intervention using a diet rich in F&V. In addition to the reduction of blood pressure in the short-term, a high consumption of F&V may also help to prevent the occurrence of hypertension in the long-term.

In the baseline assessment of the SUN cohort (Alonso, Br J Nutr 2004. 92, 311-9), we reported a prevalence odds ratio = 0.23 (95% CI 0.10, 0.55;  $P < 0.001$ ) for high blood pressure (undiagnosed hypertension) when comparing participants in the highest quintile of both vegetable and fruit consumption versus those in the lowest quintile of both food groups (F&V), after adjusting for relevant confounders. The inverse association between F&V and high blood pressure was modified by the intake of monounsaturated fatty acids (MUFA). Thus, among participants with a higher intake of MUFA (above the median) the association between F&V consumption and undiagnosed hypertension was not so apparent and almost null, whereas among those with a lower MUFA intake (below the median) we found a stronger inverse relationship with F&V consumption. The SUN cohort is conducted in Spain, and the food habits of many participants are very close to the traditional Mediterranean food pattern characterized by high F&V consumption and high MUFA intake, whose main source is olive oil. We have previously reported an inverse association between olive oil consumption and the risk of hypertension (Alonso, Lipids 2004. 39, 1233-8).

In the prospective follow-up of the SUN cohort (5939 women and 3513 men), after assessing >35,000 person-years, we identified 459 new cases of hypertension. We found that olive oil intake modified the effect of F&V. Among participants with lower consumption of olive oil we found a hazard ratio = 0.38 (95% CI 0.16, 0.89;  $P = 0.03$ ) for physician-diagnosed hypertension when comparing participants in the highest quintile of both vegetable and fruit consumption versus those in the lowest quintile of both food groups (F&V), after adjusting for relevant confounders. However, this association was not apparent among those with a higher consumption of olive oil. The statistical significance of the test for interaction (effect modification) was  $p = 0.02$ . We conclude that a high F&V consumption is able to reduce the incidence of hypertension in the context of a diet poor in olive oil. However, our results suggest that in a Mediterranean population, when olive oil intake is high (>30 g/d), there is no apparent protection against hypertension by F&V.

## **The role of fruit and vegetables in maintaining healthy bones**

**H. M. Macdonald**

University of Aberdeen, Aberdeen, UK

Low fruit and vegetable intake is one of the top 10 selected risk factors for global mortality. It is well known that a diet low in fruit and vegetables is associated with a number of chronic diseases such as heart disease, cancer, stroke and diabetes. Only recently has a link been found between fruit and vegetable intake and stronger bones. A current hypothesis, which is gaining popularity, is that fruit and vegetables produce alkaline metabolites that balance the acidity generated when consuming a Western diet (high in protein, low in fruit and vegetables). However, there are many other constituents of fruit and vegetables that could be potentially beneficial for bones. They are a rich source of vitamin C (which is necessary for cross-linking of collagen, the major structural protein in bone), vitamin K (required for post-translational modification of osteocalcin, a protein thought to be involved in bone mineralisation), folate (which can reduce circulating homocysteine levels, a factor which has been associated with increased fracture risk in some populations). Vegetables, in particular, also contain key minerals: calcium, magnesium, manganese and silicon, which could plausibly influence bone metabolism. Phytoestrogens and flavonoids are compounds only found in plant-based foods, which have been shown to act directly on bone at a cellular level and appear to possess bone-conserving properties in animal studies.

Population-based studies in Aberdeen, involving over 3200 women have shown that nutrients associated with fruit and vegetable intake were associated with improved bone mineral density measurements (BMD) in late premenopausal women and BMD was higher with increased fruit intake in early postmenopausal women. Estimated dietary acidity was associated with greater bone resorption in this population (Macdonald et al. 2005, *Am J Clin Nutr* 81 923-33). A 2-year intervention trial involving potassium citrate has recently been completed. Women were selected from the well-characterized Aberdeen cohort and randomized to high dose potassium citrate (equivalent to 9 portions of fruit and vegetables a day), low dose potassium citrate (equivalent to 3 portions of fruit and vegetables) or placebo (n=65 in each treatment arm). Results from this trial indicate that the acid balancing mechanism is unlikely to be the reason for the beneficial effects of fruit and vegetable intake in the long-term. Further work is required to find out what constituents in fruit and vegetable are important for bone health and how many portions of fruit and vegetables are required to maintain healthy bones throughout life.

## **Brassica vegetables and cancers: Molecular mechanisms underlying the observed risk reduction**

**R. F. Mithen**

Institute of Food Research, Colney Lane, Norwich, NR4 7UA, UK

Many epidemiological studies have associated Brassica consumption with a reduction in cancer risk at several sites, including colon, lung, prostate and breast. The epidemiological evidence is supported by cell and animal studies. The main mechanism proposed for the protective effect of crucifers is the activity of isothiocyanates (ITCs) derived from the metabolism of glucosinolates that accumulate within these vegetables. ITCs are generated from glucosinolates either by the action of plant thioglucosidases known as myrosinases or, if the plant enzymes have been denatured by cooking, by the action of microbial enzymes in the colon. Several studies have suggested that the extent of anticarcinogenic activity of Brassica vegetables in humans is modulated by human GSTM1 genotype. This paper reviews the several modes of action of isothiocyanates that have been proposed upon the basis of cell and animal studies. These include the induction of phase detoxification enzymes, inhibition of cell cycle, induction of apoptosis and the inhibition of histone deacetylases. The importance of isothiocyanate concentration and exposure time in these studies is discussed, and compared to normal dietary intake of these compounds.

Results of human intervention studies will be discussed that seek to examine whether there is any evidence for these mechanism occurring in humans following normal dietary intake of Brassica vegetables. Moreover, these studies suggest potential alternative modes of anticarcinogenic activity.

## **Cancer-preventive mechanisms of apples**

**S. W. Barth**

Federal Research Centre for Nutrition and Food, Institute of Nutritional Physiology, Karlsruhe, Germany

Chemoprevention has been acknowledged as an important and practical strategy for the management of cancer. Epidemiological studies have indicated that consumption of fruits and vegetables containing bioactive phytochemicals might reduce the incidence of cancers. To support these epidemiological data and further elucidate cellular mechanisms responsible for the cancer-preventive activity, numerous *in vitro* studies have already shown the antioxidant, anti-inflammatory, antiproliferative and apoptosis-inducing activities of polyphenols derived from flavonoid-rich foods such as apples. These results improved our understanding on the mechanisms by which dietary factors may control molecular signals involved in the initiation, promotion and progression of neoplasia. However, activities observed in cell culture with supraphysiological doses of the dietary polyphenols may not be relevant for the *in vivo* status because of the intestinal and/or systemic formation of (in-)active metabolites and the limited colonic and systemic availability of polyphenols. Due to these difficulties in extrapolating data from cell culture to *in vivo* cancer-prevention the possible targets of secondary plant metabolites and the mechanisms by which they act *in vivo* are unclear.

We have recently shown that a cloudy apple juice exhibited immunomodulatory activities and further antigenotoxic, antiproliferative properties in colonocytes and significantly reduced preneoplastic lesions in a rat model developed to study colon cancer (Barth et al., 2005. *Carcinogenesis* 26, 1414-21). Further these effects are not mediated by tumor-associated genes (COX-2, iNOS) or MAPK pathways. As based on these observations we have further identified the apple polyphenols and the heterogeneous cloud fraction as bioactive fractions of the cloudy apple juice. However the chemopreventive potential of the cloudy juice was higher, when compared to the fractions.

As obesity leads to a promotion of tumor-associated parameters in the colon cancer model we have further evaluated the cancer-preventive effects of the cloudy apple juice in the obese Zucker rat, which is a well established model for the human metabolic syndrome. Although the cloudy apple juice intervention significantly affected obesity-related risk factors for colon cancer, besides a significant antigenotoxic effect in colonocytes of the lean but not the obese group, the number and size of colonic preneoplastic lesions did not change.

In summary these data clearly show, that the cloudy apple juice influence cancer related processes due to bioactive substances in the polyphenolic and the cloud fractions. Further, the cancer-preventive efficacy of apple juice might be not sufficient to protect against the cancer-promoting mechanisms of obesity.

## **Quercetin: Tissue distribution and *in vivo* bioactivity**

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Epidemiological studies showed that the polyphenol quercetin is associated with reduced cardiovascular risk and, to a smaller extent, reduced lung cancer risk. *In vitro* studies have shown a wide array of effects, including activation of the metabolic regulator SIRT1. However, these studies were largely carried out with unmetabolized quercetin, which does not circulate in the body and has a different bioactivity. Quercetin is metabolized completely in the body into conjugated (glucuronides and sulphates) and partly methylated derivatives. It is known that this metabolism diminishes its anti-oxidant potential and other functional activities of this polyphenol.

To identify *in vivo* relevant effects of quercetin, we have 1) tested SIRT1 activation for physiological relevance, 2) identified target tissues and relevant metabolites and 3) applied genomics approaches to identify effects *in vivo*.

We adapted an existing assay for SIRT1 activity and analysed SIRT1 activation *in vitro* and in cells. We showed that quercetin cannot activate the metabolic regulator SIRT1 in the human colon cancer cell line HT-29 and that this is most likely due to its metabolism, since the major metabolite quercetin-3-glucuronide cannot stimulate SIRT1 *in vitro*.

We performed a long-term dietary feeding study with rats and, surprisingly, identified lung and testis as the primary target tissues of quercetin. An identical tissue distribution was observed with a 1% and a 0.1% quercetin diet.

To identify possible *in vivo* effects of quercetin, we then analysed gene expression in the lung. Using whole genome arrays, we identified small but consistent effects of dietary quercetin on lipid catabolism. These effects were confirmed using real time quantitative PCR. In addition free fatty acid levels in plasma were found to be lowered by the dietary quercetin intervention. Together, our results show that quercetin alters lipid metabolism *in vivo*.

## **Vegetables and fruits: Implications for vascular protection**

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Several epidemiological studies have indicated that regular intake of vegetables, fruit, and beverages such as red wine and green tea, is associated with a decreased global mortality due to a reduced number of cancer and coronary diseases. The protective effect has been attributable, at least in part, to polyphenols. Indeed, grape products such as red wine and grape juice contain high levels of polyphenols, which are predominantly found in skins, seeds and stems. The protective effect of grape-derived polyphenols on the vascular system is thought to include their ability to prevent oxidation of low-density lipoproteins, platelet aggregation and adhesion, and smooth muscle cell migration and proliferation. Alternatively, vascular protection might also be due to the direct action of polyphenols on endothelial cells resulting in an enhanced formation of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF), two factors playing a major role in the control of vascular homeostasis. Indeed, red wine polyphenols and grape polyphenols cause pronounced endothelium-dependent relaxations of isolated arteries, which are solely mediated by NO in the rat aorta but involve both NO and EDHF in porcine coronary arteries. The increased formation of NO and EDHF is initiated by a modest pro-oxidant response in endothelial cells, which triggers the redox-sensitive activation of the Src kinase with the subsequent activation of the PI3-kinase/Akt pathway resulting finally in the activation of endothelial NO synthase following its phosphorylation. Interestingly, the activation of endothelial NO synthase by polyphenols is a sustained event, which persists for several hours in contrast to that induced by physiological agonists such as bradykinin and acetylcholine, which occurs only for several minutes. In addition, intake of red wine polyphenols in the drinking water is able to prevent the development of endothelial dysfunction and hypertension induced by chronic infusion of angiotensin II to rats. The protective effect of red wine polyphenols involves their ability to cause persistent endothelium-dependent relaxations in pathologic arteries and also to prevent the excessive oxidative stress in the pathologic artery, in part, by blunting the expression of NADPH oxidase. Thus, grape-derived polyphenols have the dual ability to cause a modest pro-oxidant action in endothelial cells to enhance the formation of vasoprotective factors including NO and EDHF, and to evoke an antioxidant action in vascular smooth muscle cells to keep them in a quiescent state.

## **The Pro Children Study: The development and evaluation of school-based fruit and vegetable promotion across Europe**

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### Background

Although a high consumption of fruit and vegetables is considered healthful, population data suggested that children across Europe eat less fruit and vegetables (F&V) than desirable.

### Objective

The aim of the Pro Children project was to (a) develop valid instruments to assess intake levels and potential determinants of F&V across Europe; (b) to assess intake levels and potential determinants among 11 year old children and their parents in nine countries across Europe; (c) to develop and evaluate effective strategies to promote adequate consumption levels of F&V among school children.

### Methods

The project consisted of two phases. The first phase focused on development of instruments and assessing F&V intake and determinants among 11-year-old children and their parents in cross-sectional school-based studies among random population samples in nine European countries. The second phase consisted of the design, implementation and evaluation of a school-based intervention to increase F&V consumption in this age group. These theory-driven interventions aimed to improve knowledge, attitudes, self-efficacy, availability and accessibility related to F&V among children and their parents by means of a school curriculum, a school-based F&V provision scheme, computer-tailored intervention instruments, and media attention. The intervention package was implemented in schools in Norway, The Netherlands and Spain during one school year.

The effects of the intervention were examined in a school-randomised trial among 1488 children from 62 schools in Norway, the Netherlands and Spain, with measurements before, immediately after and one year after the intervention was completed. As outcome measures intake of F&V, potential personal and environmental determinants and habit strength were measured. Data were analysed using multivariate multileveling linear and logistic regression analyses.

### Results

Intake levels were below recommendations for large proportions of children across Europe. Intake levels were highest in Portugal and lowest in Iceland. Knowledge of recommended intake levels, availability of F&V, taste preferences, and parental influences were important correlates of F&V intakes.

The Pro Children intervention resulted in significant positive changes in frequency and portions of fruit intake, frequency of vegetable intake and knowledge of recommended intake levels. Country specific analyses showed in general only small differences in effect sizes between countries. No effects were found on habit strength related to F&V. Additional analyses revealed that level of curriculum implementation and students' appreciation of the project were significantly associated with intervention effects.

### Conclusion

We conclude that the Pro Children intervention is a promising means to promote F&V in European school children.

## The '6-a-Day Campaign' in Denmark and the intake of vegetables and fruits in the Danish population

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In Denmark the general population above the age of 10 years is recommended an intake of 600 g fruits and vegetables per day. The recommendation is expressed as '6-a-day' corresponding to 6 portions of approximately 100 g each. Since the recommendation '6-a-day - eat more fruit and vegetables' was launched by the Danish Veterinary and Food Administration in 1998, the awareness of the '6-a-Day' message has risen, and the mean intake of fruit and vegetables of adults has increased from 279 g per day in 1995 to 385 g per day in 2000-2001. The most recent values of intake from the national dietary survey will be presented, along with new data on awareness.

The 6-a-Day effort in Denmark is basically built on three parts working together: The *recommendation* has the following characteristics, which probably are important for the success: It is scientifically based, it is easily remembered, and it provides a concrete advise (it is easy to see if you have eaten enough or if you are far from the recommended intake). Additionally the way of expressing the recommended intake was developed in cooperation between several organizations, each dealing with public nutrition education

In 1999, a *research project* with several participating health and produce organizations was launched to develop new methods to increase the intake of fruits and vegetables in Denmark. The project focuses on the effect of improved availability of fruit and vegetables, and it has been shown that school fruit or workplace fruit systems could increase the intake of fruit by 0.4 pieces per day and 0.7 pieces per day. In the workplace canteen project interventions in workplace canteens showed that involving the entire kitchen staff in a process resulted in an exceptional increase in the mean daily consumption.

In the years 1998-2001 the different organizations that had agreed on the 6-a-day message informed the public about the message in small leaflets, etc. In May 2001, a *campaign* was launched under the heading "6-a-day – eat more fruit and vegetables". Several organizations decided to cooperate and coordinate their activities, which encourage in different ways children and adults to eat more fruit and vegetables. The campaign also advocates for the availability changes, which were proven effective in the research project. Campaign activities from the recent years will be presented.

## **WHO programmes on fruit and vegetable promotion**

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Fruit and vegetables are important components of a healthy diet, and their sufficient daily consumption could help prevent major diseases, such as cardiovascular diseases and certain cancers. Overall, it is estimated that up to 2.7 million lives could potentially be saved each year if fruit and vegetable consumption were sufficiently increased.

The WHO Global Strategy on Diet, Physical Activity and Health emphasizes the increase of fruit and vegetables consumption as one of the dietary recommendations to be considered when preparing national policies and dietary guidelines for populations and individuals.

Recognizing the increasing scientific evidence that low fruit and vegetable intake is a key risk factor for several noncommunicable diseases, WHO and FAO launched a joint Fruit and Vegetable Promotion Initiative in Rio de Janeiro in November 2003. This initiative is being developed in collaboration within the framework of the Global Strategy on Diet, Physical Activity and Health. The overall goal of this Initiative is to strengthen, promote and protect health in the context of a healthy diet by guiding the development of sustainable actions at community, national and global levels that, when taken together, will lead to reduced risk of chronic diseases through increased fruit and vegetable consumption.

In September 2004, the first Joint WHO/FAO Workshop on Fruit and Vegetables for Health was held in Kobe, Japan. At this workshop a framework to promote fruit and vegetables at country level was developed. The framework will guide the development of cost-efficient and effective interventions for the promotion of adequate consumption of fruit and vegetables. In this process, national or local production capacities, traditional agriculture and dietary practices, prevailing patterns of nutrition, the health status of the population, and existing fruit and vegetable promotion programmes need to be taken into consideration.

To support the implementation of the framework at country level, workshops are being held to develop national action plans. In 2005 the first workshop was held in Lisbon, with participants from the health and agriculture sectors from all lusophone countries. A workshop for the Spanish speaking countries was held in May 2006 and for the francophone countries a workshop is planned for January 2007.

On the European level, the second Action Plan for Food and Nutrition is under development and includes actions on fruit and vegetable promotion. The plan advocates for an increased supply of fruit and vegetables, through support to local production and local markets, price measures, more capillary distribution, as well as specific promotion actions in schools and the workplace, such as free fruit distribution schemes.

## **National Nutrition Survey II: Vegetables and fruit in German diets**

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### Objectives and Design of the German National Nutrition Survey II (NVS II)

What do Germans eat today – significant changes in food availability and in our eating habits have taken place over the last few years. It is therefore likely that our dietary intake will also have changed. Up-to-date information on dietary status, eating behaviour and fruit and vegetable consumption on which to base dietary recommendations and consumer advice in Germany is urgently required.

NVS II will provide a representative set of data concerning the current food intake and nutritional patterns of 20 000 German speaking residents 14 – 80 years old. A personal interview at a study centre will be conducted to establish details about the participant's dietary habits and body size (height, weight and circumferences). A questionnaire is handed out at the study centre to be filled in during the visit. A few weeks later, each participant will be investigated twice by telephone, to provide information on what they ate and drank in the previous 24 hours. A sub sample of 1000 participants will complete twice 4-days weighed records.

### Vegetables and Fruit in German Diets

NVS II assesses aspects of behaviour, such as frequency, amounts, timing of fruits and vegetable consumption, preparation of meals, meal structure, shopping, use of convenience foods, consumption outside the home, sources of nutrition information, knowledge about nutrition such as “five a day” and much more. Food consumption data will be calculated with the German Nutrient data base (BLS). The BLS was developed as standard instrument for evaluating nutritional epidemiological studies and consumption surveys in the Federal Republic of Germany. It contains extensive documentation on the average nutrient values for about 11 000 foods.

### Summary

Interviews began in November 2005. In order to allow for seasonal variations, the survey will be conducted over a 13-month period. Participants in about 500 randomly chosen townships in Germany were selected with the help of the local register office and then contacted. The NVS II will provide a representative set of data concerning the current vegetable and fruit consumption of 14 – 80-year-old Germans.

## **Poster Session I**

Plant food: Constituents, processing and quality

**P1**

**Antioxidant capacity and content of *Brassica oleracea* dietary antioxidants**

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Subspecies of *Brassica oleracea*, including cabbage (var *capitata*), broccoli and cauliflower (var *botrytis*), Savoy cabbage (var *sabauda*), and Brussels sprouts (var *gemmifera*) belong to Cruciferous family. Brassica vegetables contain many bioactive compounds, especially organosulfur, phytochemicals possessing anticarcinogenic activity and others phytochemicals, which are known to possess antioxidant activity. Dietary antioxidants present in these vegetables are water-soluble vitamin C and phenolic compounds, as well as lipid-soluble vitamin E and carotenoids.

In this study, we assayed content and antioxidant activity of water-soluble and lipid-soluble antioxidants in the edible portions of following Brassica vegetables: white cabbage (cv. Almanag, Tukana and Vestri), red cabbage (cv. Koda and Kissendrup), Savoy cabbage (cv. Langedijker and 60F/100), and Brussels sprouts (cv. Ajax and Filemon).

Total phenolics and total carotenoids were analysed spectrophotometrically. Phenolic profiles, vitamins C and E contents were determined by high-performance liquid chromatography. The antioxidant activity was evaluated by four different methods: a lipid peroxidation in a linoleic acid emulsion, an enzymatic method for superoxide anion radical ( $O_2^{\bullet-}$ ), and chemicals methods for the stable DPPH<sup>•</sup> radical (1,1-diphenyl-2-picrylhydrazyl) and for radical monocation ABTS<sup>•+</sup> (2,2'-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid)).

Total phenolic and vitamin C contents varied from 21 to 171 mg/100g and from 18 to 129 mg/100g, respectively. Levels of carotenoids ranged from 0.009 to 1.16 mg/100g, while  $\alpha$ -tocopherol levels - from 0.008 to 0.82 mg/100g. Red cabbage and Brussels sprouts were the richest sources of dietary antioxidants, while their content was the lowest in white cabbage. Water-soluble antioxidants were the main antioxidant compounds in the vegetables tested, and their contribution to the TEAC values was near 99%. Among phenolic compounds, anthocyanins were the main constituents in red cabbage, but hydroxycinnamic acids predominated in others vegetables. All tested phenolics extracts were capable of scavenging  $O_2^{\bullet-}$ , DPPH<sup>•</sup> and ABTS<sup>•+</sup> radicals and inhibited a lipid peroxidation in a linoleic acid emulsion. The high activity of phenolic compounds from red cabbage and Brussels sprouts, and the low activity for white cabbage phenolics were confirmed by all these methods.

This research was financially supported by a Grant from the State Committee for Scientific Research (PBZ-KBN-094/P06/2003/03).

**P2**

**Tocopherols, tocotrienols and a new radical scavenging substance in raw and processed rapeseed and rapeseed oil**

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The content of tocopherols (alpha, gamma, delta), alpha-tocotrienol and plastochromanol-8 was determined simultaneously in raw and processed rapeseed and different rapeseed oils after saponification and hexane extraction by normal phase liquid chromatography using fluorescence detection. The results show that gamma-tocopherol represents 54% and alpha-tocopherol 32% of the total tocopherols. The content of plastochromanol-8 amounted to 12% of the total tocopherols. The alpha-tocotrienol content was below 1%, it was separated with a high resolution. Applying a modified method of tocopherol analysis by decreasing the isopropanol fraction in the solvent a strong phenolic antioxidant was found in the crude rapeseed oil. The compound was identified as vinylsyringol or canolol, a decarboxylation product of sinapinic acid. For the structural identification vinylsyringol was isolated from crude rapeseed oil. Vinylsyringol was synthesized and compared with the natural compound. Vinylsyringol is present in virgin oil and in roasted rapeseed but not in fully refined oils. The vinylsyringol content increased after roasting the rapeseed. Vinylsyringol is a strong natural radical scavenger against alkyl-peroxyl radicals and peroxy nitrite radicals and it has a much greater antioxidant activity as compared with alpha-tocopherol and vitamin C.

In conclusion, crude rapeseed oil has a high content of antioxidants like tocopherols/tocotrienol, plastochromanol-8 and vinylsyringol. The present results suggest that the amount of the antioxidants especially the vinylsyringol content is reduced or completely removed after refining.

**P3**

**The antioxidative activity of watersoluble pectins in blueberries (*Vaccinium corymbosum* L.)**

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Hydrocolloids (especially pectins) have gained much attention in the discussion on the bioactivity of food ingredients. Beside their importance as dietary fibres, few studies, mainly model systems, describe an antioxidative ability of these polysaccharides. Thus, pectins with a low rate of esterification (water soluble pectin) displayed a relatively high level of activity than those with a high rate of esterification (Motomura 2002, Acta Hort., 587, 525). But the antioxidative mechanisms and the role in plant defence system have not been detected yet.

The antioxidative activity of blueberries (*Vaccinium corymbosum* L.) is mainly based on their high content of phenolic compounds like anthocyanins, flavonols and phenolic acids (Howard 2003, J. Sci. Food Agric., 83, 1238). Moreover blueberries are also popular due to their high content of pectins compared to other fruits. With regard to the antioxidative activity of fruit pectins and factors (like cultivation treatments), that could influence the content and the ability of antioxidant active pectin fractions, there is still a lack of information.

Eight year old plants of the blueberry cultivar 'Bluecrop' were cultivated on formerly used farmland in Berlin-Dahlem. The plants were cultivated in two ground cover (with and without pine bark mulch) and fertilization variants (boron foliar applications). After maturity of the fruits, mineral analysis (boron, calcium), the content of watersoluble pectins as well as the antioxidative activity (electron spin resonance (ESR) spectroscopy) of the first two harvests were determined.

Results will present the influence of boron application on the production of cell wall material and the content of watersoluble pectin (= WSP) of the fruit. Also the interaction with other minerals like calcium will be examined. The antioxidative activity of WSP will be demonstrated as well as the influence of cultivation and fertilization treatments on the antioxidant activity of WSP in blueberries will be discussed.

**P4**

**Antioxidants in fruits and vegetables and the changes in antioxidant capacity and antioxidant activity that occur during growing, processing and storing**

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The association between fruit and vegetable consumption and human health has been a great deal of interest. Oxidative stress plays a significant role in most disease processes and aging, and the potential health benefits of fruits and vegetables have been largely attributed to their potential antioxidant capacity.

Antioxidants are compounds that inhibit or delay oxidation. In another words, antioxidants are defined as substances that prevent negative effects in foods by reacting with oxygen in foods. They are agents that disable havoc-wreaking molecules called free radicals. The body generates free radicals as by products of burning fuel for energy within the cells, exercising, and fending off infections. Various environmental exposures such as pollution, tobacco smoke, the sun's ultraviolet light and radiation can also create free radicals. Oxidative damage to low-density lipoprotein (LDL) cholesterol, for example, increases the risk of atherosclerosis and heart disease. Oxidation of DNA can cause mutations that lead to cancer. If antioxidants don't mop up free radicals, the damage accumulates and fast-forwards aging and disease. Add in stresses such as infection and air pollution, the body may not be able to supply enough antioxidants to stanch free radical damage.

Plants, fruits and vegetables consist of natural antioxidants. Principal natural antioxidants that are present in vegetable sources are enzymatic systems, vitamins, phenolic compounds and nitrogen compounds. Diets rich in fruits and vegetables decrease the risk of formation of chronic diseases such as coronary artery diseases and carcinogenic events. The preventive effects of fruits and vegetables are due to the antioxidant compounds like vitamin C, E, carotenoids (lycopene,  $\beta$ -carotene) and polyphenolics. Recent studies have shown that phenolic antioxidants occurring in fruits and vegetables are principal compounds contributing to the antioxidant activity. Besides, vitamin C, E and  $\beta$ -carotene play a significant contributory role. During growing, processing and storing of fruits and vegetables there are some changes in their antioxidant capacities and antioxidant activities. It is important to know the effects of processing steps on the level and activity of these compounds in processed foods. In this study, the antioxidants occurring in some fruits and vegetables are evaluated for their antioxidant activities, and the changes in antioxidant capacity and antioxidant activity of fruits and vegetables during growing, processing and storing are also discussed.

**P5**

**Retention of key antioxidants in fresh versus minimally processed lettuce**

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Numerous studies have reported that dietary antioxidants are protective against degenerative diseases including cancer, cardiovascular disease and aging. Fruit and vegetables are key sources of antioxidants such as vitamin C, carotenoids, vitamin E and phenolics. Loss of these antioxidants can occur during processing and subsequent storage. The present study was conducted to examine the retention of key antioxidants in minimally processed lettuce. Total carotenoids and vitamin C (ascorbic acid and dehydroascorbic acid) were measured in a number of fresh lettuce types. Differences in the concentration of these antioxidants were observed among the lettuce types tested. Sliced butterhead or iceberg lettuce (50g) was packed in oriented polypropylene bags (240 x 100mm). Bags were either flushed with nitrogen (3% O<sub>2</sub> and 97% N<sub>2</sub>), sealed in air (product modified) or left unsealed. During storage (4°C) duplicate packs were analysed for total carotenoids and vitamin C. Duplicate analysis of duplicate extracts were examined. For both lettuce varieties and for all treatments total carotenoids decreased from Day 0 to Day 1. Ascorbic acid decreased for both lettuce varieties and for all treatments from Day 0 to Day 1 and from Day 1 to Day 8. Results show a decline in total carotenoids and ascorbic acid after 1 day storage of minimal processed lettuces at 4°C for all treatments studied.

**P6**

**The thermal stability of onion (*Allium cepa* L.) flavonols is influenced by plant nutrient supply**

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Plant phenolic compounds are an important constituent of the human diet. In recent years they have gained much attraction due to their antioxidative, antibacterial, and anticarcinogenic properties, leading to an enormous increase in research on cancer prevention and reduction of cardiovascular diseases. In this context, onion is an interesting vegetable plant species due to its natural high content of phenolic compounds and its widespread popularity all over the world. In comparison to other vegetables, onions can contain high levels of flavonol glycosides (mainly glucosylated quercetin derivatives). With regard to chemical changes of these compounds during harvesting, storage and food processing knowledge is still insufficient. Especially thermal processes such as boiling, frying, and deep-frying influence the flavonoid content of different vegetables. From the viewpoint of human nutrition it is desirable to define crop production systems that increase the amount of potential health promoting ingredients and lead to a flavonoid profile, which remains stable during different (thermal) processing techniques.

The aim of the present study was to test whether variations in the root environment (nitrogen form in the substrate) affect the flavonol profile of onions and its stability towards roasting. For this purpose, onions (Centurio, *Allium cepa* L.) were grown on Perlite in a greenhouse. They were provided with different ratios of nitrate and ammonium (95:5 and 25:75) as a nitrogen source. After reaching maturity, plants were harvested, cut, freeze-dried and ground. Beside common crop quality parameters (fresh and dry matter weight, bulb size, Brix value, sugars), total polyphenol content was estimated with the Folin-Ciocalteu assay. The flavonol profile was analyzed using HPLC-DAD following an extraction with methanol and solid phase extraction. For the thermal treatment samples were roasted at 180°C for 5 and 10 minutes.

Total polyphenol content illustrated a trend towards higher values when ammonium was the dominant form of nitrogen in the growth substrate. Quercetindi- and monoglucosides increased with ammonium as the dominant nitrogen source. Colonization with mycorrhizal fungi additionally increased the production of polyphenolic compounds. Thermal treatments showed that the quercetin glycosides are degraded depending on roasting time and temperature, but especially depending on the flavonol profile.

It can be concluded that both high ammonium supply and the use of mycorrhiza as a biofertilizer may support increased formation of quercetin glycosides in onion. As the flavonol glycosides are degraded during thermal food processing, a flavonol profile consisting of a high amount of diglycosides seems to be advantageous during roasting.

**P7**

**Effect of temperature on drying characteristics of green olives**

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Dried olives are used as flavouring, as an ingredient or simply as a snack or appetiser. They are especially used in Mediterranean dishes including pizza, relishes, salads, sauces or antipasto platters. The aim of this study was to investigate temperature on the drying kinetics and quality characteristics of green olives. Green olive slices were dried from 75% moisture (wet basis) to 4% in a pilot plant tray drier. The drier was operated at temperatures between 45 and 65°C, at an air velocity of 1.1 m/s. Air flowed parallel to the horizontal trays with olive samples. Drying process started when constant drying conditions were achieved. Sample weight losses were recorded every 5 minutes during drying experiments using a digital balance. Increasing drying temperature leads to lower drying time. Drying at 65°C required 290 minutes, whereas 415 minutes were needed to obtain the 4% moisture content at 45°C for the similar amounts of product. Quality of the dried green olives was evaluated according to the criteria such as colour, water activity and peroxide value. Water activity of the before and after drying process was measured 0.977 and 0.637, respectively. L, a, b colour values of fresh and dehydrated products were measured by using HunterLab ColorFlex (USA). Drying at high temperature resulted in increase in the degree of browning. Rehydration characteristics of dried samples were also determined. As a result it was found that the temperature increment increases the drying constant and decreases the equilibrium moisture content of the dehydrated products.

**P8**

## **Changes of the glycaemic behaviour of potatoes by processing**

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The temporary availability of released glucose is relevant within the metabolic role of dietary carbohydrates. Especially the rapidly digestible starch was identified to enhance the glycaemic response. In consequence of that, a food ranking according to the glycaemic index (GI) and the glycaemic load (GL) has downgraded potatoes to a more restricted intake, but concrete information of single data points (e.g. potato cultivar, specific preparation techniques) are rare. Therefore, an in vitro study of temporarily released glucose from different potato food was performed.

The preparation techniques indicated a cultivar-specific behaviour (RAG) in two of three cultivars. Chilling after boiling reduced the glucose release in one cultivar only. Dehydration and crisps production resulted in an increase of RAG, whereas in French fries a significant decrease of RAG but a significant increase of SAG was detected.

The investigation clearly indicates the need for specific considerations to prevent inaccuracies in case of potato rating.

**P9**

**Existing technologies for processing fruit and vegetables so as to preserve their nutritional value: A review**

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Chronic noncommunicable diseases are the leading causes of death in most regions of the world. The World Health Report 2003 estimated that cardiovascular disease accounted for 16.7 million deaths globally, while cancer contributed to 7.1 million deaths. There is now increasingly good evidence that fruit and vegetables protect against cardiovascular diseases and some cancers. These beneficial effects have been partly attributed to the compounds which possess antioxidant activity. These antioxidants scavenge radicals and inhibit the chain initiation or break the chain propagation. Most of the vegetables and some fruits are processed before consumption. Processing usually involves heat treatment which can disrupt the cellular matrix. The intactness of the cellular matrix determines the bioavailability of different nutrients. This is, to our knowledge the first review to identify the existing technologies for processing fruit and vegetables so as to preserve their antioxidant capacity.

**P10**

**Measuring of residue of pesticides used (endosulfan and phosalon) to control of Colorado potato beetle**

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Phosphorous pesticides because of their high degree of toxicity and organochlorines because of high stability are important agricultural pesticides which absorb to the products or environment directly and indirectly and they can enter into food chain easily.

Determining the concentration of these pesticides residue in the agricultural products and comparing them with international standards is important in the correct managing of pesticides applying in this research the effect of various concentrations of pesticides usage and the time spraying of two kinds of pesticides, endosulfan and phosalon, on the residue of pesticides in potato were investigated. The experiment was performed as completely randomized block design with two replications.

After sampling, extracting, concentrating and finally analyzing of pesticides residues were done by the GC and detectors of ECD and FTD.

The obtained results of experiment and data analyzing indicated that in the case of applying various and normal concentrations of these pesticides, their residue will not exceed standard limits.

Also, it was observed that the residue of phosalon was more than endosulfan. The results of variance analyzing showed that between the various used concentrations and also the time of pesticides applying, there is no significant difference from the point of pesticide residue in the potato.

Also the amount of pesticides residue of potato in the first of May spraying, by comparison of first of June was low significantly.

**P11****Contamination of fruits and vegetables with dioxins and public health aspects****Gh. A. Omrani, Gh. R. Jahed Khaniki**

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Dioxins are a group of chemicals formed during the burning of household and industrial waste. They also are formed during the making of some herbicides and germicides, and the bleaching of paper pulp. Dioxins also can be found as contaminants in polychlorinated biphenyl (PCB) mixtures. PCB mixtures are commonly found in electrical capacitors, fluorescent light ballasts and transformers. The burning of industrial or household waste can produce dioxins and release them into the air. Rainwater carried herbicides containing dioxins from farm fields into surface waters, and some factories discharged dioxin-contaminated waste directly into surface water. Dioxins can enter the body by eating or drinking contaminated food. Dust contaminated with dioxins may be found on the outer surfaces of fruits and vegetables. Since, dioxins last a very long time in the environment before breaking down; they may eventually find their way into the food chain. Dioxins are easily absorbed by the body and are stored in fatty tissue. For this reason, dioxins are slowly eliminated from the body. Dioxins, and dioxin-like substances like PCBs and furans, are getting into food supplies at levels that are highest in high-fat foods, and lowest in low-fat foods such as fruits and vegetables. In people, too much exposure to dioxin may cause cancer, lowered sperm count, birth deformities, headache, nervousness, dementia, irritability, depression, anxiety, loss of sleep and loss of sex drive. Also, minute amounts of these chemicals have been shown to lead to nervous system and liver damage as well as to mimic hormones that disrupt reproduction and human development. While vegetables and fruits also contained trace amounts of these chemicals, the dose was significantly less than high fat foods. Plants do not generally absorb dioxin. Small amounts of dioxin may also fall on plants when certain pesticides are used. Animals eat plants that usually are not washed. Dioxin on an unwashed plant or feed that is eaten by an animal becomes part of the animal. Japanese domestic leafy vegetables were successively investigated for levels of dioxins, including 17 dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs), four non-*ortho* co-planar PCBs (co-PCBs) and eight mono-*ortho* co-PCBs, all of which had been assigned toxic equivalency factors (TEFs) by WHO in 1997. The mean levels of dioxins in the edible portions were 0.07 pg TEQ/g in spinach, 0.13 pg TEQ/g in garland chrysanthemum, 0.01 pg TEQ/g in mitsuba (marsh parsley) and 0.01 pg TEQ/g in chingentsuai (*Brassica Campestris* var. *chinesis*). The dioxin levels in the leaves were found to be higher than those in the stem and red collar, but they were much lower than those found in the primary and secondary roots, which are considerably affected by the soil, which is recognized as a sink of airborne dioxins. The dioxin levels in edible portions (leaves, stem and red collar) were obviously lower than those in non-edible portions (primary and secondary roots). The Food and Drug Administration regulates these foods products to make sure that people are not exposed to harmful levels of dioxin.

**P12**

**Risk assessment of furan in German nutrition**

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Furan, a very volatile and colourless liquid, has been classified by the International Agency for Research on Cancer as a possible human carcinogen. Due to its widespread occurrence in many types of food products, concerns were raised that furan might become a serious problem similar to acrylamide.

Furan generation in food is multifactorial. A number of model tests have shown that furan is produced through heating of reducing carbohydrates through the Maillard reaction. Furan generation was also determined in the presence of ascorbic acid or certain amino acids. The oxidation of poly-unsaturated fatty acids may also represent a means of generation. In this study, we analysed a large number of samples as basis for risk assessment of furan in the German nutrition.

Up to 4600 µg/kg of furan are produced in roasted coffee beans. Espresso powder and powdered coffee on the market contain between 1700 and 1920 µg/kg, instant coffee up to 910 µg/kg of furan. The furan content in various typed of coffee drinks depends on the type of preparation. During preparation with a "normal" coffee machine and by hand pouring, about 20 µg/l of furan could be found in the beverage, about 50 µg/l with a French press. The highest furan values were found in coffee beverages that came from fully automatic coffee machines.

In comparison to other food groups, there were very relatively low furan contents found in beer. Notable concentrations were found in crisp bread (38 µg/kg), baked snack foods (10 µg/kg), and pear-apple cabbage (22 µg/kg). In the majority of the examined alcohol-free beverages (e.g. tea, cola drinks), furan was not detectable. However, furan was detectable in a number of baby food products. The highest contents were found in baby jars with a high vegetable content, while almost no furan is found in baby beverages.

So far a „no-observed-effect-level“ of 2 mg per kilogram of body weight in animal testing can be derived. Under the assumption of a safety factor of 1,000, an ADI of 2 µg per kilogram of body weight results. For an average adult these hypothetical ADI values would be 150 µg/day. For small children this value would be about 30 µg/day and for babies about 12 µg/day. An adult drinking coffee from the regular household coffee machine would hardly reach this ADI. However, by drinking about eleven cups per day from an automatic coffee machine the limit could be reached.

**P13****Trace metal contaminants in edible green vegetables in Zanjan city, Iran****Gh. R. Jahed Khaniki<sup>1</sup>, Gh. A. Omrani<sup>1</sup>, A. Eslami<sup>2</sup>**

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Vegetables constitute essential components of the diet, by contributing protein, vitamins, iron, calcium and other nutrients which are usually in short supply. These plants can contain both essential and toxic elements over a wide range of concentrations. Chronic low level intakes of heavy metals have damaging effects on human beings and other animals, since there is no good mechanism for their elimination. Metals such as lead, mercury, cadmium and copper are cumulative poisons. These metals cause environmental hazards and are reported to be exceptionally toxic. A study was accomplished to evaluate trace metal contaminants in edible green vegetables in Zanjan City of Iran. Roots and leaves of radish (*Raphanus sativus* L), leek (*Allium ampeloprasum* L), sweet basil (*Ocimum basilicum* L), and parsley (*Petroselinum crispum*) are the most common green vegetables consumed by people living in Zanjan city. Four heavy metals (cadmium, lead, zinc, chrome and arsenic) were determined in some green vegetables cultivated in Zanjan city. Atomic absorption spectrophotometry was used to estimate and evaluate the levels of these metals in the vegetables. Vegetable samples were analyzed by flame atomic absorption spectrophotometry for three heavy metals: Lead (Pb), Cadmium (Cd) and Zinc (Zn). The mean and standard deviation concentrations of Pb levels in roots and leaves of radish, leek, sweet basil, and parsley were  $6.95 \pm 1.92$  and  $19.16 \pm 6.23$ ,  $10.22 \pm 2.04$ ,  $9.46 \pm 1.49$ , and  $7.44 \pm 2.16$  mg/kg dry weight, respectively. Also, the mean and standard deviation concentrations of Cd levels at these vegetables were  $8.89 \pm 3.12$  and  $7.65 \pm 1.99$ ,  $7.43 \pm 5.49$ ,  $9.59 \pm 7.73$  and  $11.55 \pm 4.28$  mg/kg dry weight, respectively. The mean and standard deviation concentrations of Zn were  $71.73 \pm 37.08$  and  $107.55 \pm 52.51$ ,  $72.17 \pm 14.73$ ,  $101.27 \pm 18.92$  and  $85.79 \pm 18.41$  mg/kg dry weight, respectively. The results show that leaves of leek has ability to accumulate more lead and zinc than the other vegetable studied. The high levels of daily intake of lead and cadmium may cause public health problem. Monitoring heavy metals is imperative during culture and production of vegetables for reduction of public health concerns.

## **Poster Session II**

Biological effects in vitro

**P14**

**Antioxidants of fruits and vegetables: *In vitro* stability in gastric and duodenal conditions mimicking the human upper digestive tract**

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Health benefits of diet rich in fruits and vegetables are nowadays clearly established in regard to epidemiological studies (New SA Proc Nutr Soc 2003, 62: 889; Trichopoulou A *et al.* Int J Vitam Nutr Res 2003, 73: 63; Johnsen SP Curr Opin Clin Nutr Metab Care 2004, 7: 665). Fruits and vegetables are especially rich in various antioxidants such as vitamins C and E, carotenoids and polyphenols that contribute to their protective effects against cardiovascular diseases (Stanner SA *et al.* Public health Nutrition 2003, 7: 407). Numerous studies suggest a synergistic effect of all these components but there is a lack of knowledge of their behaviour along the digestive tract.

The aim of this study was therefore to assess the susceptibility to oxidation of various combinations of dietary antioxidant microconstituents submitted to *in vitro* digestion.

A full factorial design was used to establish the effect of different combinations of antioxidants on their stability. The chosen molecules were: vitamins C, E (RRR- $\alpha$ -tocopherol and RRR- $\gamma$ -tocopherol), a carotenoid mixture ( $\beta$ -carotene, lycopene and lutein) and a polyphenol mixture (gallic acid, caffeic acid, (+)-catechin and naringenin). Sixteen test meals were then prepared, and digested *in vitro* under gastric (Pafumi *et al.* J Biol Chem 2002, 277: 28070) and duodenal conditions mimicking human digestive tract. Antioxidants were extracted and measured by HPLC. The experiments were performed in triplicate, and statistical analyses were done using generalized linear model.

The results showed great differences in stability behaviour among the antioxidant structures. Vitamin C was not detected in any condition tested suggesting that this vitamin is the first to act as antioxidant. Carotenoids were very sensitive to oxidation during either gastric or duodenal digestion. By contrast, polyphenols displayed a higher stability, except for gallic acid.  $\alpha$ -Tocopherol appeared to be more oxidizable than  $\gamma$ -tocopherol. In binary combinations, vitamin C exhibited a deleterious effect on the stability of carotenoids. Nevertheless, this effect was abolished when polyphenols or vitamin E were added, suggesting the ability of phenolic structures to inhibit the breakdown of carotenoids. This could be due to the fact that ortho-diphenolic structures (gallic and caffeic acids, (+)-catechin) are able to chelate metals such iron that is known to catalyze oxidations.

The present results support that a single antioxidant should be combined with other antioxidants to achieve optimal protective effects. Synergism between antioxidants could explain the beneficial effects of fruits and vegetables.

Supported by INSERM and INRA (ATC Nutrition 2002, project A02256AS).

**P15****Combination effects of the broccoli compounds sulforaphane and 3,3'-diindolyl-methane on cell growth inhibition of cultured 40-16 colon carcinoma cells****G. Pappa, H. Bartsch, C. Gerhäuser**

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A diet rich in cruciferous vegetables like broccoli can reduce the risk of developing colon cancer. The compounds responsible for the anti-cancer activity of these plants are glucosinolate cleavage products like isothiocyanates and indole derivatives. Apart from other mechanisms, these compounds have been shown to possess growth-inhibiting and apoptosis-inducing activities in various cancer cell lines *in vitro*. Recently, we have demonstrated that isothiocyanates like sulforaphane (SFN) are cytotoxic, whereas indoles like indole-3-carbinol or its condensation product 3,3'-diindolylmethane (DIM) are acting by a cytostatic mechanism in human colon cancer cell lines (Pappa *et al.*, 2006, Mutation Res. 599, 76-87).

So far, exclusively single pure broccoli compounds or crude extracts have been studied regarding their anti-proliferative potential, but little is known about interactions between different glucosinolate cleavage products that occur concomitantly in the plant. Therefore we were interested in investigating how defined combinations of both would affect cell proliferation. Cultured 40-16 colon carcinoma cells were treated with SFN, DIM and combinations of both (ratio 1:4, 1:2, 1:1, 2:1 and 4:1) at eight serial two-fold dilutions for 24, 48 and 72 hours, respectively. Cell proliferation was assessed by Sulforhodamin B staining.

Calculations of combination effects were based on the method of Chou and Talalay (Chou and Talalay, 1984, Adv. Enzyme Regul. 22, 27-55). Median-effect plot analyses revealed that SFN and DIM were mutually non-exclusive, confirming distinct modes of action. Dose-response interactions were expressed as a combination index (CI) for the affected fractions.  $CI < 1$ ,  $CI = 1$  or  $CI > 1$  represent synergism, additive or antagonism, respectively.

Interestingly, at a total drug concentration of 2.5  $\mu\text{M}$ , all combinations of SFN and DIM were antagonistic and inhibited cell proliferation less than each single compound alone. The strongest antagonistic effects at this concentration were observed with an excess of DIM at the 1:4 and 1:2 combinations after 24 hours of treatment (mean CI values of 4.2 and 3.6, respectively). With increasing concentrations, the antagonistic effect gradually turned into a synergistic interaction at the highest combined concentration of 40  $\mu\text{M}$  (mean CI values ranging from 0.7 to 0.9, exception 1:4, 72 h,  $CI = 1.5$ ).

These results indicate that cytotoxic concentrations of SFN:DIM combinations affect cell proliferation synergistically. At low concentrations, which might physiologically be more relevant, the combined broccoli compounds showed antagonistic interactions in terms of cell growth inhibition. Thus, the transfer of promising findings achieved with single food components to whole foodstuff should be done with caution.

**P16*****In vitro* biological activities of red cabbage anthocyanins**

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Red cabbage is a source of the anthocyanins, which belong to flavonoids, and are used as natural, red food colorants. Red cabbage contains over 20 different anthocyanins which are cyanidin derivatives highly conjugated with sugars (glucose and xylose) and acyl groups (caffeoyl, *p*-coumaroyl, feruloyl, *p*-hydroxybenzoyl, sinapoyl and oxaloyl). The major acylated anthocyanins in red cabbage are cyanidin 3-diglucoside-5-glucoside derivatives (Wu and Prior, 2005, J. Agric. Food Chem., 53, 3101-3113). The health benefits of anthocyanins, such as cardioprotective effect and anticancer activity, have been partly attributed to their antioxidant capacity. There have been many reports about biological activity of anthocyanins from fruits, especially from berries and grapes, but relatively little has been published regarding the acylated anthocyanins from red cabbage.

The aim of this study was isolation, identification, and determination of biological activities of the red cabbage anthocyanins. The pigments were purified on a C-18 solid-phase cartridge, and then fractionated on Sephadex LH-20. The fractions obtained were characterised using HPLC and MALDI-TOF assays. Biological activities of the fractions were evaluated *in vitro* by a measurement of an antioxidant activity (ABTS and FRAP methods), apoptosis induction in HL-60 human acute leukemia cells (DAPI assay), and an influence on lipid peroxidation in hypercholesterolemia and normal erythrocytes (thiobarbituric acid assay).

Eight fractions, which contain 0.27 – 1.14 mg anthocyanins (as cyanidin 3-glucoside) per 1 ml, were isolated from red cabbage and studied with regard to their biological activities. These fractions were found to be efficient free radical scavengers towards ABTS<sup>•+</sup>, TEAC values ranged from 2.16 to 6.29  $\mu$ moles of Trolox/mg anthocyanins. On the contrary, ferric reducing antioxidant capacity (FRAP) of fractions varied slightly (5.67 – 8.93  $\mu$ moles of Trolox/mg anthocyanins). In the red blood cells system, at 10  $\mu$ M concentration, inhibition of TBA reactive substances was 44.7 - 77.8%. Only one of these fractions has shown significant proapoptotic activity towards HL-60 cells and after 24 h incubation generated 90% of the apoptotic cells.

The data show that the *in vitro* biological activities of red cabbage anthocyanins fractions are highly dependent on their structure. The most efficient are fractions, which contain cyanidin 3-(sinapoyl)glucoside-5-glucoside and two anthocyanins with molecular weight about 1300.

This research was financially supported by a Grant from the State Committee for Scientific Research (PBZ-KBN-094/P06/2003/03).

**P17**

**Modulation of gene expression in peripheral blood mononuclear cells (PBMC) by carob fibre extract and gallic acid**

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Introduction: Detoxicating effects of vegetables and fruits are attributed partly to polyphenols and in part to metabolites of dietary fibre. Carob fibre, a product of the Mediterranean carob pods (*Ceratonia siliqua* L.), contains insoluble lignin, cellulose and hemicelluloses as well as tannins, gallic acid and other polyphenols. The components may contribute to chemoprotective effects of carob fibre by enhancing expression of genes involved in toxicological defence.

Aim: An aqueous polyphenols-rich extract of carob fibre and gallic acid were studied for effects on patterns of expression of genes related to drug metabolism and to stress response using PBMC as surrogate tissue.

Methods: PBMC were incubated with carob fibre extract (50 - 20000 mg/l) and equivalent concentrations of gallic acid (0.4 - 160 µM). Viability was assessed with the CellTiter-Blue™ assay, which measures the conversion of resazurin to resorufin as an indicator of metabolically active cells. Non-toxic doses of carob fibre extract and corresponding concentrations of gallic acid were used to determine the effects on gene expression after treatment for 24 hours with two pathway-specific cDNA-macroarrays. Expression of modulated genes was reassessed with quantitative Real-Time PCR (qRT-PCR).

Results: High concentrations of carob fibre extract (> 4 g/l) and gallic acid (> 48 µM) were cytotoxic to PBMC significantly. Of the 96 genes spotted on each cDNA macroarray, 38 (drug metabolism) and 41 (stress response) genes were expressed in the medium control. None of the stress-related genes was significantly regulated by either treatment. In contrast, 4 genes of drug metabolism (glutathione S-transferase [GST] A4, GSTT2, cytochrome P450 [CYP] 2C9 and CYP4F3) were significantly enhanced by carob fibre extract (500 mg/l) whereas 3 genes (N-acetyltransferase 5, microsomal GST3 and histamine N-methyltransferase) were suppressed by gallic acid (4 µM). Up-regulation of GSTA4 and GSTT2 by carob fibre was not confirmed by qRT-PCR, and confirmatory analysis for CYP4F3 and other genes is ongoing.

Conclusion: Carob fibre extract and gallic acid were cytotoxic only in non-physiological high concentrations, but low concentrations of carob fibre induced genes related to drug metabolism. Some of the alterations point to an improved detoxifying capacity of the cells. Altogether the findings indicate a potential suitability of using PBMC as surrogates for *in vivo* studies. Additional confirmatory analyses with qRT-PCR and with methods detecting protein expression and enzyme activities, however, are still needed to better judge the predictivity and functional consequences of results obtained with cDNA arrays.

**P18**

**Analysis of signal transduction pathway activation by apple extract using a phosphorylation specific antibody array**

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Cellular response to external and internal stimuli is mediated by a network of signal transduction pathways. Although expression of the participating proteins is an essential prerequisite for activation of a particular pathway, transient modifications on the protein level, like phosphorylation, play a crucial role in their activation. Phosphorylation allows changes in the activation state of proteins without changing their overall abundance. Thus, the level of expression of such proteins gives only partial information on their activity. Only knowing the phosphorylation state of these expressed proteins allows the decision - active or inactive - for a given signal transduction pathway.

In order to detect changes in signal transduction pathway activation in response to apple juice ingredients the colon cancer cell line HT29 was treated with apple extract for short time periods. In order to maintain appropriate growth conditions that may allow an *in vivo* like response, cells were maintained in medium with low concentration of FCS during treatment. In addition to the analysis of changes mediated directly by apple extract treatment, we also analysed changes in protein phosphorylation in response to EGF after pretreatment with apple extract.

The most prominent results were a strong activation of the Erk1/2 MAPKinases and to a lesser extent also of MAPKinase p38.

The results obtained clearly indicate that apple extract has an immediate effect on various signal transduction pathways, the mechanism of which remains still elusive and may go beyond a simple toxic response. Nevertheless, the strong activation of Erk1/2 suggests that apple extract is a strong inducer of an immediate cellular stress response.

Acknowledgements: We thank our colleagues and partners in the network "Nutrition Net" for continuous support and encouragement. <http://www.nutrition-net.org/>

This work is funded by the Bundesministerium für Forschung und Technologie (BMBF), Germany (BMBF FKZ.01EA0103) within the network.

**P19**

**Neuroprotective effect of cocoa flavonoids *in vitro***

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Objectives

Cocoa is a rich source of flavonoids that among other functions can act as antioxidants. The production of reactive oxygen species (ROS) activate an array of intracellular cascades, namely mitogen-activated protein kinases (MAPK), that are closely associated with cell death or survival pathways. We study here the role of a cocoa extract and its main flavonoid, (-)-epicatechin, on an *in vitro* model of oxidative stress induced in a neuronal cell line. We analyzed ROS production and the MAPK activation pathways including extracellular signal-regulated kinases 1/2 (ERK 1/2) and c-Jun N-terminal kinase (JNK) and p-38.

Material and Methods

ROS production was evaluated by fluorometry (dichlorofluorescein assay) and MAPK phosphorylation by Western blot analysis. Cells were incubated with cocoa extract (0.1-30 µg/mL) or (-)-epicatechin (0.3-29 µg/mL) for 30 min. After removing flavonoids, cells were incubated with dichlorofluorescein diacetate (DCFH-DA) for 30 min. Oxidative stress was induced by adding H<sub>2</sub>O<sub>2</sub> plus FeSO<sub>4</sub>. Fluorescence, proportional to the amount of ROS, was monitored. Cell lysates were stored at -80°C until Western blot determination.

Results

Cocoa extract and (-)-epicatechin reduced ROS production in a dependent-dose manner, reaching a 35% inhibition. pJNK and p38, involved in apoptosis, were down-modulated by cocoa extract and (-)-epicatechin, showing the cocoa extract the highest inhibition on p-38 (up to 70%).

Conclusions

Cocoa extract and (-)-epicatechin exert a neuroprotective action by reducing ROS production and modulating MAPK activation.

**P20**

## **Modulation of key elements of the Wnt-pathway by apple polyphenols**

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Glycogen synthase kinase-3 $\beta$  (GSK3 $\beta$ ) is one of the key elements of the Wnt pathway, involved in the regulation of  $\beta$ -catenin homeostasis. The inhibition of GSK3 $\beta$  kinase activity might lead to the onset of  $\beta$ -catenin/TCF/LEF-mediated gene transcription, representing a potentially mitogenic stimulus. Recent studies showed that apple polyphenols possess several biological properties which might be of interest with respect to the prevention especially of colorectal cancer. Objective of the study was to elucidate whether apple polyphenols affect key elements of the Wnt-pathway, which might limit their usefulness in the prevention of colon carcinogenesis.

The effect of a polyphenol-rich apple juice extract (AE02) on immunoprecipitated GSK3 $\beta$  from HT29 cells was determined in a kinase assay as the phosphorylation of a specific GSK3 $\beta$  substrate peptide. AE02 was found to effectively inhibit isolated GSK3 $\beta$  as well as intracellular GSK3 $\beta$  kinase activity in HT29 human colon carcinoma cells. The inhibition of enzyme activity occurs at polyphenol concentrations corresponding to the concentration of these compounds in the original apple juice, which represented a consumer-relevant apple juice blend. In accordance with the inhibition of GSK3 $\beta$  kinase activity by AE02, treatment of HT29 cells resulted in a significant decrease of phosphorylated  $\beta$ -catenin measured by Western Blot analysis. However, unexpectedly, also the total intracellular  $\beta$ -catenin level was found to be diminished, indicating that the interference of the apple constituents with GSK3 $\beta$  was not associated with a stabilization of  $\beta$ -catenin in HT29 cells. In line with these results, TCF/LEF-mediated gene transcription remained unaffected by treatment with AE02 as shown in a reporter gene approach. The results let assume that at consumer-relevant concentrations apple polyphenols do not mediate growth stimulating effects in HT29 cells via the Wnt-pathway.

The study was performed as part of the NutritionNet funded by the grant 01EA0101 of the German Ministry of Research and Education.

**P21**

**Phenolic apple juice extracts: Modulation of glutathione-synthesis by transcription of  $\gamma$ -glutamate-cysteine ligase in human colon cell lines**

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Epidemiological studies indicate that a diet rich in fruits and vegetables is associated with a lower risk of colon cancer and of inflammatory bowel disease. Bioactive dietary polyphenols have been claimed to exhibit a potential to prevent bowel diseases. In addition to the direct antioxidative capacity of the compounds, modulation of redox-sensitive gene expression, e.g. of  $\gamma$ -glutamate-cysteine ligase ( $\gamma$ -GCL), which catalyzes the rate-limiting step of glutathione synthesis, is of particular interest.

In this study, we investigated modulation of  $\gamma$ -GCL transcription by two phenolic apple juice extracts (AE02, AE03, from table and cider apple varieties), known to elevate the glutathione level in HT-29 and Caco-2 cells [Schaefer et al., 2006, Mol Nutr Food Res, 50, 24-33; Schaefer et al., 2006, Mol Nutr Food Res, 50, 413-417]. The aglyca quercetin (Qu) and phloretin (Ph) and two known inducers of  $\gamma$ -GCL (menadione, Mn; sulforaphane, Sul), were also studied. mRNA levels of  $\gamma$ -GCL (catalytic and modulatory subunits) were analyzed by TaqMan PCR after 6 and 24h incubation.

Distinctly increased  $\gamma$ -GCL transcription was observed in Caco-2 cells after incubation with Sul (5/10  $\mu$ M) and Mn (10  $\mu$ M). Qu (10/30  $\mu$ M) and AE03 (50/100  $\mu$ g/ml) also elevated  $\gamma$ -GCL expression, but to a lower extent; Ph and AE02 (50/100  $\mu$ g/ml) were not effective. The known increase of glutathione level by Qu and AE03 [Schaefer et al., 2006] correlates well with  $\gamma$ -GCL gene expression. In HT-29 cells, Sul and AE03 strongly induced  $\gamma$ -GCL transcription, the other compounds were less effective (Mn > Qu, Ph > AE02); induction of  $\gamma$ -GCL by the aglyca corresponded to the distinct increase of glutathione level.

The results confirm the known induction of  $\gamma$ -GCL expression by Mn and Sul. Modulation of gene expression by aglyca and AEs varied with cell type and structure of the compounds: e.g. Qu and AE03 (rich in quercetin glycosides) are most effective, elevating  $\gamma$ -GCL transcription. A relation between  $\gamma$ -GCL transcription and glutathione level was partially observed, e.g. with Qu and AE03 (both effective in Caco-2 cells) and AE02 (not effective in HT-29 cells).

Support: BMBF grant no. 01EA 0501 and Land RLP, Schwerpunktförderung A4, „Darmgesundheit und Ernährung“

**P22**

**Assessment of UDP-glucuronosyltransferase (UGT) induction by apple polyphenols in the human colon adenoma cell line LT97**

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Purpose

UDP-glucuronosyltransferases (UGT) are phase II enzymes that have a major role in the glucuronidation and subsequent elimination of potentially toxic xenobiotics, endogenous compounds (e.g. bilirubin) and exogenous carcinogenic compounds (e.g. heterocyclic amines, polycyclic aromatic hydrocarbons). An enhanced expression of UGT family members may thus contribute to metabolic deactivation and facilitate excretion. Recent observations suggest that UGT inducers include dietary polyphenols, such as chrysin (5,7-dihydroxyflavone), quercetin and apigenin. It is the objective of this study to investigate other dietary flavonoids, such as those contained in apples, for their effects on expression of specific UGT-mRNA isoforms and UGT-enzyme activity in human colon adenoma (LT97) cells, which represent an early premalignant stage of tumor development.

Methods

LT97 cells were treated with a polyphenols-rich extract of apples (apple extract, AE) delivering 3.75 - 15  $\mu$ M phloridzin to the cell culture medium for 24 h. The RNA was extracted and used to synthesize labeled cDNA. This was hybridized to 2 different c-DNA arrays spotted with genes related to drug metabolism and to stress response. One (Superarray®) contained probe sets for 5 UGTs and the second (self-designed custom array) contained 17 additional UGTs. Differential gene expression was analyzed by comparing treated cells to control cells. Genes of interest were evaluated by qRT-PCR and UGT enzyme activity was measured using 4-methylumbelliferone (4-MU) which is a substrate for UGTs 1A1, 1A6, 1A7, 1A8, 1A10, 2B7 and 2B15.

Results

Superarray and custom array analysis showed that treatment of LT97 cells with AE highly modulated ( $\geq$  1.5-fold) several UGTs (UGT1A1, UGT2B7, UGT2B10, UGT2B11, UGT2B28) in LT97 cells. Directional changes of significantly modulated genes were confirmed by qRT-PCR (UGT1A1, UGT2B7, UGT2B10, UGT2B11 at 1.8, 3.4, 2.1 and 4.1 fold respectively). Microsomes from AE-treated LT97 cells, revealed a marked induction (1.7-fold) of the total UGT-enzyme activity, which may reflect enhanced expression of UGT1A1, and UGT2B7, UGT2B11.

Conclusions

In the present study we determined the ability of apple polyphenols to induce specific UGTs and UGT enzyme activity in a colon adenoma cell line. Induction of specific UGTs such as UGT1A1, UGT2B7 and UGT2B11 might facilitate glucuronidation and potential detoxification of the xenobiotics such as N-hydroxy-2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine or benzo[a]pyrene (substrates for UGT1A1) and opioids, androgens or mycophenolic acid (substrates for UGT2B7). In theory these findings may be thus related to mechanisms of chemoprevention, but the specific functional consequence of this UGT-induction by apple polyphenols, needs to be explored in more detail.

**P23**

**Induction of GST-T2 expression in HT29 colon epithelial cells by apple polyphenols**

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Apple polyphenols have been shown to protect colon epithelial cells from genotoxic damage and to attenuate colon carcinogenesis in rodent models. The underlying mechanisms for the protective activity are only partially understood. Expression array analysis revealed the regulation of multiple genes by treatment of colon epithelial cells with apple polyphenols *in vitro*. Among other genes, the one encoding glutathione-S-transferase T2 (GST-T2) was consistently upregulated in both HT29 colon carcinoma, and LT97 colon adenoma cells (S.Veeriah, T.Kautenburger, N.Habermann, J.Sauer, H.Dietrich, F.Will, B.L.Pool-Zobel, Mol. Carcinog. 2006 45:164-74, and S. Veeriah et al., unpublished data). Elevated GST-T2 activity might mediate some of the protective effects of apple polyphenols. Here, we addressed the issue whether expression regulation of GST-T2 can be recapitulated with a reporter containing GST-T2 promoter sequences (construct kindly provided by Dr. P. Buckland, Cardiff, UK). Apple polyphenol extracts consistently activated transcription of this reporter in HT29 cells 2- to 3-fold. We also tested a series of pure compounds, which are known components of apple polyphenol extracts, for their activity on the GST-T2 reporter. Among them, only chlorogenic acid stably induced reporter activity, albeit to a lesser extent than the complete polyphenol mixtures. In conclusion, chlorogenic acid and potentially further apple polyphenols activate the GST-T2 promoter. Future work will aim at the identification of involved promoter elements, and of further potential inducers contained in the polyphenol extract.

**P24**

**Modulation of cell growth and HDAC activity by colonic fermentation products of dietary fibre and apple juice polyphenols**

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Fermentation of nutritional compounds by the gut flora leads to different biologically active products, most of which are unknown. In the presence of dietary fiber sources i.e. pectin, a high amount of short chain fatty acids (SCFA) is formed. Butyrate, a major SCFA, is potentially chemoprotective by acting as inhibitor of histone deacetylases (HDAC). 3-Phenylpropionic acid, a phenolic carboxylic acid, was recently identified in human fecal water and is correlated to nutritional polyphenol intake.

Pectin, two different apple juice extracts (AE02; and the polyphenol-enriched APE03) and mixtures of apple juice extracts and pectin were fermented for 6 and 24 h with human faecal slurries *in vitro*. Fermentation supernatants (FS) were analysed for SCFA. Growth of HT29 cells, a human colon carcinoma cell line, was determined after treatment with different FS, SCFAs (butyrate, propionate, acetate, isobutyrate, isovalerate, valerate) or aromatic acids (phenylacetate, phenylpropionate, homo vanillic acid, p-coumaric acid, trans-cinnamic acid, 3,4 dihydroxyphenylacetate, 3-hydroxyphenylacetate and 3-(4-hydroxyphenyl)propionate), respectively. A reporter gene assay using transfected Hela cells and a fluorescence-based assay with isolated nuclear cell extracts of HT29 were performed to investigate HDAC activity.

FS are potent sources of SCFA and had only slight effects on the growth of HT29 cells up to a concentration of 5%. From the tested aromatic acids, only 3,4-dihydroxyphenylacetate was cytotoxic.

In HT29 nuclear extracts, treatment with propionate, butyrate and with a variety of aromatic acids as well as FS of pectin/AE02/APE03 resulted in a concentration dependent HDAC inhibition with butyrate being the most potent HDAC inhibitor. In intact Hela cells, butyrate was the most potent HDAC inhibitor followed by valerate. The other test compounds were inactive or showed lower potency, while all FS derived from fermentations of AE02, APE03, pectin or mixtures thereof were active. The SCFA content could not fully explain the observed effects found after incubation with FS. Our results indicate that other HDAC inhibitors are formed and/or act synergistically together with SCFA during fermentation of polyphenols and dietary fiber in the human colon. Additionally, the results suggest that HDAC inhibition can be obtained with aromatic acids added to HT29 nuclear extracts but not to intact Hela cells. This finding indicates that aromatic acids derived from nutrition may have limited access to intracellular targets such as HDACs. Nevertheless, further analysis of FS is indispensable to identify the active compounds in FS.

**P25****Activation of arbutin by human gut bacteria****A. Braune**, S. Wunderlich, P. Sauer, H. Schneider, H. Glatt, M. Blaut

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Arbutin (hydroquinone- $\beta$ -D-glucopyranoside) occurs naturally in various food plants such as pears and onions. Its aglycone, hydroquinone, is mutagenic and carcinogenic. Yet, little is known about the fate of arbutin following its ingestion. We investigated whether hydroquinone may be released from arbutin under conditions encountered in the human gastro-intestinal tract. Arbutin was stable in artificial gastric juice and, therefore, *in vivo* would reach the small intestine. Fecal slurries from nine human subjects completely converted arbutin (2 mM) into hydroquinone. No further degradation of hydroquinone was observed in any of the samples. We subsequently tried to identify intestinal bacteria that catalyze the release of hydroquinone from arbutin. The species selected were representatives of major bacterial population groups known to occur in the human gut. Four of the nine species investigated, namely *Eubacterium ramulus*, *Enterococcus casseliflavus*, *Bacteroides distasonis*, and *Bifidobacterium adolescentis*, deglycosylated arbutin at rates of 21.08, 16.62, 8.43 and 3.59 nmol  $\times$  min<sup>-1</sup>  $\times$  (mg protein)<sup>-1</sup>, respectively. In contrast, homogenates from small intestinal mucosa and cytosolic fractions from colon mucosa deglycosylated arbutin at substantially lower rates: 0.50 and 0.09 nmol  $\times$  min<sup>-1</sup>  $\times$  (mg protein)<sup>-1</sup>, respectively. Arbutin, unlike hydroquinone, did not induce gene mutations in Chinese hamster V79 cells in the absence of an activating system. However, in the presence of cytosolic fractions from *Eubacterium ramulus* or *Bacteroides distasonis*, arbutin was strongly mutagenic. Cytosolic fraction from *Escherichia coli*, showing no arbutin glycosidase activity, was not able to activate arbutin in this model system.

The high arbutin  $\beta$ -glucosidase activity detected in human fecal slurries and in pure cultures of intestinal bacteria together with the demonstration of the mutagenicity of the released hydroquinone toward eukaryotic cells clearly indicate the potential genotoxicity of dietary arbutin and the possible role of intestinal bacteria in its activation. As arbutin in fruit is matrix-bound, it is conceivable that a considerable proportion of dietary arbutin may reach the distal part of the human intestinal tract, where it would be a potential substrate for gut bacteria. The hydroquinone released in the intestinal tract may trigger mutations in epithelial cells of the mucosa. Whether this is of relevance, depends on whether and to which extent the liberated hydroquinone is detoxified or toxified by host enzymes.

**P26**

**Butyrate-induced apoptosis is mediated via the extrinsic and intrinsic pathway in colon cells from different stages of carcinogenesis**

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The short chain fatty acid butyrate, a product of bacterial fermentation in the colon, has previously been shown to induce apoptosis in colon cancer cells. Thus, butyrate is considered to play an important role in secondary chemoprevention. Controversial results are available regarding the mechanism of apoptosis induction by butyrate. To further elucidate the chemoprotective properties of butyrate and the mechanism of apoptosis induction, we analysed its effects on cell proliferation and determined molecular parameters of apoptosis in two colon cancer cell lines. We used LT97 cells, a colon adenoma cell model representing an early stage of carcinogenesis, and HT29 carcinoma cell line, a model for cells in the later stage of the cancer process. Both cell lines were incubated with different concentrations of butyrate, and effects on cell proliferation were examined. Induction of apoptosis was analysed by detection of PARP cleavage on Western blots and of caspase activity with Apoalert® Caspase Assay Plates (Clontech). Butyrate reduced cell number in a time- and dose-dependant manner in both LT97 and HT29 cells. The determined EC<sub>50</sub> values after 48 h incubation with butyrate were 2.8 mM for HT29 cells and 0.8 mM for LT97 cells. Thus, LT97 were more sensitive to butyrate-treatment. Furthermore, butyrate clearly induced apoptosis, detected by amount of cleaved poly(ADP-ribose)-polymerase, in LT97 cells incubated for 24 h with 2 mM butyrate. In HT29 cells, in contrast, only minor effects on apoptosis were detected using these conditions. Elongated incubation periods (48 h) or increased butyrate concentrations (10 mM) induced apoptosis also in HT29 cells. Next, the influence of butyrate on activities of caspase-2, -3, -8 and -9 was analysed to obtain more information on the mechanism of butyrate-induced apoptosis. All analysed caspases were significantly activated by treatment with butyrate in both cell lines. Since caspase-8 is a key enzyme of extrinsic apoptosis and caspase-9 a key enzyme of intrinsic apoptosis, butyrate apparently induced apoptosis by activation of both pathways, a finding which provides new insights on the possible mechanism of apoptosis induction. Moreover since adenoma cells were more susceptible than the cancer cell line, the findings indicate for the first time that butyrate may also be active in the earlier stages of the carcinogenesis process. Hence, butyrate's important function in secondary chemoprevention is supported. Since butyrate levels are increased by consumption of dietary fibres, the presented results illustrate the importance of dietary fibres in a healthy diet.

**P27**

**Fermented apple juice extracts reduce oxidative stress in human colon carcinoma cell line Caco-2**

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Cancer and other degenerative diseases have been associated with chronically increased formation of reactive oxygen species (ROS). For colorectal cancer, a leading cause of cancer death in Western countries, studies have shown an inverse correlation between fruit and vegetable consumption and disease risk. Apples and apple juice represent a major dietary source of polyphenols. Polyphenol-rich apple juice extracts have been shown to protect colon cells against oxidative cell damage in-vitro [Schaefer et al., 2006, Mol Nutr Food Res, 50, 24-33; Schaefer et al., 2006, Mol Nutr Food Res, 50, 413-417]. Since a major part of ingested flavonoids/polyphenols are metabolised by the gut microflora, products/ metabolites generated might well contribute to the antioxidative efficacy.

In this study, two polyphenolic apple juice extracts (AEs) have been subjected to anaerobic fermentation for 6 and 24 h, using fresh human faeces (fermentation supernatants, FSAE<sub>6h</sub> and FSAE<sub>24h</sub>). For each fermentation period, a control (fermentation without AE, FS Blank) was included. The antioxidant capacity of the FSAEs was determined with cell-free ABTS-decolourisation assay (Trolox Equivalent Antioxidant Capacity, TEAC). After 24 h treatment of Caco-2-cells with FSAEs, cellular ROS-level was determined by Dichlorofluorescein (DCF)-Assay, using tert-Butylhydroperoxide (TBH, 250 µM, 40 min) to generate increased oxidative stress. Fermentation decreased antioxidant capacity of both AEs from 4.2 / 4.0 mM to 2.7 / 3.0 mM (6 h) and to 2.1 / 2.9 mM Trolox (24 h), respectively. The FS Blanks showed only a marginal antioxidant capacity (≤ 0.3 mM). After incubation with FSAEs (concentrations equivalent to 0.5 – 50 µg AE/mL), TBH-induced ROS-level was decreased in a concentration-dependent manner. At higher concentrations, FSAEs reduced the ROS-level more efficiently than the original AEs. FSAEs and the corresponding FS Blanks were almost similarly effective; after 6h fermentation, the FSAEs decreased the ROS-level to a slightly higher extent, compared to the FS Blank.

In conclusion, fermentation of phenolic apple juice extracts increases their potential to reduce cellular ROS-level in human colon carcinoma cells, even though the cell-free antioxidant capacity is clearly diminished. Comparing the results of FSAEs and FS Blanks suggests that microbial fermentation of the AEs contributes to their preventive effects in colon cells.

Support: BMBF grant no. 01EA0501

**P28**

**Impact of degradation products and H<sub>2</sub>O<sub>2</sub> formation on the growth inhibitory properties of quercetin in human colon tumour cells**

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Quercetin (QUE) has been reported to interfere with cellular signaling cascades, inhibiting the growth of human tumour cells in-vitro. However, depending on the respective experimental conditions and the apparent limited chemical stability of quercetin, at least a partial degradation can not be excluded. Under in-vivo conditions in the gut, extensive microbial degradation of flavonoids has been postulated. Therefore, we addressed the question whether potential degradation products of QUE, 3,4-dihydroxyphenylacetic acid (HPA) and phloroglucinol (PHG) might contribute to the cellular effects of QUE. Furthermore, cell incubation with quercetin has been associated with the formation of H<sub>2</sub>O<sub>2</sub>, which might also contribute to the growth inhibitory properties of this compound.

Effects of QUE and its respective degradation products on the growth of the human colon cancer cell line HT29 were determined in the sulforhodamine B assay in the absence or presence of catalase (CAT) or sodium ascorbate (ASC) to eliminate H<sub>2</sub>O<sub>2</sub>. The microbial degradation of QUE led to two products with growth inhibitory properties. Whereas PHG failed to reach an IC<sub>50</sub>-value up to 300 µM, HPA effectively inhibited the growth of HT29 cells, comparable in its potency with the parental flavonol. The presence of CAT or ASC does not affect the growth inhibitory properties of QUE. However, a contribution of H<sub>2</sub>O<sub>2</sub> to the growth inhibitory properties of the potential degradation product HPA can not be excluded.

We previously showed that QUE represents a potent inhibitor of the epidermal growth factor receptor (EGFR), an effect which might at least contribute to the growth inhibitory properties of the compound. In contrast, the potential degradation products HPA and PHG were found to possess only marginal inhibitory properties on the activity of the EGFR.

In summary, the results let assume that the degradation of QUE generates a phenolic acid with substantial growth inhibitory properties. This phenolic acid appears to differ from the parental quercetin with respect to its impact on cell signaling cascades, as exemplified for the EGFR. Merely formation of H<sub>2</sub>O<sub>2</sub> seems not to be responsible for the growth inhibitory properties of the test compounds; however a contribution could totally be excluded. The mechanism of action responsible for the inhibition of tumor cell growth by HPA is not known so far and demands further investigation.

The study was performed within the FlavoNet, funded by the Deutsche Forschungsgemeinschaft, grant MA 1659/4-1.

## **Poster Session III**

Animal and human intervention studies: Biological effects

**P29**

### **Tissue distribution and metabolism of the flavon hispidulin in animals**

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We have investigated the distribution of hispidulin (4',5,7-trihydroxy-6-methoxyflavone) to brain, kidneys, liver, spleen and peritoneal fat, in relation to the levels of the circulating hispidulin forms in blood, following intraperitoneal administration of radio-labelled hispidulin. The resulting data are obtained from the total radioactivity analysis, high performance liquid chromatography (HPLC) with online autoradiography detection, HPLC electrospray tandem mass spectrometry (HPLC-ESI-(MS)MS/MS), high resolution gas chromatography mass spectrometry (HRGC-MS) as well as from the autoradiography studies of the organs investigated. For the first time our studies demonstrated that hispidulin crosses the blood brain barrier *in vivo*. Shortly after the intraperitoneal injection of hispidulin to mice, the analyte and three other metabolites are detectable in blood and in different tissues under study. In brain, fat and in spleen, only hispidulin was detectable. Hispidulin monoglucuronide was identified by hydrolytic enzyme treatment and HPLC-ESI-MS/MS methods in blood, liver and kidney samples. This glucuronide was formed via phase II liver metabolism and imitated *in vitro* formed by incubation with rat liver hepatocytes and human recombinant UDP-glucuronosyl transferase. In liver, kidney and blood a methylated derivative, circimaritin (4',5-dihydroxy-6,7-dimethoxyflavone), and in kidney and blood extracts a polar metabolite of hispidulin was observed.

**P30**

**Effect of cocoa flavonoids intake on spleen immune function in young rats**

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Objective

Previous studies *in vitro* showed the down-regulating influence of cocoa flavonoids on lymphocyte activation. These results led us to establish the influence of a cocoa enriched diet on the spleen function of young rats.

Material and Methods

An extract of natural cocoa (*Forastero*), containing 32 mg flavonoids/g, was daily administered by oral gavage (3.5 % w/w food intake) to Wistar rats, just after weaning and during 3 weeks. In 6-week-old rats, blood was obtained by cardiac puncture and spleen was removed to isolate lymphocytes. Cell phenotype was determined by double-staining with fluorochrom conjugated monoclonal antibodies followed by flow cytometry analysis. Immunoglobulin (Ig) A-secreting cells were quantified by ELISPOT technique and IgM secretion was determined after 3 and 6 days of cell culture by ELISA. In addition, the response of lymphocytes to an unspecific stimulus was also studied. Cells were plated and stimulated with phorbol miristate acetate plus ionomycin (PMA+I) for 20 h. Then, supernatants were collected and interleukin (IL)-2, a lymphocyte activation marker, was quantified by ELISA.

Results

Although cocoa-fed rat spleen showed a lower proportion of T helper/T cytotoxic ratio, the levels of IL-2 secreted by spleen cells in response to PMA+I were not modified. Therefore, the ability to produce IL-2 by spleen T helper cells was enhanced after cocoa intake. Spleen IgA secreting capacity was similar in both groups of animals, but IgM was significantly reduced in cocoa fed group, especially after 3 days of cell culture. However, sera IgA and IgM were not affected by cocoa supplementation.

Conclusion

These results suggest the influence of cocoa flavonoids on certain immune functions. The consequences of these effects on physiological status remain to be elucidated.

**P31**

**A chronic consumption of cocoa increases the antioxidant status in thymus in young rats**

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Objective

Cocoa is a rich source of antioxidants called flavonoids. The present study is focused on establishing whether chronic consumption of a cocoa enriched diet influences antioxidant capacity and cellular antioxidant enzymes of young rats.

Material and Methods

Wistar rats were fed with diet containing 3.5% (w/w) of natural cocoa powder (*Forastero*) containing 32 mg flavonoids/g, just after weaning and during 3 weeks. In 6-week-old rats, blood was obtained by cardiac puncture and thymus, spleen and liver were removed to determine the total antioxidant capacity (TAC) and catalase (CAT) and superoxide dismutase activities. Tissues were homogenized in cold phosphate buffer and frozen at -80°C until assays were performed.

TAC was determined by colorimetric ABTS assay using Trolox as standard. Catalase activity was established by the ability to produce formaldehyde in the presence of methanol and H<sub>2</sub>O<sub>2</sub>. This formaldehyde was spectrophotometrically measured using purpald as chromogen. Superoxide dismutase activity was quantified using a tetrazolium salt to detect superoxide radicals generated by the addition of xanthine oxidase. One unit of SOD is defined as the amount of enzyme needed to exhibit 50 % dismutation of the superoxide radical. Catalase and superoxide dismutase activities were expressed as μmol/min or units per mg of protein, respectively. Protein concentration was quantified by Bradford assay using bovine serum albumin as standard.

Results

Cocoa enriched diet highly improved the TAC in thymus, being the catalase and superoxide dismutase activities also enhanced. TAC was also increased in spleen, but there were no significant differences in catalase and superoxide dismutase activities in this tissue. Liver TAC, catalase and superoxide dismutase activities were not affected by cocoa supplementation.

Conclusion

The chronic consumption of cocoa during the youth enhances the antioxidant status of rats. Therefore, cocoa supplementation could play an important role in the prevention and amelioration of certain diseases associated with high production of free radicals.

**P32**

**A cocoa-enriched diet induces antiinflammatory potential in young rats**

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Introduction and Objective

In previous studies, we observed in vitro that secretion of proinflammatory mediators by macrophages was significantly inhibited by cocoa extract. Exactly, cocoa flavonoids diminished, among others, tumor necrosis factor alpha (TNF $\alpha$ ) produced by stimulated macrophages. TNF $\alpha$  is considered a key mediator of innate immunity since it activates neutrophils, promotes adhesion molecule expression on vascular endothelial cells, and enhances the secretion and synthesis of other proinflammatory cytokines and acute phase proteins. TNF $\alpha$  also acts as an endogenous pyrogen. In this study we determined the secretion of TNF $\alpha$  by peritoneal macrophages obtained from young rats after 3 weeks of a cocoa enriched diet.

Material and Methods

Wistar rats were fed daily with natural cocoa (containing 3.2 g polyphenols/100 g) at doses of 3.5% or 10% food intake. Cocoa was administered by oral gavage (about 3.5% w/w food intake) or in the chow (10% w/w) just after weaning and during three weeks. In 42-day-old rats, peritoneal macrophages were isolated by peritoneal lavage with 40 mL of cold sterile PBS. Peritoneal cells were plated at  $1.2 \times 10^6$ /mL in RPMI supplemented with 10% FCS. Non-adherent cells were removed by washing three times with PBS after 5 h of culture. The attached macrophages were stimulated by addition of LPS (1  $\mu$ g/mL). After 24 h of stimulation, cells were harvested to determine cell viability and supernatants collected and stored at  $-80^\circ\text{C}$  for further TNF $\alpha$  quantification. Levels of TNF $\alpha$  were quantified by ELISA using rat TNF $\alpha$  OptEIA sets from BD Pharmingen (Madrid, Spain).

Results

In vitro, peritoneal macrophages produced TNF $\alpha$  although they were not LPS-stimulated. In this condition, macrophages from cocoa-fed rats (3.5 or 10%) and standard-fed rats secreted the same amount of the proinflammatory cytokine. However, after 24 h of LPS-stimulation, macrophages obtained from cocoa-fed animals produced significantly less amount of TNF $\alpha$  than cells obtained from reference animals. This result was observed in macrophages from animals with a cocoa-enriched diet of 10% but not when cocoa only represented 3.5% of food intake. This difference could be related to cocoa dose or to the effect of time elapsed between cocoa intake and macrophage isolation.

Conclusion

These results suggest the potential value of cocoa as antiinflammatory bioactive nutrient.

**P33**

**Influence of apple juice on inflammatory changes in the rat colon**

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With the aid of animal models it is possible to simulate the human intestinal bowel diseases ulcerative colitis (UC) and Crohn's disease (CD) by chemical damage of the intestinal mucosa. While UC is mostly limited to the colon CD is able to affect the whole intestinal tract from the oral cavity to the anus. Mostly the inflammation is limited to the gut while often several parts are affected.

Up to now the exact causes for IBD are unsettled. Besides genetic factors, the intestinal flora and environmental factors like stress and nutrition are discussed as causative factors. The increasing incidence of IBD in the western industrial countries is associated with a carbohydrate rich, fat rich and low fibre diet.

It was the aim of this study to investigate the influence of apple juice on a dextrane-sulfate-sodium (DSS) induced colitis in the adult male rat. For this purpose, drinking water containing 3 % DSS (MW 36.000-50.000) was offered to the animals for ten days ad libitum. Subsequently, drinking water was offered for seven days ad libitum. Animals were sacrificed and tissue samples were taken from day 1-17 of the treatment. To investigate the influence of apple juice on the inflammation process cloudy or clear juice instead in drinking water was offered to the animals ad libitum seven days before and after DSS treatment.

Macroscopically, the inflammation got visible at the eighth day of the DSS-treatment and was still visible after the end of the DSS-treatment. The colitis was alleviated by clear apple juice but especially by cloudy apple juice. The following parameters were improved: Body weight change, ratio between bowel weight and bowel length, whole tissue myeloperoxidase activity and levels of transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1).

In conclusion the application of apple juice had a positive influence on a DSS induced colitis in adult male rats. Cloudy apple juice showed a stronger anti-inflammatory effect than clear apple juice.

Financed by the Bundesministerium für Bildung und Forschung, Bonn.

**P34**

**Cloudy apple juice does not decrease the colonic formation of 1,2-dimethylhydrazin-induced aberrant crypt foci which are enhanced by obesity in Zucker rats**

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To investigate the link between genetic and nutritional factors in the development and prevention of colon cancer we have characterised the metabolic parameters in lean (fed ad libitum) and obese Zucker rats (fed ad libitum and pair fed) and examined how these factors affect colon carcinogenesis induced by 1,2-dimethylhydrazin (DMH).

Ten-weeks pair feeding, corresponding to an energy reduction of about 30% compared to ad libitum feeding, did not prevent the development of obesity, but led to a significant reduction in body weight, serum free fatty acids, total cholesterol and triglyceride concentrations in obese Zucker rats. Obese ad libitum rats had a higher cumulative energy intake, weight gain and serum concentrations of free fatty acids, total cholesterol and triglycerides than the lean Zucker rats. Serum levels of glucose were higher in obese pair fed rats than in either of the other groups.

The DMH-induced formation of aberrant crypt foci (ACF) in the colon was most pronounced in obese ad libitum rats. Pair feeding significantly reduced the formation of ACF while lean rats showed the lowest level of number/size of ACF. There were no differences between the groups in the levels of DMH-induced genotoxic damage or proliferation of colonocytes.

Intervention with cloudy apple juice led to a significantly elevated final weight in obese ad libitum rats, as compared with obese ad libitum control rats consuming an energy adjusted control drink. The weight of the obese pair fed and the lean Zucker rats did not reveal any differences between the apple juice and the control group. Intervention with apple juice applied within a pre-initiation protocol did not affect DMH-induced DNA-damage or the number/size of ACF in the distal colon in any group.

In summary these data show the strong obesity-associated promoting influence on cancerous processes in the colon as well as the beneficial effect of energy reduction on colon carcinogenesis in obese rats. Consumption of apple juice has no effect on those analyzed colon cancer-related biomarkers which are enhanced by obesity in this animal model.

This study was supported by the project grant -01 EA0105- from Federal Ministry of Education and Research of Germany.

**P35****The effect of beetroot juice fermented by probiotic bacteria of *Lactobacillus* sp. on intestinal microflora of rats**

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The fermented red beetroot juice (technology developed by the authors) not only displays nutritive value typical of vegetables, i.e. it supplies vitamins, macro- and microelements and has high antioxidative potential, but also contains live cells of two probiotic bacterial strains *Lactobacillus casei* and *Lactobacillus brevis* ( $3.5\text{--}4.0 \times 10^9$  CFU/ml). This juice was supplemented with apple pulp (10% w/v) to improve sensory and rheological attributes. The effects to health of the fermented beetroot juice were tested using Wistar rats. Three groups of rats (8 males in each group) were administered for 4 weeks with 3 different volumes of the juice (1.5, 3 and 6 ml) every day. The control group of rats was fed with the basic (casein) diet. The volumes of juice samples were calculated on the basis of assumption that humans will ingest 500 ml of juice every day (7 ml/kg of body weight). This in turn was equivalent to 1.5 ml of juice per day for the model laboratory rat because the average weight of each of them was 200 g. Larger aliquots (3 and 6 ml) of juice per day were administered to the rats to evoke the explicit reaction of gastrointestinal tract. Composition of caecal microflora was examined by the routine agar plate tests for *Lactobacillus* sp., *Bifidobacterium* sp., *Clostridium* sp., *Bacteroides* sp., *Enterococcus* sp., *Enterobacteriaceae* and the total number of anaerobic bacteria. The intestinal epithelium histology was carried out by light and electron microscopy methods.

The number of *Lactobacillus* cells was changed in none of the examined groups of animals and was maintained on the level of  $1.5 \times 10^6$  –  $4.6 \times 10^6$  CFU/g. In contrast, the population of *Bifidobacterium* sp. was reduced from  $10^5$  CFU/g to  $10^3$  CFU/g. A drop in a number of *Bifidobacterium* cells could have resulted from the antagonistic activity of *Lactobacillus* strains ingested with the juice.

Everyday consumption of 1.5 and 3 ml of the fermented beetroot juice caused a significant decrease in population of *Bacteroides* sp. (by one order of magnitude) from  $8.5 \times 10^5$  (the control group) to  $1.5 \times 10^4$  -  $2.1 \times 10^4$  CFU/g. Also the *Enterobacteriaceae* sp. were affected and their total number was lessened from  $9 \times 10^4$  CFU/g (control group) to  $1.8 \times 10^3$  CFU/g. The suppression of both the genera is advantageous because these bacteria are involved in synthesis of carcinogens. The intestinal histology of rats from these two groups showed no aberration from the normal state.

In case of the third group of rats, i.e. these which were administered 6 ml of the fermented beetroot juice, only the number of *Enterobacteriaceae* cells was decreased (to  $5 \times 10^2$  CFU/g) and the number of *Bacteroides* sp. cells was maintained at approximately  $10^6$  CFU/g. Histological examination of the ileum showed increased number of lymphocytes in lamina propria of this group.

Ultrastructural analyses presented injured intestinal microvilli and intraepithelial junctions, i.e. structures responsible for mucosal barrier.

Thus, the fermented red beetroot juice containing live cells of *Lactobacillus* sp. and administered in aliquots of 1.5 and 3 ml per day benefited the intestinal microflora of rats and reduced the number of bacteria capable of producing carcinogens. Because of microscopical changes of intestinal mucosa within the group of rats with a high intake of juice (6 ml), the determination of the safe dose of the juice is a prerequisite. Studies focused on identification of factors responsible for the observed pathology of the intestinal mucosa are in progress.

Presented studies have been partially supported by the grant (no PBZ-KBN-094/P06/2003) from the State Committee for Scientific Researches.

**P36****Antioxidative capacity in human faeces after intervention with black currant seed press residues****D. Ulbricht, A. Wagner, S. Schulze, G. Jahreis**

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In a human intervention study 18 female smokers consumed subsequently to a baseline period (period I) 10 g seed press residues of black currant per day for four weeks (period II). They continued for another four weeks with 20 g/d (period III). Black currant seed press residues are rich in tocopherols (17.5 mg/100 g total tocopherol) and also contain anthocyanins (118 mg/100 g); both act as antioxidants. At the end of each period faeces have been collected for three days while a standardised diet was administered. Total faeces was mixed, lyophilised, grinded and stored at -20 °C. For sample preparation faeces was incubated with phosphate buffer containing salicylic acid and iron ions for 18 h at 37 °C. After centrifugation, samples have been filtered and measured using HPLC-DAD. Thereby the main principle of the reaction is the aromatic hydroxylation of salicylic acid in the presence of ephemeral hydroxyl radicals to 2,3- and 2,5-dihydroxybenzoic acid (DHBA) which is stable and can be detected to judge the antioxidative capacity of faeces.

Our results show a significant reduction in 2,5-DHBA from the baseline period ( $291 \pm 169 \mu\text{mol}$ ) to the intervention period III  $218 \pm 129 \mu\text{mol}$  when based on 100 g dry matter of faeces ( $p < 0.05$ ). This reduction is also significant between period II ( $280 \pm 178 \mu\text{mol}$ ) and III ( $p < 0.05$ ). Concerning the 2,3-DHBA formation there is only a trend in reduction at period II to III ( $311 \pm 207 \mu\text{mol}$  to  $272 \pm 155 \mu\text{mol}$  in 100 g dry matter of faeces) ( $p < 0.1$ ). Summing up the both generated DHBA the reduction is significant from period II to III ( $591 \pm 383 \mu\text{mol}$  to  $490 \pm 278 \mu\text{mol}$  in 100 g dry matter) ( $p < 0.05$ ) and there is a trend in reduction from period I to III ( $606 \pm 352 \mu\text{mol}$  to  $490 \pm 278 \mu\text{mol}$  in 100 g dry matter) ( $p < 0.1$ ).

The formation of DHBA from salicylic acid represents the power of antioxidative capacity of the faecal matrix after provoked hydroxyl radical formation. Our results show an enlarged capacity to scavenge those radicals when 20 g/d black currant seed press residues are consumed. This beneficial effect can be due to tocopherols and anthocyanins of the black currant seed.

**P37**

**Comparison of organically and conventionally produced carrots: Carotenoid content, bioavailability and antioxidant capacity**

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A diet rich in fruits and vegetables exerts protective effects against cancer and cardiovascular diseases. These beneficial health effects are imparted by the antioxidant properties of carotenoids. Carotenoids are secondary metabolites and the most widespread group of pigments in nature, with at least 600 members. They are present in all photosynthetic organisms and responsible for most of the yellow to red colours of fruits and flowers.

The aim of the present study was to compare the carotenoid content and antioxidant capacity of carrots grown under organic or conventional conditions (harvest 2005) *in vitro*. Furthermore the influence of consumption of cooked carrots from organic or conventional production systems on the antioxidant and carotenoid status in humans was assessed.

In order to measure the antioxidant activities in carrots (Narbonne, Nerac) and in human plasma three different *in vitro* test systems were used: FRAP-, ORAC- and TEAC-assay. The carotenoid contents in carrots and human plasma were identified and quantified using HPLC/DAD/MS. Furthermore, a double-blinded, randomized human intervention study was conducted. 36 healthy volunteers (2 intervention groups, 1 control group, n=12, respectively) consumed organically or conventionally produced carrots (Narbonne, 2 weeks, 200 g daily).

Organic carrots had significantly higher lutein contents (Narbonne 28%, Nerac 16%) and higher antioxidant capacities (Nerac 10%; FRAP, Narbonne 20%; TEAC) than conventionally produced carrots. Additionally, the analysis of the two varieties Narbonne and Nerac showed, that Narbonne exhibited a higher  $\beta$ -carotene content (10%) and antioxidant activity (FRAP, 20%) than Nerac.

In the human intervention study no significant differences in the antioxidant capacity of human plasma after consumption of carrots (FRAP, ORAC, TEAC) were observed. The plasma content of vitamin C was significantly reduced in all groups (2 interventions, 1 control). The impact of carrot consumption on the concentration of carotenoids in plasma is currently analysed.

In conclusion, the different cropping systems had inconsistent effects on the carotenoid content and antioxidant capacity of carrots. Carrot consumption further did not affect the antioxidant status in humans. The decrease in vitamin C is caused by the low intake of fruits and vegetables during the intervention trial.

Supported by the "Bundesprogramm Ökologischer Landbau"

**P38**

**Watercress supplementation reduces lymphocyte DNA damage and alters blood antioxidant status**

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A number of epidemiological studies have provided evidence that consumption of cruciferous vegetables (CVs) is associated with a decreased risk of several cancers. Members of the *Cruciferae* family have been shown to contain high amounts of phenolic compounds. The CV, Watercress (*Rorippa nasturtium-aquaticum*) in particular contains one of the highest concentrations of glucosinolates per gram weight of any vegetable as well as containing high levels of folate, lutein and  $\beta$ -carotene. The anti-carcinogenic properties associated with *Cruciferae* have been in part associated with such phytochemicals.

*In vitro* study- HT29 human colonocyte cells were used to investigate the protective effects of a watercress extract (0-50 $\mu$ l/ml) on DNA damage and the cell cycle. The extract (50 $\mu$ l/ml) inhibited DNA damage ('comet' assay) induced by two genotoxins hydrogen peroxide ( $p=0.011$ ) and faecal water ( $p=0.039$ ). Watercress extract (20, 50 $\mu$ g/ml) caused an accumulation of HT29 cells in the S phase of the cell cycle ( $p\leq 0.05$ ).

*In vivo* study was to determine effects of watercress supplementation on biomarkers related to cancer risk. A single blind, randomised crossover study was carried out with 60 healthy subjects, equal numbers of males, females, smokers and non smokers were used {mean (range) age – 33 (19-55) years}. Subjects consumed 85g of raw watercress daily for 8 weeks in addition to their habitual diet. The effect of supplementation was measured on a range of endpoints including DNA damage in lymphocytes ('comet' assay), activity of detoxifying enzymes (glutathione peroxidase, superoxide dismutase) in erythrocytes, plasma antioxidants ( $\beta$ -carotene, lutein, retinol,  $\alpha$ -tocopherol, ascorbic acid), plasma lipid profile and plasma total antioxidant status (FRAP assay). Watercress consumption (active vs control phase) was associated with decreases in basal DNA damage (by 17%,  $p=0.03$ ), in basal plus oxidative purine DNA damage (by 23.9%,  $p=0.002$ ) and in basal DNA damage in response to *ex vivo* hydrogen peroxide challenge (by 9.4%,  $p=0.07$ ).  $\beta$ -carotene and plasma lutein were significantly increased by 33% and 100% respectively ( $p\leq 0.001$ ) as a result of consumption. Greater changes were observed in smokers compared to non-smokers. The results support the theory that consumption of watercress may be linked to a reduced risk of cancer via decreases in damage to DNA and possible modulation of antioxidant status.

**P39**

**Bolus ingestion of white or green tea stabilizes the extracellular pro-/antioxidant balance but does not protect from H<sub>2</sub>O<sub>2</sub>-induced DNA strand breaks in leukocytes**

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Introduction

Unfermented teas (green tea, GT; white tea, WT) are nutritive sources of highly active antioxidants like flavan-3-ols. The effects of WT and GT consumption on pro-/antioxidant balance and oxidative stress have, however, not been completely understood. Hence, the aim of this randomized, controlled intervention study was to investigate the effects of bolus ingestion of WT or GT on plasma antioxidant capacity and parameters of oxidative stress in healthy volunteers.

Methods

Sixty-eight non-smokers (21 m, 47 f; 24±4 yrs; BMI 22.0 ± 2.2 kg/m<sup>2</sup>) were stratified by sex and randomized to consume a single dose of 600 mL WT (226 mg catechin equivalents/L) or 600 mL GT (395 mg catechin equivalents/L) or 600 mL water (controls) after an overnight fast. Twenty-four hours before the study, participants were asked to renounce from foods rich in polyphenols and antioxidant vitamins (handout). The study drinks were consumed within 15 min and blood samples were taken before, 2 and 5 hours (hrs) after ingestion. Antioxidant capacity was measured as TEAC. DNA strand breaks of leukocytes (SB) were measured *in vivo* and *ex vivo* (H<sub>2</sub>O<sub>2</sub> challenge) by Comet Assay. Plasma-8-Iso-prostaglandin-F<sub>2α</sub> were analysed by ELISA, total phenolic content (TPC) and uric acid by photometry, vitamin C by HPLC.

Results

(Table, means ±SD). Baseline levels were identical in all groups except for uric acid (Mann-Whitney-U-test). TEAC was not influenced by tea consumption but decreased in controls. In all groups SB *in vivo* were higher 2 hrs after ingestion compared to baseline and to 5 hrs. Vitamin C increased 2 hrs after GT and water consumption and returned to baseline level in both groups. Changes in 8-iso-prostaglandin-F<sub>2α</sub> occurred only in control subjects, with a tendency towards increase after 2 hrs (p=0.053) and a subsequent decline after 5 hrs.

	White tea (n=23)			Green tea (n=22)			Water (n=23)		
	0h	2h	5h	0h	2h	5h	0h	2h	5h
TEAC [mM TE]	1.67 ±0.07	1.70 ±0.06	1.69 ±0.07	1.67 ±0.09	1.67 ±0.08	1.69 ±0.10	1.69 ±0.07 <sup>a</sup>	1.70 ±0.12 <sup>b</sup>	1.64 ±0.05 <sup>ab</sup>
SB <i>in vivo</i> [TM]	2.1 ±1.3 <sup>c</sup>	3.6 ±1.5 <sup>cd</sup>	1.8 ±1.4 <sup>d</sup>	2.7 ±1.4 <sup>e</sup>	4.7 ±2.0 <sup>ef</sup>	2.2 ±1.7 <sup>f</sup>	2.6 ±1.5 <sup>gh</sup>	3.8 ±1.9 <sup>eg</sup>	1.5 ±1.4 <sup>eh</sup>
SB <i>ex vivo</i> [TM]	0.8 ±1.5	0.6 ±1.6	0.6 ±1.4	0.8 ±1.4	0.3 ±1.1	0.8 ±1.2	1.1 ±1.4	0.6 ±2.0	0.5 ±1.4
TPC [mg CE/L]	22.5 ±3.5	22.9 ±3.6	22.4 ±3.8	22.5 ±3.0	22.9 ±2.9	22.7 ±2.9	22.2 ±3.4	22.2 ±2.4	21.8 ±3.1
Vitamin C [mg/L]	9.6 ±2.6	9.8 ±2.8	9.6 ±2.6	10.2 ±2.7 <sup>i</sup>	11.2 ±2.9 <sup>ij</sup>	10.2 ±3.3 <sup>j</sup>	10.2 ±2.7 <sup>i</sup>	10.9 ±3.2 <sup>i</sup>	10.3 ±2.9
Uric acid [mg/dL]	3.20 ±1.12	3.12 ±1.01	3.24 ±1.05	3.03 ±0.93 <sup>l</sup>	2.95 ±0.98 <sup>ai</sup>	3.30 ±1.03 <sup>a</sup>	3.70 ±0.91	3.60 ±0.90	3.63 ±0.67
8-Iso-prosta- glandin-F <sub>2α</sub> [pg/mL]	9067 ±3564	9254 ±3820	9435 ±3526	8829 ±3125	9008 ±3446	9252 ±2943	8500 ±3024	9306 ±4053 <sup>l</sup>	8289 ±2611 <sup>l</sup>

TEAC: Trolox equivalent antioxidant capacity; TE: Trolox equivalents; SB: DNA strand breaks, TPC: total phenolic content; CE: catechin equivalents. Identical letters: significant differences within each group: c, d, h, j, k, l:  $p \leq 0.05$ ; a, f, g, i:  $p \leq 0.01$ ; b, e:  $p \leq 0.001$  (Wilcoxon signed rank test).

#### Discussion

After an one-day antioxidant poor diet, bolus ingestion of unfermented tea, in contrast to water, maintains the antioxidant defence transiently probably due to the supply of flavan-3-ols. Interestingly, GT was not superior to WT despite its higher flavan-3-ol content. The lacking effects on *ex vivo* SB might be explained by a generally high level of antioxidant protection of these healthy subjects.

**P40**

**The effects of carrot and hazelnut supplementation on super oxide dismutase (SOD) activity and some lipid parameters**

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The effects of dietary factors on SOD activity were investigated. 38.0±6.9 years of age 28 healthy volunteers were randomly selected and beside their daily diet they were supplemented with carrot and hazelnut for 75 days. Blood samples were taken from subjects both in the initial and the final of the study and analysed for Hb, SOD, T. cholesterol, triglyceride, HDL, LDL, VLDL cholesterol and fasting blood glucose levels. During the study dietary records were recorded 3 different times and energy values, nutrient consumption levels were calculated. At the end of the study, SOD activity was found to have increased in the diet supplemented with carrots group ( $p<0.05$ ). A significant positive relation ( $p<0.05$ ) was determined between the SOD activity and dietary iron, calcium, zinc, vitamin B group,  $\beta$ -carotene, vitamin A, E and C. SOD activity was not changed in the diet supplemented with hazelnut group. The findings indicated that nutrient composition of foods effect the antioxidant system of metabolism.

**P41**

**Effect of *Spinacia oleraceae* L. and *Perilla frutescens* L. in a human intervention study**

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*Perilla frutescens* L. is a vegetal food frequently used as one of the most popular garnishes and edible colorant in some Asian countries such as China and Japan. Several parts of the plants are used: seeds and leafs are used for cooking, the seed oil and Perilla leaf extract are utilized in traditional Chinese medicine. The leaves of *P. frutescens* possess detoxicant, antitussive, antibiotic and antipyretic properties and are used in folk medicine for treating intestinal disorders and allergies. Main phenolic compounds are rosmarinic acid besides small amounts of flavonoids and phenolic acids, e.g. apigenin or caffeic acid.

We recently demonstrated high contents of carotenoids and high antioxidative capacity in extracts from Perilla leaves. For this reasons, *Perilla frutescens* L. is a promising species with high nutritional or even medical value. Therefore it could provide a new vegetable in European diet to support healthy nutrition and to meet the directions of the "5 a day" campaign.

The aim of this study was to compare spinach, a widespread European vegetable which is considered to be rich in SPM, with this new asia salad. For this purpose we analysed polyphenolic content, carotenoid composition and the according antioxidative capacity of these two leafy vegetables.

Additionally an intervention study was conducted to determine the effect of perilla and spinach on several parameters of human blood plasma. Besides standard blood parameters main antioxidants (e.g. uric acid), antioxidant enzymes (e.g. SOD) and markers of oxidative stress (e.g. malondialdehyde) were analysed. First results of this study will be presented.

Acknowledgements: This study was kindly supported by the Horst-Müggenburg Stiftung, Germany.

## **Poster Session IV**

Consumer research and epidemiology

**P42**

**The bioactive substances in Food Plants Information System (EuroFIR-BASIS)**

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The Bioactive Substances in Food Plants Information System (BASIS) is being developed within the European Food Information Resource Network (EuroFIR), whose strategic objectives are to integrate European expertise and resources in food composition database systems and to disseminate and exploit new scientific and technological knowledge. EuroFIR-BASIS aims to develop a web-based database system, focusing on health-promoting bioactive constituents in plant based foods that are commonly consumed in European countries. It will contain critically evaluated data obtained from peer reviewed publications on the content and biological effects of bioactive compounds and an up to date comprehensive list of about 340 plant names and plant parts in 12 EU languages. A comprehensive coverage of both compound classes and food plants will be provided, thereby facilitating calculations of dietary exposure to bioactive compounds such as flavonoids, phenolic acids, phytosterols, carotenoids, isoflavones and lignans.

The structure of EuroFIR-BASIS includes a management group and three experts working groups (Plant List Group, Compositional Evaluators Group, Biological Evaluators Group) integrated by scientists with appropriate expertise from various research institutes across Europe. A web-based system has been developed to facilitate data inputting and knowledge transfer.

To date, the Plant List Group has completed a list on European food plants (Revised Nettox-list) including both Latin and common names and a description of the plant part used. A more comprehensive list on major European food plants, together with an exotic and health food plants list are under development. Based on the European food plants list, the Compositional Evaluators Group has been developing an electronic input form including a quality assessment scoring system to collate data from original research papers on bioactives. The quality evaluation will be based on the information pertaining to the description of the plant, the sampling process, the compound, the analytical method and the method performance. The scoring system serves to provide an indication of the quality of the compositional data. The Biological Evaluators Group is currently developing a system to allow the inclusion of critically evaluated published data on the biological effects of bioactive compounds, including data from human studies, animal models and *in vitro* studies.

In conclusion, EuroFIR-BASIS will facilitate research into the relationships between plant bioactive intake and human health by providing a single database linking data on the contents of bioactives in plants and foods and data describing the biological activities of the plant bioactives. It is expected that the main users will be researchers (e.g. in epidemiology, nutrition sciences, diet and health), food consumption database managers, food regulatory affairs and risk assessment institutions, and product development departments.

**P43**

**Associations between dietary flavonoids and bone mineral density (BMD) in Scottish postmenopausal women**

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Flavonoids are bioactive compounds found in plants. Common dietary sources in our postmenopausal population of Scottish women are tea, wine, chocolate, fruit and vegetables. Little is known about their effect on bone health in humans, but studies in this cohort (Aberdeen Prospective Osteoporosis Screening Study) have shown that higher intakes of fruit and vegetables are associated with higher bone mass and reduced bone turnover (Macdonald *et al*, Am J Clin Nutr 2004; 79: 155-65). The aim of the study was to compare dietary flavonoids from all sources with those solely from fruit and vegetables with respect to BMD in postmenopausal women.

The subjects had been recruited in 1990-3 for the Aberdeen Prospective Osteoporosis Screening Study, and the majority returned  $6.3 \pm 0.6$  y later (mean age at baseline (SD) 54.7 (2.2) y). 3238 subjects completed a food frequency questionnaire (FFQ) and had bone density scans of the lumbar spine (LS) and hip (FN) (Norland XR26/36 scanner).

The diets were analysed for flavonoid intake using a food composition database (Kyle & Duthie, Flavonoids in Foods 2006) developed for a similar version of our FFQ. We have now validated our FFQ for estimating dietary flavonoid intake in early postmenopausal women using 218 4-day food diaries. Pearson correlations for energy adjusted total flavonoids, flavonols, catechins and flavanones were 0.76, 0.75, 0.66, 0.60 respectively ( $p=0.001$ ).

The mean intake of flavonoids solely from fruit and vegetable sources was  $84.29 \pm 49.58$  mg/d, which was 27% of the total flavonoid intake. Sources of flavonoids not included in these categories were tea, chocolate, wine, beer and honey. Energy adjusted intakes of all flavonoid groups except flavones were correlated with FN BMD ( $p \leq 0.01$ ). LS BMD was correlated with all groups except flavanones and flavones ( $p \leq 0.01$ ). After adjusting for menopausal status, HRT use, age height and weight these relationships were still significant at the hip with the exception of flavonols (flavanones  $p=0.036$ , catechins  $p=0.017$ , procyanidins  $p=0.024$ , all flavonoids  $p=0.004$ ).

Although flavonoids from fruit and vegetables are a small constituent of total flavonoid intakes they appear to contribute to bone health in postmenopausal women. This data adds to the evidence that fruit and vegetables are beneficial for bone and supports animal and cellular studies that show flavonoids reduce osteoclast action and positively affect bone strength. Further work is required to elucidate the mechanism and determine precisely which components are important.

**P44**

**Fruit and vegetable consumption and cancer: A review of Japanese studies**

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The most abundant evidence for an effect of diet on cancer incidence has been related to a lower risk with greater intake of fruit and vegetables. World Cancer Research Fund-American Institute for Cancer Research concluded that there was convincing evidence that high intake of vegetables decreases the risk of most cancer types. However, some recent results of epidemiologic studies do not support the hypothesis of the protective role of fruit and vegetables in the etymology of cancer. The aim of the study is to review the relation between frequency of consumption of fruit and vegetables in Japanese diet and the risk of cancer types. Review of data with specific focus on epidemiological studies in Japan. Fruit and vegetable intake even in lower amounts was related to reduced cancers of the digestive tract, stomach, bladder, oral, liver, and lung. In conclusion fruit and vegetable consumption in Japanese populations appears to provide protection against several types of cancers.

**P45**

## **Interference between alcoholic beverages and the 5-a-day concept**

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Alcoholic beverages are currently a typical component of the diet in Europe. The recommendations to consume at least five servings of vegetables and fruits per day lead to the question if there may be synergistic or antagonistic effects by additional consumption of alcohol or if a complete prohibition would be advisable.

Besides the negative effects of excessive alcohol consumption, positive nutritional effects are known from the literature, e.g. the French paradox that was suggested to be caused by France's high red wine consumption as a primary factor.

In this study, we conducted a thorough literature review about constituents (e.g. vitamins, minerals, polyphenols) and physiological effects of alcoholic beverages and made a comparison to vegetables and fruits by means of multivariate data analysis using the software Unscrambler V.9.2 (CAMO Process AS, Oslo, Norway). Principal component analysis was used to visualize the data in simple two-dimensional coordinate systems. By this way, it was possible to identify key relationships in the data, in other words, to find similarities and differences between the nutritional properties of alcoholic beverages and fruits and vegetables. In addition, the interaction between alcohol and the effects of vegetables and fruits was evaluated.

It can be shown that small quantities of beverages like beer and wine do not interfere with the 5-a-day concept. However, due to the known effects of alcohol itself, a moderate consumption should be stressed.

**P46**

**Purchase, consumption and quality perception of fresh and preserved vegetables and fruit in Austrian**

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Objective

Despite the propagation of constitutional qualities of fruit and vegetables and the “5 a day campaign” consumption of these food groups does not meet recommendations of nutritional societies.

Methods and Subjects

To investigate fruit and vegetable consumption of Austrian adults a questionnaire has been performed. During spring and summer 1338 questionnaires were analysed; 60% of study subjects were female and 40% male. The mean age of interviewee was 38.7±11.0 years. Fifty-six percent do have education without university entrance diploma and 30.6% with university entrance diploma and 13.8% high school diploma. Fifty-four percent of interviewee live in Eastern Austria and 46% in Western Austria.

Results

8.6% of study subjects eat vegetables several times a day, 30.2% daily and 22.9% 4-6 times a week, 31.9% 1-3 times a week, 5.9% seldom and 0.5% do not consume vegetables at all. 23.2% eat several times a day fruit, 32.3% once a day, only 0.5% never consume fruit. No difference in vegetable and fruit consumption was observed between spring and summer as well as between Eastern and Western Austria. Women consumed significant more vegetables and fruit than men and higher educated ate significant more vegetables than lower educated ( $p < 0.001$ ).

78.3% of interviewee buy most frequently fresh vegetables, while only 31.1% get deep frozen vegetables frequently. No difference in consumption of deep frozen vegetables was observed between spring and summer. Mean reasons for buying deep frozen vegetables were all-season availability, long preservation and quick preparation. More than half of the participants (57.6%) considered the quality of fresh vegetables being better compared to deep frozen.

Canned and glass vegetables were bought seldom (43.1%, 42.5%). Reasons for the purchase of tanned and glass vegetables were equal to deep frozen vegetables. The quality was scored less as compared to fresh vegetables. Most interviewee consumed tanned vegetables (52.1%) and glass vegetables (47.3%) only every second week or seldom.

Most of investigated subjects (86.5%) buy their fruit fresh, deep frozen fruit as well as tanned and glass fruit are bought seldom or never.

Conclusion

Women, as well as older and higher educated participants consume more often vegetables and fruit, especially fresh products. Deep frozen, tanned and glass vegetables and fruits are eaten more frequently by men. More information should be given on quality of preserved vegetable and fruit products. Strategies on vegetable and fruit consumption should be addressed more specific to target groups.

**P47**

**The proportion of fruit and vegetables in the diet of 10- to 12-year-old schoolchildren - Results from the Austrian Pro Children cross-sectional survey**

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Aim

The present work gives an overview of the food consumption pattern of 10- to 12-year-old children in Austria with focus on fruit and vegetable consumption.

Methods

The study covering 851 participants was part of the Pro Children cross-sectional survey on fruit and vegetable intake of 10- to 12-year-old children carried out in 2003. Single 24-hour recalls were used to assess total energy intake and food group consumption patterns. The children's food consumption patterns were compared to the German food-based dietary guidelines (DGE Food Guide Circle) and the recommendations for an Optimized Mixed Diet (OMD).

Results

The Austrian children showed comparatively good results for cereals, dairy products and fats. However, the children consumed too much meat and meat products, as well as too many sweets and sugar products.

The proportion of vegetable consumption was low (8% of total food intake in grams, 1% of total energy intake). The proportion of fruit intake was acceptable if fruit juice was included (22% of total food intake in grams, 8% of total energy intake). By excluding fruit juice, the proportion of fruit declined significantly in the overall diet (9% of total food intake in grams, 4% of total energy intake).

Conclusion

Fruit and especially vegetable intake is low in Austrian schoolchildren. With regard to healthy food choices, it is advisable to reduce the proportion of meat and sugar products in the children's diet in favour of adequate fruit and vegetable intake levels.

**P48**

**Personal factors predict daily fruit and vegetable consumption in 14 - 19 y old students of secondary schools**

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Objective

To examine the associations of nutritional knowledge, interest in dietary information and lifestyle with daily fruit and vegetable consumption in 14 - 19 y old students of secondary schools in Vienna.

Material and Methods

A sample of 261 students (131 girls and 130 boys) with a mean age of 16.0 y (SD 1.2) was enrolled in a cross-sectional survey of diet and nutrition which took place in six different secondary schools in Vienna. Participants completed a 58-item food frequency questionnaire (FFQ), including questions on lifestyle (alcohol consumption, smoking, physical activity, TV/PC consumption), interest in dietary information and a standard test about nutritional knowledge. Data on lifestyle were aggregated into a summary lifestyle index and FFQ data on fruit and vegetable consumption were coded binomially (either daily consumption or not). Logistic regression was used to model the associations of daily fruit and vegetable consumption with the selected factors.

Results

Fruits and vegetables were consumed at least once a day by 49% and 40% of the respondents respectively. Between girls and boys significant differences in frequency of daily fruit consumption were found (57% vs. 41% resp.) ( $p=0.011$ ) but not for daily vegetable consumption. The probability to consume at least once a day vegetables increased with increasing nutritional knowledge ( $p=0.000$ ), with interest in dietary information ( $p=0.003$ ) and a healthier lifestyle ( $p=0.005$ ). Daily fruit consumption was associated with interest in dietary information ( $p=0.002$ ), and a healthier lifestyle ( $p=0.000$ ) but not with nutritional knowledge ( $p=0.100$ ).

Conclusion

Nutritional knowledge, interest in dietary information and a healthy lifestyle are strong predictors of daily fruit and vegetable consumption in students of secondary schools. Improved nutritional knowledge, stimulated interest in dietary information and the promotion of a healthy lifestyle may help to increase daily fruit and vegetable consumption in young people.

**P49**

## **The role of vegetables and fruit in the diet of Austrian adults**

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### Objective

The objective of the present analysis was to examine the attitudes of Austrian adults towards the consumption of vegetables and fruit and the campaign “5 a day, for a better health”.

### Sample

A sample of 5 400 adults, with an age between 19 to 64 y, was enrolled in the present cross-sectional survey, covering all regions of Austria and all seasons. Participants completed a self-administered food frequency questionnaire, including general questions on vegetables and fruit and the campaign “5 a day, for a better health”.

### Results

Compared to 2001 the proportion of respondents who answered to consume fruit “just because fruit is available” increased from 9 to 30 %, a reason which was found to be pronounced in males. Nevertheless, the reasons “taste” and “health” remained the most important reasons to consume fruit in both sexes. The main obstacles to consume more fruit were found to be “forgetfulness to consume fruit” and “believes to consume already sufficient amounts of fruit”, particularly in males. Regarding the reasons for vegetable consumption no differences were found between the years 2001 and 2005. “Taste” and “health” remained the most important reasons to consume vegetables.

Compared to fruit, vegetables are more likely to be consumed “just because vegetables are available”. The main obstacle to consume more vegetables was found to be “believes to consume already sufficient amounts of vegetables”.

About 30 % of men and 60 % of women answered to know the campaign “5 a day”. Among respondents who are familiar with this campaign 8.2 % only achieve the recommended amounts of vegetables and fruit. Generally, among Austrian adults 5.5 % only adhere to this food based dietary guideline. However, Austrian adults are willing to eat “plus one” portion a day.

### Conclusion

Health promoting potential of vegetables and fruit is still not recognized by the consumers. The results reveal background for promising intervention programs to improve nutrition and health status in Austria.

**P50**

### **Fruit and vegetable consumption**

**“How many portions of fruit and vegetables did you eat yesterday?”**

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There exist very little reliable data on fruit and vegetable consumption as propagated in the campaign “5 am Tag” (five-a-day) in Germany. Continuous household budget surveys and sample survey of income and expenditure do not show fruit and vegetable consumption on a quantity bases per person and day. National consumption studies which provide this information are conducted in long time intervals. Aim of this study is to gain information on the fruit and vegetable consumption at comparatively low expenditure using the campaign’s terminology, and to find out to what extent the claims made by this campaign are fulfilled by the nutritional behaviour of the population.

In the frame of a representative multiple purpose (omnibus) survey in November 2002 several questions concerning fruit and vegetable consumption were asked. Target group was the German speaking population of the age of 14 years and older, the sample (corrected) comprised 2000 people. Participants in the study were asked for their fruit and vegetable consumption the day before. The questioning started with “What kind of fruit (vegetables) did you eat yesterday” (out of a list) followed by “How many portions did you eat?” (of the respective item). A “portion” was defined according the 5-a-day-campaign’s specifications in Germany.

The results show that 75 % of the participants in the study had eaten fruit, 79 % had eaten vegetables and 33 % had drunk juice the day before. 92 % had had either fruit and/or vegetables and/or juice (one portion of juice was taken into account). Diversity in chosen vegetables was higher than in chosen fruit. Mainly named fruit were apple (44 %), banana (27 %), orange (22 %) and grapes (10 %), and mainly mentioned vegetables were tomato (27 %), leafy vegetables (19 %), cucumber and carrots (17 % each) and pepper 13 %.

Most frequently one and two portions of fruit and vegetables respectively were eaten. One portion of fruit (vegetables) was eaten by a quarter (a third), two portions of fruit (vegetables) were eaten by 25 % (23 %) of the respondents. Taken into account all fruit, vegetable and juice consumption, 12 % of all respondents had eaten only one portion, 17 % two and three portions and 15 % had eaten four portions the day before. Five portions were declared by 10 % and over five portions by 21 % of the people questioned.

In the main the participants in this representative study have chosen fruit and vegetables according to the season. Only 31 % of the respondents meet the requirements which are claimed by the campaign “5 am Tag” but at least nearly two thirds eat three and more portions of fruit and vegetables a day.

**P51**

**Dietary habits and physical activities of the obese children in different schools in Kigali city**

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Obesity among children is rapidly increasing in many countries and becoming a concern for public health professionals. Though Rwanda is among the developing countries and on struggle fighting hunger and poverty by raising the standards of living of the population, the economic status increases, hence the nutrient consumption is also increased and this may lead to a double burden of obesity and hunger at the same time. The research was carried out to study the dietary habits of obese children belonging to 5 schools. They comprised of both sex and were found in the range of 4 to 14 years. They comprised of 58 girls and 29 boys. Majority of the respondents (18.39%) were in the range of 11-12 years while 17.24 % were in the range of 12 to 13 years and 16.29% were in the range of 8 to 9 years. The results revealed that 50% of the parents worked in private organization, 26% were government employees, while 9% worked for NGO's and 15% had no jobs they are helped by relatives. The BMI of children varied depending on sex and age, and was calculated using the metric method of BMI calculation where  $BMI = \frac{\text{weight}}{[\text{height}]^2}$ . The frequency of meal pattern per day determined, revealed that 58.7% ate normally 3 times/day while 33.33% ate 4 times/day and 12.64% ate 5 times/day. It is evident from the results that children who eat 4 and 5 times had higher BMI, hence contributing to additional weight. Majority of the children consumed bulky food and fatty food. Cereals such as rice, macaroni, maizeposho were regularly consumed by 89.8%, 96.5% consumed meat; 90.8% ate breakfast cereals such as wheat bread, cookies, corn flakes while chapatti were consumed by 90.8% and 75% consumed home made potato finger chips. Milk and its products were consumed by 75% while 70% consumed legumes. Green leafy vegetables and fruits were consumed by only 57% and 31% respectively. The physical activities that children were engaged were not much at home or at school. Swimming was the activity done mostly at home during weekends. Boys were more engaged in physical activities than girls and running was the activity done mostly at school daily.

**P52**

**Food habits of the elderly people in Gasabo district, Kigali**

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Ageing increases the inability of a person's body to maintain itself and to perform its operation as it once did. Since food is the main source of nutrients, the food habits of the elderly were assessed. Fifty adults, comprising of 21 males and 29 females belonging to low-income group in Gasabo district, Kigali were taken for the study. Highest (48%) percent of the respondents belonged to the age range of 60 - 69 years. The study indicated that 30% were involved in moderate activity. The frequency of meal consumption varied and 50% consumed food 2 times a day and 20% consumed food 3 times a day while 18% consumed food once a day. The elderly consumed various kinds of foods. Among the cereals, maize was consumed once a while by 60.7% while 18.37% consumed it more than 3 times a week and a same percent of respondents never consumed it. Wheat flour was never consumed by 78% of them. Millet flour was consumed once a while by 40.43% of the respondents but 55.32% did not consume it. Rice was consumed by 53.19% while it was not consumed by 19.15%. The study revealed that milk, a well-loved food, was consumed once a while by majority of them - milk by 42.86%, milk products by 47.73% and only milk by 48.94%. Beans was consumed everyday by 48% while 30% and 20% consumed them more than 3 times a week and once a while respectively. Greens and vegetables were consumed once a while by 80% and only 6% consumed everyday. Fruits were considered to be foods for children and therefore never consumed. Roots and tubers were consumed everyday by 26.53% while 51.02% consumed it more than 3 times a week. Non-vegetarian foods such as fish was not consumed by 63.83% but 25.53% consumed it once a while. Meat was consumed once a while by 46.81% while it was not consumed by 38.30%. This may be due to fact that they were expensive. However, they all consumed alcohols - 44% consumed local alcohol made from sorghum while 34% consumed other forms of local alcohol and 8% consumed branded alcohols such as Primus and Mutzig.

**P53**

## **Assessment of Vegetable and Fruit consumption by the National Nutrition Survey II**

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### Objectives and Design of the National Nutrition Survey II (NVS II)

The nationwide survey will provide representative data on food consumption patterns. About 20 000 German speaking persons between 14 and 80 years will be interviewed. Data on nutrition intake and behaviour are collected on an individual level starting with a computer assisted personal interview (CAPI) using a dietary history followed by a 24 hour recall applied by two computer assisted telephone interviews (CATI). A sub sample of 1000 participants will complete twice a 4 days weighed record.

### Collection of Consumption Data

Frequency of food consumption of the last 4 weeks is the emphasis of the dietary history method. "Usual" nutrition and dietary habits for those 28 days is being assessed with the help of standardised tableware and a picture book. The record which food is not eaten at all is being acquired, too. Meal patterns of the participants can be connected with the amount of consumed vegetables and fruits.

At least 2 weeks after the personal interview participants are being called by phone on a randomly chosen day to complete a 24-hour recall. Advantage of this instrument is the improved memory of the consumed quantities as well as the completeness of eaten food on the passed day. The program includes a very specific and differentiated adaptation of food items and recipes. This detailed depiction offers several steps to specify the eaten vegetables and fruits.

For about 1000 participants completing the weighed records is the most complex and time-consuming way to measure food consumption. But the exact indication of consumed portion sizes, specific quantities and preparation style is important to reassess present assumed portion sizes and necessary for the intake of hazardous substances. It will also be possible to see in practice the portion sizes of participants with a high consumption of vegetables and fruits (5 a day).

### Summary

Due to the different study instruments the NVS II will reveal exact statements on the frequency, amount and kind of consumption of fruit and vegetable. On the basis of the design the results can be differentiated for regional and seasonal variety. The broad spectrum of nutrition habits and lifestyle differences, which will be assessed by sociodemographic data, allows diversifying the fruit and vegetable consumption for special population groups, like smokers, singles and/or athletes. So the NVS II guarantees the best possible record of vegetable and fruit consumption within the German population.

**P54**

**Promotion of fruit and vegetable consumption in school-age children and teen-agers: an interactive multidisciplinary project**

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An interactive project to introduce knowledge on fruits and vegetables, their quality and beneficial properties has been carried out and it is still ongoing in order to promote the increasing of consumption of those type of food in nursery, primary and first-level secondary school of Udine city, North-East of Italy. Working team was composed by evolution psychologists, a physical education teacher, communication experts, the city marketplace Society and food scientists. The planned activities were related mainly to the knowledge of different type of fruits, especially apple and season's fruits available at the local marketplace in the different period of the year belong the scholastic time (from September to June). The activities were carried out firstly in nursery schools, working with 3-6 years-old children, by means of animators able to involve the class in observation of simple phenomena related to the fruit and the basic study of the behaviour (i.e. browning, softening, etc.) of pickled fruits when prepared for eating by simple processes. Emphasis was put to the discovering of different tastes, colour, shapes with the main goal to promote the curiosity of the kids together with explaining the beneficial properties for human health. Every "taste laboratory" where finalised to eating fruit together in the class and to promote the daily consumption of different type (selected by colour and depending to the season time) also into the family environment.

Another action of the project were carried out with 9-10 years old kids (primary school) where in the taste laboratory workplan the concept of five-a-day fruits and vegetables consumption and the explanation, by means of scientific tools, of five-colour-relation to protective and beneficial properties of fruit and vegetables was introduced. This project section was completed by visiting the city open marketplace where the daily exchange of fruit and vegetables takes place under the control of a public-controlled society assuring food quality and safety (in terms of commercial grade, provenience, residue limits, etc.). Also in this case the activities were monitored by questionnaires and interviews. Actions have been fully appreciate by students, teachers and families that had the possibility to be introduced in deep and with good knowledge in the food chain understanding, other than to the beneficial of increasing the daily consumption of fruit and vegetables.

Third action of the project, addressed to the first cycle of secondary school (11-14 years old students) is planned for the incoming scholastic year (2006-07).

**P55**

### **Criteria for products with fruit and vegetable claims**

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Current intakes of fruit and vegetables (F&V) are well below the recommendations of the WHO (400 gram F&V per day) and local food authorities. Processed foods, providing F&V are an option to bridge the gap in the diet by offering attractive options in a responsible way and so boosting F&V consumption.

There are no well-accepted criteria available for F&V claims in processed foods. Unilever has developed criteria to substantiate these claims based on 6 key nutrients. As key nutrients in F&V were defined: vitamin C, fibre, total carotenoids, total polyphenols, folate, and potassium. Two approaches were investigated to assess the nutrient retention of these key nutrients in industrial products. Analytical data of the product was compared with 1) analytical nutrient data based on the recipe using the average of 4 food composition tables; and 2) analyses of home-prepared products using the same recipes.

This approach was used in the underpinning of the claim of the Knorr Vie shots: 'Provide the goodness of at least half the recommended daily intake of 400 gram'. Knorr Vie shots compared well with expected nutrient values based on nutrient databases and the home prepared products, with the exception of fibre, which was lower to keep the product drinkable. It can be concluded that Knorr Vie provides half the daily intake in F&V without added sugar, sodium or other additives.

Knorr Vie is meant to be consumed in addition to and not as replacement of habitual F&V consumption. Market research supports that consumers understand Knorr Vie is a boost and not a replacement for F&V.

Based on above prescribed approach Unilever is developing guidelines for the underpinning of F&V claims of other products as well. These guidelines aim to help in the development of healthy F&V options.

**P56**

## **Consumers' attitudes towards functional foods based on GMOs**

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As opposed to their traditional counterparts, functional foods have an increased health benefit for the consumer, for instance because they are enriched with specific ingredients like vitamins or pre- and probiotic substances. Within the joint research project "Improvement of the health promoting properties of foods by increasing and modifying their carotenoid content", which was financially supported by the German Federal Ministry of Education and Research (BMBF) under the contract number 0312248I, our task was to assess the consumers' attitudes towards functional foods enriched with carotenoids and produced by means of genetic modification of the raw materials.

Functional foods are well-appreciated by the consumers in some segments of the food market and offer a high potential for innovations for the industry. However, the market share of functional foods in Germany and Europe is still below 1 to 2 % of the total food market. The success of a new product highly depends on the perception of its attributes by the consumers.

Lycopene is a red pigment that can be found in tomatoes and tomato products. Because of its high antioxidative potential, lycopene can protect biological systems against free radicals and therefore has the potential to contribute to the prevention of some types of cancer and coronary heart disease.

Using the example of lycopene-enriched carrot products, 204 consumers assessed the different attributes of these products. The data were analysed using the conjoint analysis; an accompanying questionnaire completed the data collection. The conjoint design consisted of 19 product profiles; it included 3 holdout cards, which were used to determine a good reliability value of  $\tau = 0.95$  ( $p < 0.0001$ ).

Consumers have a sophisticated understanding of functional foods. The production process using genetic modification of the raw materials turned out to be the strongest obstacle for purchasing such products even if they offer a strong health benefit. The promised health effect was the second most important factor for the purchasing decision, followed by the price and the colour of the product (lycopene-enriched carrots are dark red). Additional information from trustworthy sources is of outstanding importance for the consumers.

It is concluded that new product concepts must be based on a conclusive and transparent presentation of the products' benefits for the consumer. The plant breeders' expectation that GM plants with clear benefits for the consumers would decrease public concerns about genetically modified plants is not supported by this survey.

This conference was supported by:

Federal Ministry of Food, Agriculture and Consumer Protection

Dr. Rainer-Wild-Stiftung

"5-am-Tag"-Association, Germany

Institut Danone für Ernährung e.V.

Schwartauer Werke GmbH

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## **Notes**









