EGEA

FIRST EDITION

International Conference on Health Benefits of Mediterranean diet

Highlights on Cancer & Cardiovascular Diseases

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Preface

Nutrition is recognized as a major health determinant. Unhealthy diets and sedentary lifestyles are critically implicated in cardiovascular diseases and various cancers, and underpin the increasing burden of chronic diseases in Europe /worldwide - notably, the escalating prevalence of obesity among children and adults. This Symposium on the Mediterranean Diet, organized by Aprifel, is therefore timely in addressing issues of critical significance to public health. It is also timely given the state of public debate between scientists, policy-makers and citizens on appropriate nutritional recommendations for health. On the one hand, the prospects of a coherent nutrition policy (for Europe) appear tantalizing close with the convergence of evidence based policy recommendations from the EURODIET project, the Action Plan produced by the SFSP during the French Presidency of the EU in 2000, the promise of a Community Action Plan on Nutrition embodied in the Commission's White Paper on Food Safety and, most recently, the WHO Action Plan on Nutrition. On the other hand, in the eyes of many citizens, the credibility of the science underlying accepted guidelines is currently being challenged by the widely publicized controversy as to the health benefits of high-fat vs low-fat dietary recommendations. The Symposium provides us with an opportunity to evaluate the most recent scientific evidence on the health properties, traditionally associated with the "Mediterranean diet" and in so doing to re-assess/debate the bases for a coherent approach to the promotion of healthy diets and lifestyles.

The location of the Symposium in Crete is particularly appropriate since the Mediterranean diet of Crete has a richly documented history stretching back over 4000 years to the Minoan era. Moreover, the health properties attributed to the traditional Cretan diet have been – and continue to be - the source of scientific and popular interest for nearly half a century. The Symposium addresses a broad range of themes : components of the Mediterranean diet; epidemiological, clinical and biochemical studies of cancers and of cardiovascular diseases; interactions between the Mediterranean diet and genetic and lifestyle factors; the current status, prospects and dietary recommendations to be derived from the Mediterranean diet. We are confident that the outcomes of this Symposium will contribute significantly to the development of coherent nutritional recommendations for good health.

Anthony Kafatos, MD
Professor, Director of Preventive Medicine and Nutrition Clinic
University of Crete School of Medicine

An official opening talk was held prior to the sientific lectures
on Monday, June 5th, 2003 featuring :

L. Damiens ; Director of Aprifel
and S. Barnat ; Head of the Scientific Department of Aprifel,
who welcomed their distinguished guests before giving the floor to :
Pr Siafakas ; Vice Rector of the University of Crete
and Pr Zoras ; Deputy Dean of the Medical School of Crete

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Why we need a European nutritional policy

Anthony KAFATOS
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Welcome to Crete. In addition to the scientific benefits of this meeting, I hope you will have the time to relax here and the opportunity to enjoy the beaches and mountains of our beautiful island.

We are here this morning to discuss a highly topical question: why do we need a European nutrition policy ?

Talking first in terms of food and regulatory mechanisms, it is apparent that food policy has characterised the European Union since its inception. We begin with a history of policies dealing with food supply, starting with the Common Agricultural Policy. Following the BSE and dioxin crises we now also have emergent European policy and institutions dealing in an increasingly comprehensive manner with food safety. The critical missing dimension is a European nutrition policy.

The reasons why we need a European nutrition policy seem clear-cut given that nutrition is now widely recognised as a key health determinant. Poor diets are directly related to the increasing burden of chronic, non-communicable diseases (NCDs) in Europe, notably cardiovascular diseases and various cancers, obesity and its co-morbidities, and osteoporosis. The challenge for public health is how to influence the trends in dietary choices for effective NCD prevention. This necessarily confronts the fact that food and nutrition are 'cross-cutting' issues in a fundamental sense. That is, effective action requires recognition of the complex interaction of factors impacting directly or indirectly on food consumption. Dietary choices are affected not only by cultural influences and individual food preferences, but also by socioeconomic and environmental factors which are, in turn, shaped by a wide range of national and Community policies (eg policies impacting on the availability of foods and prices, quality, safety and so on). A European nutrition policy is a critical requirement to kick-start the development of comprehensive, coordinated, multi-sectoral strategies

In looking at the possible 'roadmap' for European policy it is instructive to look at existing national nutrition policies and strategies in Europe. I have chosen 2 contrasting examples: Finland, which offers a prime example of effective national health and nutrition policy, and Greece, where the development of nutrition policy is in its infancy.

Finnish policy is characterised by multiple specific mechanisms and measures including :

- Legislation on a wide range of issues including free school meals; food fortification; selenium supplementation of fertilisers; labelling; food additives; and specific regulations governing maximum sugar content.
- A National Nutritional Council (established 1954)
- Education and training of professionals
- Research and monitoring

It should be noted that these measures were not necessarily the product of 'top-down'initiatives. Many would regard the impact of the North Karelia Project, a community-based nutrition intervention program launched in 1972, as the most significant catalyst (Pekka et al., 2002). Comprehensive interventions in the community were eventually supported by national activities – from expert guidelines and media activities to industry collaboration and policy, with the involvement of stakeholders in the health sector, the educational system and the food industry being essential components. Monitoring is also a critical element in any policy, and the Finnish National Nutrition Surveillance System (FNNSS) provides the tools for impact assessment through comprehensive surveys at regular 5 years intervals. The aims of the FNNSS are (1) to collect, interpret, evaluate and distribute data on nutritional status and food consumption in Finland and (2) to promote nutrition and health policies

There are, in other words, multiple levels and areas of activity impacting directly and indirectly on nutrition and food consumption in Finland. This activity has developed alongside the formal written dietary recommendations and guidelines issued by public authorities or committees in Finland. There have been a plethora of these, with the first population guidelines being issued in 1968. This was eventually followed by recommendations for dietary guidelines in1987 and the National Nutrition Council Action Plan published 1989. Apart from the population guidelines, there are many guidelines aimed at specific target groups including, for example, schoolchildren, students in higher education, hospital patients, the elderly, infants and their mothers.

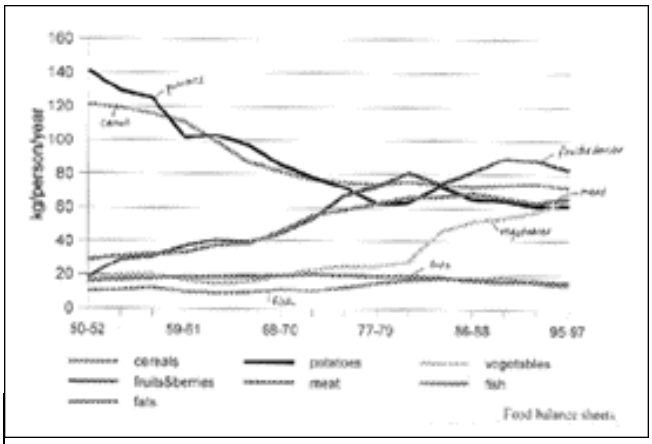
In contrast – in Greece the development of nutrition policy is in its infancy. We have to date a few limited initiatives in terms of the education and training of professionals, ad hoc research and monitoring initiatives and local interventions. At national level, in 1999 the Greek Ministry of Health endorsed nutrition guidelines for adults and more recently, under the stimulus of WHO initiatives in this area, there is at least talk of nutrition policy and action plans.

Have these alternative policies of multiple interventions (Finland) versus benign neglect (Greece) had a discernible impact ?
In terms of food consumption patterns, food balance sheets for Finland indicate dramatic changes in dietary choices between 1950-1997 in all the major food groups. Specifically, more reliable data from 5-year dietary surveys (Pietinen et al., 2001) indicate that in Finland between 1972 to 1992 there has been a two-to-three fold increase in fruit and vegetable consumption and reductions in salt consumption. There has also been a modest reduction in total fat content of the Finnish diet (from 38% energy to 34%) but dramatic change in composition of fats consumed :

- saturated fat from 21 to 16%,
- polyunsaturated fat from 3-5%
- and intake of cholesterol decreased by 16%

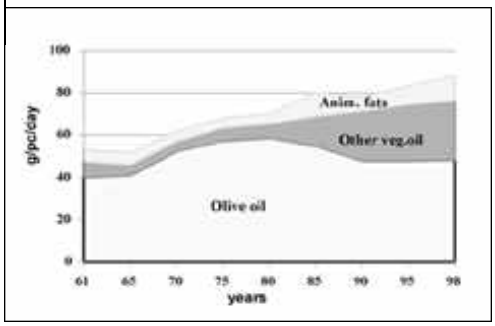
Food consumption patterns in Greece have also changed dramatically over the same period, but not towards healthier dietary choices. The rapid transition from traditional consumption patterns to more energy dense 'Westernised' patterns is clearly illustrated by changes in the consumption of vegetable oils and the increasing proportion of saturated fat.

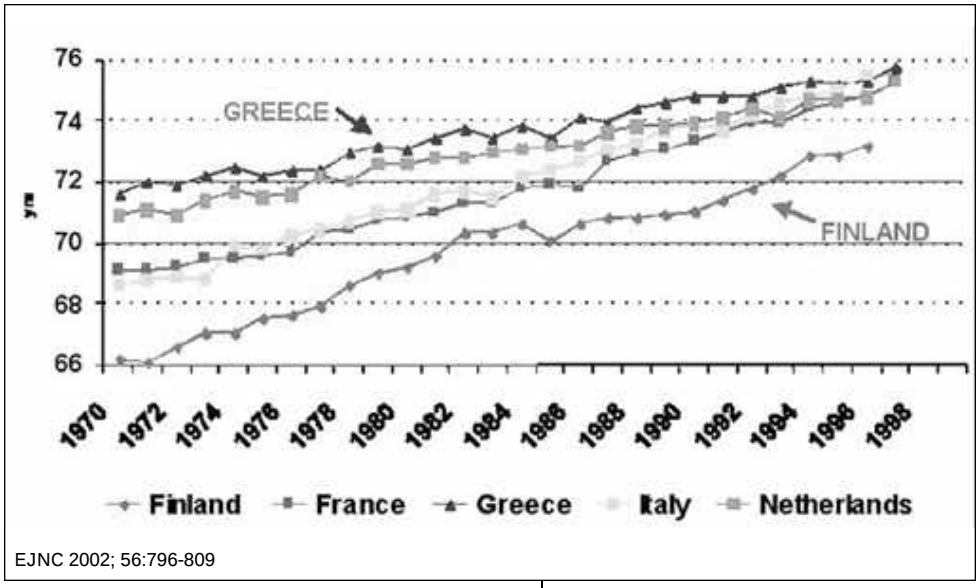
In terms of health, we find these changes reflected in the health statistics, particularly for cardiovascular disease risk factors



Food consumption in Finland 1950-1997 (KTL)

Time trends in consumption of olive oil and other vegetable oils in Greece 1961-1998 (FAO, 2000).





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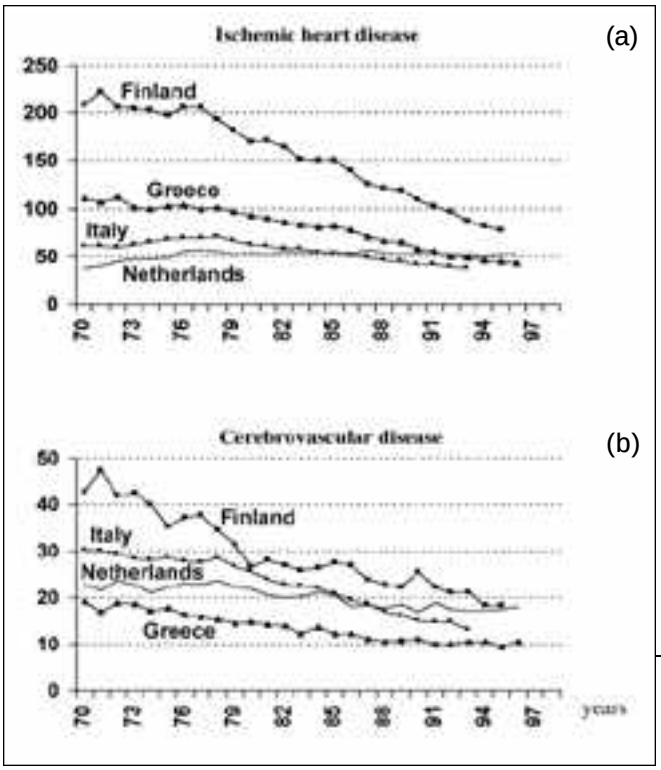
Life expectancy at birth, in years, males, in selected EU countries (WHO 2000)



Age standardised premature death rate (SDR) trends in Greece, Finland, the Netherlands and Italy for (a) heart disease and (b) cerebrovascular disease (WHO 2000)

(serum cholesterol, blood pressure, BMI). The most impressive form of policy impact assessment is provided by life expectancy charts and particularly the trends in ischaemic heart disease and cerebrovascular disease.

As indicated, in 1970 life expectancy in Greece was the longest in Europe whereas Finland was amongst the worst. Since then Finnish policy has contributed to the largest percentage improvement in life expectancy and the gap in premature death rates due to and ischaemic heart disease and cerebrovascular disease has all but disappeared. Most notably, ischaemic heart disease mortality in the Finnish working age population declined by 65% from 1971 to 1995 (Pekka et al., 2002).

There is no room for complacency in either country. In Greece we are sitting on a time-bomb whereby we can expect the food choices and lifestyles current today to result in the near future in much higher rates of morbidity and premature death from non-communicable diseases (NCDs) unless radical measures are taken. In Finland also there are persistent concerns and efforts to facilitate healthier consumption patterns (eg relating to fibre, salt intake and alcohol consumption).

While the scope for improvement in terms of national nutrition policies obviously varies widely within Europe, it is also apparent that national policies are inherently limited. We do not live in closed national economies. Food choices are affected by socioeconomic and environmental factors, by global market forces affecting food availability (quality, quantity, safety, pricing) and by the legal and regulatory framework of EC membership. By the same token we need broader strategies to mitigate the rise in nutrition-related NCDs – with the rise in obesity throughout Europe as arguably the most obvious and immediate global public health challenge. A European nutrition policy could provide the guidelines to prompt the multi-sectoral actions required over a wide range of policy decisions on agriculture and fisheries, health and welfare, social affairs, education, trade, commerce and finance which directly or indirectly impact on food consumption. This also requires a serious investment of political will given that the solutions required will collide with established trade and financial practices or interests.

There have of course been a number of recent initiatives recognising the need for a European nutrition policy. Politically the most significant to date is the Commission's White Paper on Food Safety (2000), followed by a Council Resolution on health and nutrition (23.01.2001). But momentum within the EU appears to have stalled and rather than the anticipated action plan geared to 'the development of a comprehensive and coherent nutrition policy' we have a Status Report on the European Commission's work in the field of nutrition in Europe (October, 2002).
We have, however, commitment in another context : in the recently endorsed WHO European Region Action Plan for food and nutrition policy, which provides a framework of 3 inter-related strategies for food safety, nutrition and food security.

In short : the need for a European nutrition policy is clear-cut. At issue now are the components of that policy and the measures/administrative structures required in formulating and implementing it in such a way as to effectively 'protect and promote health and reduce the burden of food-related diseases, while contributing to socio-economic development and a sustainable environment'(WHO, 2001).

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Diet and overall survival

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Dear colleagues and friends, it is a pleasure and a privilege for me to talk to you this evening. The topic of my presentation is Diet and Overall Survival.

The role of diet as the cause of many diseases, including coronary heart diseases and several forms of cancer, has been extensively studied, and many food groups and nutrients have been identified as having beneficial or adverse effects on health. However, dietary patterns, rather than single foods, may play a more important role in health and disease.

Dietary patterns are important because they have the ability to integrate the interactive effects that are observed between the various food groups. Moreover, simple dietary analyses are hindered by correlations that exist between the various groups. Dietary exposure patterns can be developed in two ways: the first is to use previous knowledge – we know the effects dietary constituents may have – and we call this approach 'a priori approach'. The second is to use existing data as a basis, and this is called 'a posteriori approach'. In the latter case, two main techniques are used: factor analysis and principal components analysis.

The "a priori" Mediterranean dietary pattern has received more attention, and this is due to the fact that several ecological and analytical studies provided evidence that the Mediterranean diet may have beneficial effects on health.

I will present you four studies referring to the Mediterranean dietary pattern. We have, developed a simple score: if you have all the characteristics of this diet, you have the highest score and thus a better diet. The score is assessed on a scale of 8. If you have no characteristics of the diet, the score would be 0.
Specifically, 1 unit is added when an individual has:

- high olive oil consumption
- high consumption of legumes
- high consumption of cereals
- high consumption of fruits
- high consumption of vegetables
- moderate ethanol consumption (but not high or very low)
- moderate consumption of dairy products mostly as cheese and yoghurt
- low consumption of meat and meat products

In the studies, I will refer to it was hypothesised that a diet with more of these components has beneficial effects, whereas a diet with fewer of these components would be less healthy. As indicated, these assumptions are based on the collective epidemiological and biological evidence as summarised in the report of the USANational Academy of Science and other critical overviews.

The Greek study:

In the first study, data collected in three Greek villages provided the opportunity to evaluate prospectively the role of diet in the survival of elderly Greeks.
The data were collected from 182 elderly residents as part of an international cross-cultural study of food habits in later life. Diet was assessed with an extensive semi quantitative questionnaire on food intake. A one-unit increase of the score, devised a priori on the basis of the eight components characteristics of the traditional diet common in the Mediterranean region, was associated with a significant 17% reduction in overall mortality. This study provided the first direct evidence that Mediterranean diet favourably affects life expectancy among elderly people.

Results of studies of Mediterranean diet in Mediterranean populations, however, may be confounded by the likely association of adult diet with early life nutritional patterns and culture-specific psychosocial variables like social support. But a similar study performed in Denmark provided similar results.

The Danish study:

This study examined the association of a Mediterranean dietary pattern with total mortality in a cohort of elderly people living in a North European Community. Diet and nutritional status were studied among 202 elderly men and women born between 1914 and 1918 and living in a Danish Municipality in 1988. They were followed for 6 years. A diet score, with seven dietary characteristics of the Mediterranean diet, was associated with a significant reduction in overall mortality. A one-unit increase in the diet score predicted a 21% (95% confidence interval 2-36%) reduction in mortality. The authors agreed with us that a Mediterranean diet score predicts survival in a north European population.

We have also undertaken a study in Australia among elderly Anglo-Celts and Greek-Australians with two objectives :
(1) to examine whether the results from the studies in rural Greece and Denmark could be replicated in an urban setting in Australia, and (2) to examine whether the apparent benefits of the Mediterranean diet are transferable to population groups with very different lifestyle.

The Australian study:

This study was undertaken in Melbourne Australia and involved 141 Anglo-Celts and 189 Greek-Australians of both sexes aged 70 years or more. The objective was again to evaluate whether adherence to the principles of Mediterranean diet affects survival of elderly people in a non-Mediterranean country. A one unit increase in the diet score was again associated with a 17% reduction in overall mortality (two-tailed p-value 0.07). Mortality reduction with increasing diet score was at least as evident among Anglo-Celts as among Greek-Australians.

The Spanish study:

Finally, the fourth study comes from Spain. Once again, it was found that an increase of one unit was associated with a significant 31% drop in the mortality rate among subjects aged less than 80 years old. The authors did not find any association for subjects over 80 years of age, and this is a point that needs to be investigated.

So we can conclude that a diet that adheres to the principles of the traditional Mediterranean one is associated with longer survival.

Two important questions remain to be clarified; is the Mediterranean Diet an integral entity or the sum of identifiable components that can and should be separately considered? Is the Mediterranean Diet or its major components transferable to all

populations, irrespectively of ethnic group and socio-cultural coordinates ?

The traditional diets of Mediterranean countries are based on fruits, vegetables, seafood, legumes and unprocessed cereals and, of course, olive oil.

So, what is the best diet? Most of the existing knowledge, translated into various guidelines or recommendations, relies on the associations between foods and nutrients on the one hand, and specific diseases on the other. The health effects of overall dietary patterns may be of critical importance; however, it is much better to speak about dietary patterns, rather than single food groups or nutrients, because there are many interactions between the various food groups. So the use of dietary patterns could be a powerful tool towards our understanding of the role of diet health and longevity and on drafting guidelines. For example, our group has calculated that if Northern populations shifted to the Mediterranean diet, several forms of cancer could be affected. It was estimated that 35% of colorectal cancer incidence, 15% of breast cancer incidence and 10% of prostate cancer incidence could be prevented.

Clearly, more research is needed in this area and I would like to draw your attention to the fact that last year, the European Commission approved a project with the objective to study the association of dietary patterns, including the Mediterranean dietary pattern, with longevity among elderly Europeans (the EPIC elderly project).

We have concentrated on the Mediterranean diet, but there may be another dietary pattern in other European countries with health beneficial properties. It could be an alternative, but we have no supportive data, at this time. The aim of this project, just mentioned, is to study the possible role of dietary patterns on the longevity of elderly people and to identify an optimal overall dietary pattern. In the EPIC elderly database we use, we have more than 100,000 subjects aged 60 years or over from ten European countries. The results will be available soon.

The value of this cohort lies in the presence of many people who follow the Mediterranean diet in the cohort. With respect to Mediterranean people, however, science is supported by culture and tradition.

After half a century of commercial pressure on Mediterranean people to change their nutritional style, they have to be reassured that they actually have one of the best dietary patterns.

We scientists are never absolute. It is not certain that the Mediterranean diet can fully explain the good health of Mediterranean people nor is it definite that it represents the best diet for all people. Nevertheless, it seems likely that this diet plays an essential role in the good health and longevity of Mediterranean people.

But remember what Hippocrates said: “Life is short, art is long, experience disappointing and judgement difficult”.

The epidemiology of cardiovascular diseases in Europe

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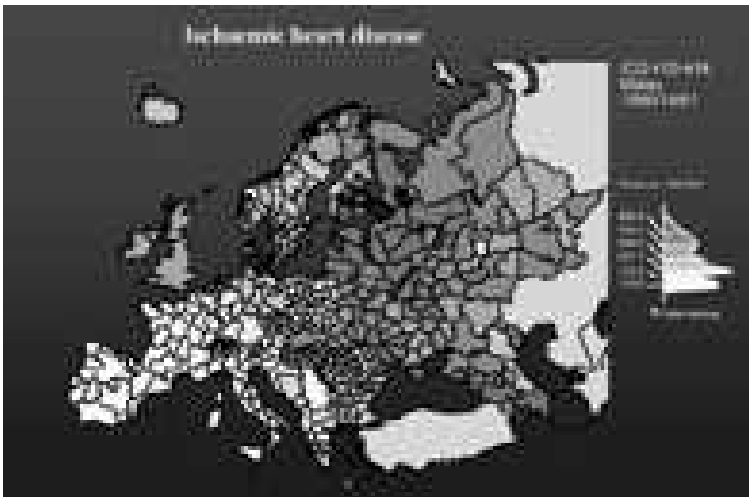
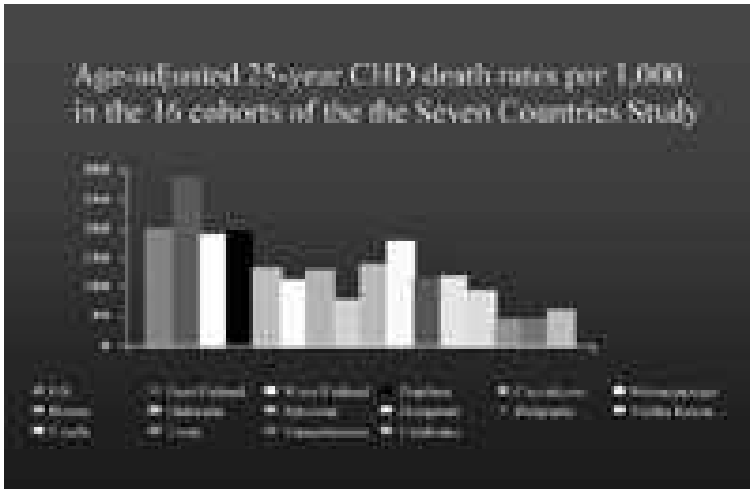
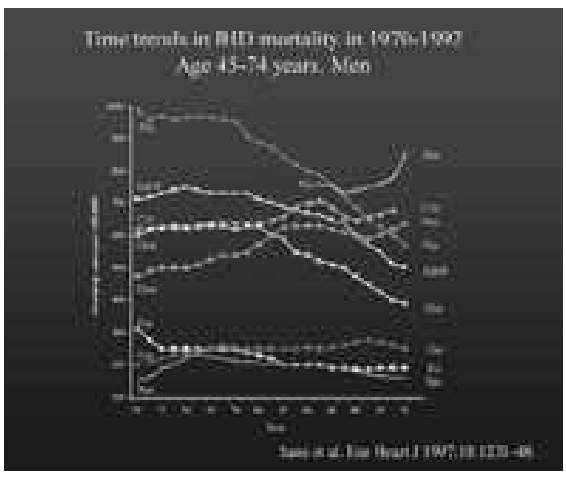
Within Europe large differences exist in mortality from coronary heart disease and stroke. These diseases show a clear West-East gradient in both men and women. Mortality rates are five times higher in Eastern compared with Western European countries. In most Western European countries a downward trend in age-adjusted coronary heart disease and stroke mortality is noticed since 1970. During that period an increasing trend is observed in Central and Eastern European countries e.g. Hungary and the Russian Federation. In recent years an increase was also observed in Greece.



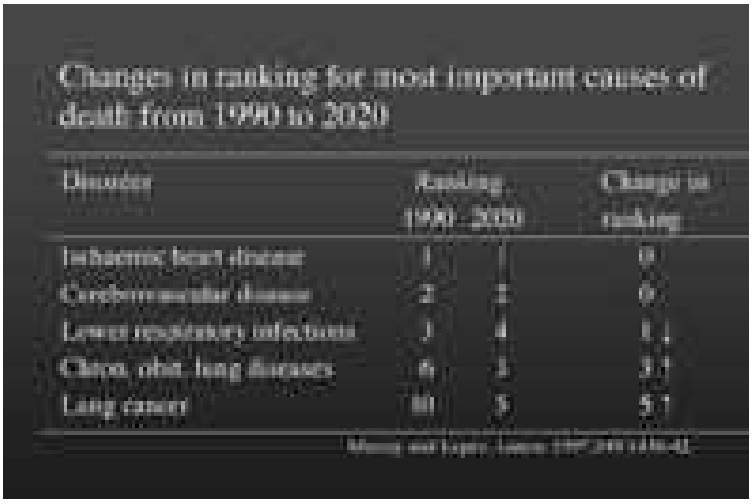
In the Seven Countries Study mortality data were collected prospectively in 16 cohorts of originally 12, 763 middle-aged men. During 25 years of follow-up about 6,000 men died, 1,500 from coronary heart disease and 800 from stroke. The 25-year coronary heart disease mortality rate was highest in East-Finland and lowest on the island of Crete. There was a six-fold difference between these two populations. For the mortality rate from stroke a three-fold difference was observed between Zrenjanin (Serbia) and the Dutch town of Zutphen (The Netherlands). At the beginning of the Seven Countries Study the coronary heart disease mortality rates were high in Finland and The Netherlands and low in Serbia and Greece. Time trends showed that the curves for the annual increment of coronary heart disease mortality in Finland and the Netherlands tended to flatten out at the end of the follow-up period. The curve for Greece increased at a regular rate at a low level and the curve for Serbia overcrossed all other curves during 25 years. For stroke all curves tended to rise during 25 years.



From a public health point of view information about the burden of cardiovascular diseases in Europe is needed. In spite the decreasing trend in age-adjusted cardiovascular disease mortality in Western European countries an increase in the number of cardiovascular patients is expected because of the ageing of the population. In many Central and Eastern European countries the number of cardiovascular patients will increase because of the increasing trend in these diseases. We therefore conclude that the number of cardiovascular patients in Europe will increase in the next decades. Consequently also the health care cost for these diseases will increase.



In conclusion, cardiovascular diseases are and will stay the most important cause of death in Europe during the next decades. Large changes in age-specific cardiovascular mortality rates occurred during the past 30 years with decreasing rates in Western and Northern Europe and increasing rates in Central and Eastern Europe and recently also in Greece.



Components of the mediterranean diet

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Dear colleagues and friends, it is my pleasure to chair today's session together with Dr. Schatzkin. I was asked to make a short introduction to this session, but before I do that, it is really my pleasure to present two of the speakers: Nadia Slimani and Ada Naska.

You have their curricula vitae in your folders, so I will not speak any more of their education and their scientific publications except to say that both of them are talented colleagues.

Nadia works at the International Agency for Research on Cancer (IARC) and Ada Naska is working with us at the Medical School of the University of Athens. I have personal experience in collaborating with both of them for almost ten years.

We always see young people as the continuation of our efforts, and they both showed very promising signs, many years ago. And the early signs were highly predictive. I do not want to add any more and I will let them present their work to you, after a short introduction from myself.

The MD can be thought of as having 8 components:

- **high olive oil consumption**
- **high consumption of legumes**
- **high consumption of cereals**
- **high consumption of fruits**
- **high consumption of vegetables**
- **moderate ethanol consumption**
- **moderate consumption of dairy products mostly as cheese and yoghurt**
- **low consumption of meat and meat products**

In most countries, Dietary guidelines have been widely perceived as indicating that total fat should be reduced. "Total fat" however, is not a very useful term, because fats and oils are distinct categories in the broad group of lipids. For southern Europe "fat" means mainly olive oil, although for north and central Europe the word fat is associated with animal fat. By recommending "fat" reduction, we may have had, as a result, the reduction of olive oil consumption in southern Europe and the concomitant reduction of vegetable and legume intake, (vegetables are consumed as salads as well as main dishes cooked in olive oil). I realise that for north America and north and central European consumer the term "fat" identifies animal fat, but for a document which addresses all people, including southern Europeans, subtile linguistic changes may have serious unintended consequences.

During the last 20 years, there has been an accumulation of scientific evidence about the different role of different types of "fat" and carbohydrates, on health and disease. The debate is still open, but cannot be ignored. It should be made clear that the evidence for the adverse effects of dietary fat, such as it is, applies to saturated and not to the monounsaturated fat that dominates olive oil. Recent studies have provided a new insight on this topic.

When "fat" in the diet is reduced, it is replaced with other foods. There lies an unanswered question. Is a low-fat diet in Mediterranean countries (essentially a low olive diet) as high in antioxidants and fiber as is the traditional Mediterranean diet? The low-fat snacks prominent on supermarket shelves certainly are not.

Low fat and high carbohydrate diets lower not only LDL cholesterol but also HDL cholesterol levels. HDL cholesterol levels are lowered by both sugars and complex carbohydrates (starch), and the depression of HDLcholesterol lasts for as long as the low-fat diet is eaten. The effect of carbohydrates on HDL cholesterol is a cause for concern.

Moreover, reducing "fat" intake and replacing it by even complex carbohydrates without specifying that they should be rich in fiber might lead to the intake of a lot of starch which tends to increase post-prandial hyperglycemia and its cascade of metabolic consequences.

Fat and obesity

Another argument allegedly supporting "low fat" intake is that "fat" is conducive to obesity. For several decades it has been recommended that a healthy diet provides less than 30 % of total energy from fat. Two schools of thought have expressed different views about the optimal level of overall lipid intake. However, there are no conclusive findings from controlled studies on the appropriate amount of fat in the diet to promote long-term weight reduction and maintenance. Although data are limited, no strong association has been demonstrated between dietary "fat" and human adiposity. It has been found, instead, that the proportion of fat and carbohydrate in a fully -controlled energy-restricted diet does not materially affect weight loss.

Energy intake has occasionally been perceived by the public as adversely affecting health. In reality, when body mass index (BMI) is adjusted for, higher energy intake is associated with lower cardiovascular and total mortality – because, in this instance, energy intake equals energy expenditure, which is partially determined by physical activity. In fact, it is physical inactivity and obesity that adversely affect health, the former by increasing the risk of cardiovascular diseases, osteoporosis, colorectal cancer and possibly other forms of cancer, and the latter by increasing the risk of non-insulin depended diabetes mellitus, hypertension and dyslipidemia. In other words, between two persons with the same BMI, the one who consumes more food is likely to be healthier than the one who consumes less food. It is noted that central (male-type) obesity is generally considered more disease-conducive than peripheral (female-type) obesity. Obesity is primarily a disorder of energy balance, rather than a consequence of "fat" intake.

There are several additional questions: Is a diet of very low "fat" (~ 20% of energy intake) a realistic option for Western populations long accustomed to substantially higher dietary "fat" intake? What happens when an affluent sedentary population is offered a variety of foods low in fat, abundant in the US and European markets? Usually such foods don't succeed in changing the body weight and the long-term health effects are poorly understood.

The evidence for the adverse health effects of dietary "fat" do not apply to olive oil and perhaps other types of plant oils. In any

case, special emphasis should be given to the specific properties of various types of fat and oils and on how these should affect the recommended quantities. For the prevention of coronary heart disease the guidelines should emphasise reduction of saturated fat, and for the prevention of obesity reduction of total energy intake. In both instances, and in several others, a central recommendation is to increase physical activity.

The Mediterranean diet could offer a healthy alternative approach to low animal fat diet. Moreover its expanded range of options could promote adherence, particularly over the long term. Finally, the intense focus on total fat intake may or may not be beneficial but it certainly distracts people from lifestyle changes that can have real benefit.

Vegetable and "fat"

There is strong evidence that antioxidants, largely derived from vegetables and fruits, contribute to the protection against coronary heart disease and, probably, cancer and other diseases. Recent findings suggest that polyphenolic compounds in vegetables are endowed with several beneficial biologic activities.

The campaigns to increase fruit and vegetable consumption have been relatively successful in terms of increasing fruit consumption, but only a minority of people in developed countries consume adequate quantities of vegetables. However, how can one consume considerable amounts of vegetables unless they are cooked or seasoned in oil?

It appears that consumption of olive oil in conjunction with vegetables can convey a substantial degree of protection against a wide range of chronic diseases. The consumption of raw, steamed or boiled vegetables is not very attractive, especially if an objective of 300 g daily intake is to be met.

In the Mediterranean parts of Europe more than 300 g of vegetables per capita per day are consumed mainly mixed with or cooked in olive oil and with addition of tomatoes, garlic, onion and many herbs. If "fat", that is olive oil, were to be reduced in these population groups, vegetable intake would also be reduced, since boiled or steamed vegetables are not part of their dietary tradition. In Greece, 40% of energy is provided from "fat" but most of it is olive oil and Greeks have been characterised by one of the highest vegetable consumption in the Western word. A balanced increase in consumption of fiber-rich foods, antioxidants and unsaturated fat, as in the Mediterranean diet, is the most rational way to replace foods rich in saturated fat and cholesterol.

Fruits and vegetables in the same basket?

As fruits and vegetables have partially different nutritional attributes. I think it is appropriate, from a health point of view, to segregate the recommendations concerning the two food groups. Targeted interventions that focus specifically on vegetables may have to take priority, because it is with respect to vegetables that the deficit is more substantial. The "more than 400g a day", the "5 servings" and the "eating more fruit and vegetables" recommendations are open to different interpretations. Agreement is further required with respect to foods such as pulses, potatoes and nuts, the classification of which is ambiguous.

In a recent study, fruit and vegetable availability patterns in 10 European countries have been described and their compliance with current recommendation was examined. Under the light of the segregated criteria, a discrepancy was revealed. The populations surveyed presented a pattern of fruit availability different from that of vegetables. In almost all cases the percentages of low fruit consumers were significantly smaller than those of vegetables, indicating a preference of European populations towards the consumption of fruits.

Thank you very much. I hope that Nadia Slimani and Androniki Naska's presentations will give you more information for discussion.

Mediterranean diet at present

Androniki NASKA
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Thank you, Madam Chair, for those very nice words. Thank you also to the organising committee for their kind invitation.

As explained this morning, my topic will be the Mediterranean diet at present.

Ladies and gentlemen, I am sure you are all aware of the increased attention the Mediterranean diet has received due to ecological and analytical evidence of its inverse association with coronary heart diseases and several forms of cancer. The underlying observation behind that is that adults living in areas bordering the Mediterranean Sea have displayed favourable health statistics, and they have done so since at least the early 1960s.

Such statistics could not be readily explained by a difference in education levels, financial status or health care systems, simply because in this area in the 1960s, socio-economic indicators were much lower than those in industrialised countries. Thus attention has focused on diet as one of the key explanatory factors.

Since the 1960s, several research questions of prime importance have emerged, particularly concerning the Mediterranean diet and its effects on health. The today's topic is 'In what way is this diet changing?'

In order to be able to answer this question, we need to know what the Mediterranean diet is.

At least 16 countries border the Mediterranean Sea. Their populations vary in terms of culture, religion, economic and political status, and several other factors that may influence dietary intake. However, the diet of these countries share many characteristics which are centred around the consumption of olive oil, together with a prevailing intake of fruit and vegetables.

Thus, a global definition of the Mediterranean diet may derive from observations and from reports and results of studies made in this area in the 1950s and 1960s.

The first systematic attempt to investigate dietary patterns in the Mediterranean region took place as early as 1948 on the island of Crete. At that time, the Greek government was worried about the post-war conditions and invited the Rockefeller Foundation to undertake an epidemiological study in order to advise the government on how to raise the standard of living of the population. L.G. Allbaugh was the appointed epidemiologist and he led a comprehensive survey that included the members of 128 Cretan households.

The study report was published in 1953, and I quote from this report: "olives, cereal grains, pulses, fruits, wild greens and herbs, together with limited quantities of goat meat and milk, game and fish, consist the basic Cretan food….no meal was complete without bread. Olives and olive oil contributed heavily to the energy intake. Food seemed literally to be "swimming" in oil." The Rockefeller survey data also indicated the frequent wine consumption.

This description was more or less repeated in the Seven Countries Study report, which actually set the basis for bringing the Mediterranean diet to the limelight.

L.G. Allbaugh advised the Greek government by saying that the food consumption levels "…were surprisingly good". On the whole, their food patterns and food habits were extremely well adapted to their natural and economic resources, as well as their needs".

What is interesting, however, about this study is that they asked the survey respondents to judge their daily diet. Allbaugh mentions in his report that only one in six families judged their typical diet to be satisfactory. He quoted one family as saying "We are hungry most of the time." Meat, rice, fish, pasta, butter and cheese, in that order of priority, were the foods most desired by the survey respondents in order to improve their diet. Meat was the first priority food, as it was mentioned by 72% of the studied sample. Please keep this point in mind because it will be our bridge from the past to the present. Those Greeks who were not satisfied with their diet in the 1960s implemented meat as their favourite food when economic conditions improved – when post-war conditions were overcome, they had the opportunity to make their dream come true.

Putting the results together from studies undertaken in this area in the 1950s and 1960s, we can say that the Mediterranean diet can be defined as a dietary pattern based on plant foods. Olive oil was used as the main, if not the only, added lipid. This diet was low in saturated fatty acid intake, contributing 7% to 8% of total energy intake. The total lipid intake ranged from 25% to 40%, and according to the data recorded at that period, the daily life of Mediterranean people and their work in the fields enabled them to have regular physical activity.

Variations in the Mediterranean diet do exist. However, for this presentation it will be convenient, if not fully accurate, to define the Mediterranean diet as the pattern recorded in the olive-growing areas of the Mediterranean basin in the late 1950s and early 1960s.

I will make use of the nine components shown earlier by Madam Chair, which I will use in my presentation as the definition of what constitutes the Mediterranean diet. You may have already heard about these components yesterday, so I will be very brief.

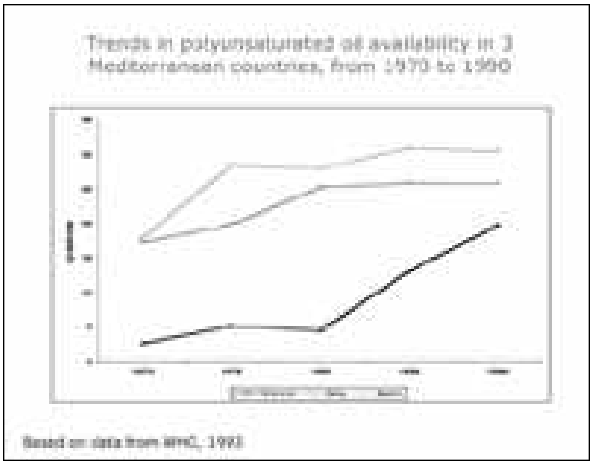
High consumption of olive oil, vegetables, fruits, non refined cereals (including bread) and legumes; moderate consumption of milk and dairy products (mostly as cheese and yoghurt); moderate to high consumption of fish; moderate wine consumption, and low consumption of meat and meat products. This is another point worth keeping in mind.

Coming now to the present, national surveys in the regions do exist, but they are of limited comparability when it comes to international comparisons because of the variable methodologies used. However, they generally agree that Mediterranean populations have significantly increased their consumption of meat and dairy products, especially cheese, while consumption of olive oil and fruits has decreased.

When it comes to international comparisons, one should rely on data gathered by FAO and collected in food balance sheets (FBS), or from data retrieved from the DAFNE databank, including information from national household budget surveys. Unfortunately, there has been a limited number of food consumption surveys undertaken in international settings, using

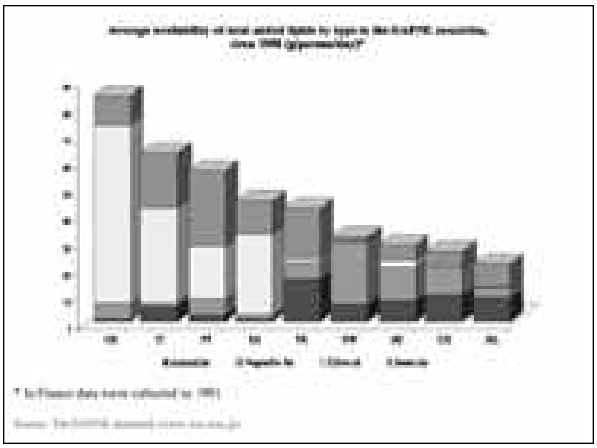
standardised methodologies, such as the EPIC study, on which Nadia Slimani will give a more detailed presentation today.

Caveats about the use of FBS data are well known. However, I myself will refer to these data in this slide, which presents changes in the supply of polyunsaturated vegetable oils in three Mediterranean countries: Greece in dark blue, Italy in purple, and Spain in light blue. You can clearly observe a sharp increase in the supply of polyunsaturated vegetable oils occurring in Greece in the 1980s. This picture is the result of a very successful campaign addressing the Greek population about the potential health benefits of seed oils. I can assure you that during this period, olive oil consumption has decreased in Greece – not sharply, but it has decreased.



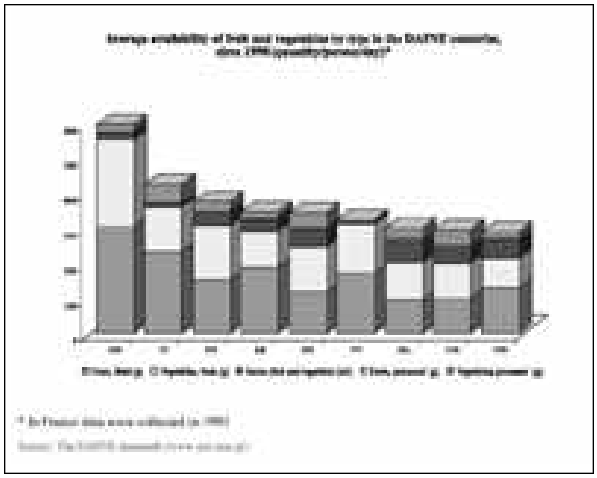
This graph presents data retrieved from the DAFNE databank (www.nut.uoa.gr) and collected in the 1990s.

The DAFNE databank includes information on food availability among nationally representative samples of European households. Four types of added lipids are displayed. The dark purple is animal fat, the light blue is vegetable fat, the yellow bar is olive oil, and the light purple is vegetable oils other than olive oil, like corn oil.



Once again, Mediterranean countries have the highest lipid availability. The picture of differences in the type of lipids consumed in Southern and Northern Europe persists. Olive oil provides almost the totality of added lipids availability in Greece, and it is the main added lipid in Spain and Italy. However, seed oils, other than olive oil, have become increasingly popular in Portugal.
In France, the consumption of olive oil is not frequent. France covers a large area from NorthWestern Europe to the Mediterranean shores. Furthermore, the cultivation of olive trees, which is an important characteristic for the Mediterranean countries, has not been important in France as most of the olive groves were destroyed by frost in the 1950s.

This graph, again retrieved from the DAFNE databank, presents the daily availability of fruits and vegetables in the same nine European countries. The data were collected in the 1990s. Mediterranean countries still record the highest availability of fruits and vegetables. You may concentrate on the yellow and the light blue, which are fresh vegetables and fresh fruits respectively. As I have already said, countries bordering the Mediterranean basin still record the highest fruit and vegetable availability values, but they have clearly departed from the high values recorded in the 1960s.



The DAFNE databank also enables food availability monitoring within the regions of a country. On this map, you can see the meat availability of 13 regions in Greece in mid 1990s. For the majority of them, meat availability exceeds 140g per person per day. Daily availability exceeds 160g/person/day in Crete, while in the seven countries studied in the early 1960s, meat consumption was 35g per person per day. It is quite an increase.

National studies have also been undertaken on how well populations adhere to the traditional Mediterranean diet. One of the first to address this question was Trichopoulou and her colleagues in a BMJ publication in 1995 (Trichopoulou A. et al., "Diet and overall survival in elderly people" BMJ 1995;311:1457-60). This publication refers to a study undertaken in the late 1980s on elderly males and females, all residents of three Greek villages. I will refer again to this publication, but from another perspective. Trichopoulou and her colleagues constructed an a priori defined diet score to assess adherence to the traditional Greek-Mediterranean diet, based on eight characteristics. Only 19% of the total were found to have two or fewer of the eight components, whereas 57% were found to have

four or more of the eight desirable components, reflecting at that time the attachment of elderly rural Greeks to their traditional diets.

A paper has recently been publisched, based on data collected through the Greek participation in the EPIC study (Costacou T. et al. "Tracing the Mediterranean diet through principal components and cluster analyses in the Greek population" EJCN 2003; 57(11):1378-85). The Greek EPIC data were collected in regions from all over Greece and the studied sample consisted of volunteers. In terms of energy density, no substantial differences were observed between the two genders, with the sole exception of alcohol contributing 5% of daily energy intake among males and only 1% among females. However, the percentage of energy deriving from saturated fat has increased from 8%, reported in the Seven Countries Study, to 13%

In the same publication and using the same data, the investigators applied principal component analysis to identify the dietary patterns of the Greek participants in the EPIC study.

Four components were identified. The first one was associated with high vegetable, legume, fruit, fish and olive oil intake, which approximated the Mediterranean diet. The second one mostly reflects a vegetarian diet, with emphasis on seed oils, rather than olive oil. The third one reflects a preference for sweets, and the last one reflects a western-type diet.

The investigators further evaluated the association of these four components with the previously defined Mediterranean diet score. The score was strongly positively associated with component 1, essentially unrelated to components 2 and 3, and negatively associated with component 4. These results give confidence to the interpretability of both component 1 and the Mediterranean diet score.

Furthermore, component 1 was used in analyses to evaluate its dependence on several socio-economic, demographic and lifestyle variables.

So, component 1 was positively associated with female gender, age, education, physical activity and non-smoking status, indicating that this type of diet mostly represents the choice of educated and health-conscious contemporary Greeks.

Component 1 was also higher in the islands, which are essentially rural, whereas lower scores were evident in Attica and in Northern Greece, which included the large cities of Athens and Thessalonica.

Similar findings on the characteristics of people who apply the Mediterranean diet were also reported in a Spanish publication, presenting data retrieved from the SUN project – a prospective study undertaken in Northern Spain using university-level participants with the aim to study the effect of Mediterranean diet to health (Sanchez-Villegas A. Eur J Nutr 2002 Dec;41(6):249-257). The investigators have recently published the results of a cross-sectional analysis to determine the characteristics of people who follow the traditional diet. In accordance with the Greek results, they reported that women were more compliant than men, and that in both genders, compliers were older and more physically active.

In another publication, in Spain, healthy volunteers from 29 to 69 years old from regions covering Northern and Southern Spain – all participants in the Spanish EPIC cohort – were studied to assess adherence to Mediterranean diet (Gonzalez CA. et al., Gac Sanit 2002 May-Jun; 16(3):214-21). The investigators used a Mediterranean diet score, including nine food components: vegetables and garden products, fruits, pulses, cereals, red meat, fish, olive oil, milk and milk products and wine. They reported that in this case, no variation in adherence according to the educational level of the participants was found, whereas they noted that adherence was lower for young adults and women and was slightly higher in the south than in the north of Spain.
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A North South gradient in adherence to the Mediterranean diet was also reported in Italy. It is true that Northern Italians eat more like Northern Europeans. Unfortunately, however, it seems that trends in the eating practices of the Southern Italians are at the risk of impairing the comparative advantage given by the Mediterranean diet.

In this study undertaken in a rural village in Sicily (Barbagallo CM. et al., J Am College Nutr 2002; 21(5):523-29), the investigators reported a significant increase in total and saturated fatty acid intake among young and highly educated people. They also reported that physical activity – an important component of the Mediterranean pattern – was lost.

Lastly, an interesting study, also recently published, compared the plasma phospholipid fatty acid composition in pre-school children from Porto and Munich as a biomarker of their fatty acid intake (Guerra A. et al., Ann Nutr Metab 2001; 45(2):78-81). In both groups, plasma phospholipid contents of total saturated fatty acids were similar and, surprisingly, the Portuguese children had lower values of monounsaturated fatty acids. Interestingly enough, the results indicated that the food habits of Portuguese children were even less close to the traditional Mediterranean diet than those of German children.

Overall dietary patterns are the result of the continuous interaction between traditional foods and the assimilation of new foods. There is unanimous agreement that the Mediterranean diet is healthy. However, it is important to try to understand what the diet is now, what it used to be, and in what way it is changing. For populations who are not familiar with this type of diet, efforts are needed to educate them on its health benefits. As for the Mediterranean populations efforts are needed to reverse the change, not only as a need to promote health in our populations but also as one of the few ways to preserve our tradition.

Thank you very much.

Questions

***Antonia TRICHOPOULOU** – School of Medicine - University of Athens*
Are there any questions or comments?

Member of the Audience
Thank you for your presentation, it was very interesting.
I would like to comment on the point that we also need multi pre-correspondent analysis. We found two patterns: the Mediterranean type and the fast-food type.

The essential socio-economic determinacy was essentially urbanisation and the presence of large supermarkets and high-distribution types of stores. We also found that the Mediterranean type belongs to the rural areas, whereas the fast-food type was around Montpellier.

It is in line with what you have said, except that because our socio-economic indicators were higher in France in the 1960s than in Greece, it is globalisation, if I may say so, and not the desire for better food which has brought this change in our regions.

My second remark is more a question. I am very surprised by the Portuguese study because monounsaturated fat in the serum is not really an intake marker because it can be synthesised, so I do not know if you can really say in this study that they were consuming less monounsaturated fat. Monounsaturated fat comes from meat, and monounsaturated fat in Germany may essentially come from meat and pork.

Androniki NASKA– University of Athens – Medical School
As for your first comment, I was also quite surprised when going through the literature because the changes are quite similar in the different Mediterranean countries.

As for your question, you are right. However, I have decided to present these results because I find it very interesting to show that monounsaturated levels were high in German children, not as an indicator of high olive oil consumption in Germany – as you correctly mentioned, this kind of fat comes from pork, which is quite commonly consumed in Germany – but as an indication of the low consumption of olive oil in Portugal.

According to the DAFNE databank, it seems that the traditional olive oil consumption is getting lost in Portugal: the consumption is becoming lower and lower and other vegetable oils are becoming more popular.

Dietary patterns in Europe

Nadia SLIMANI
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First of all, I would like to thank the organisers for inviting me to give this presentation. As you will see, my presentation will consist of asking a question, or at least answering a question: whether we have varying dietary patterns across the ten Western countries participating in the EPIC study.

The Mediterranean diet and its specific beneficial effect on health cannot be fully understood if it is not compared with other existing dietary patterns throughout the world. Comparison of dietary patterns within and between countries is essential in order to identify different forms of the Mediterranean diet and other existing diets, and also to understand how they evolve over time and how differently they can affect the association between diet and disease.

The aim of my presentation is to identify different dietary patterns across the countries participating in the EPIC study and to determine whether the study actually benefits from a sufficiently large heterogeneous type to optimise the diet-disease association analysis, particularly for cancer and cardiovascular diseases.

The European Prospective Investigation into Cancer and nutrition, the EPIC study, is the largest prospective study on diet in cancer. It represents a total cohort of half a million subjects from 22 centres in 10 European countries. The geographical spread of this population is very large: from Southern countries from the Atlantic and Mediterranean areas, like Spain, France, Italy and Greece to Nordic countries, like Sweden, Norway and Denmark, and including some Central European countries, like the UK, Netherlands and Germany.

The main objective of this presentation is to determine the nature and magnitude of the difference in dietary patterns within and between the EPIC countries. My presentation will consist of trying to highlight the contrasts across these countries, rather than focusing only on the quantitative aspects.

The population involved in this study consists of a representative sample of middle-aged men and women representing 5% to 12% of the active cohort and representing a total sample of about 36,000 subjects. In France, Norway, and two centres respectively in the Netherlands and Italy, only women were recruited. In the UK, two kinds of cohorts were recruited, one from the general population, and another one in the Oxford centre that represented a quite heterogeneous group of vegan overlapping to vegetarian, fish eating and milk. In total, we considered 27 redefined centres or regions in the analysis.

The dietary information was collected using a single 24-hour recall per subject with the purpose of estimating mean intake consumption at a central level. This information was obtained using a highly standardised computerised programme, called EPIC-SOFT, during a face-to-face interview conducted by 80 trained interviewers throughout EPIC. In Norway, where the population is scattered all over the country, the interviews were conducted by telephone using a very similar method adapted for telephone interview.

The 24-hour recall is a dietary method that, because of its open-ended nature, provides a high level of detail on individuals and recipes, thus allowing more comparable data. To further improve the comparability of consumption data across countries, the recipes were systematically broken down into ingredients and further classified according to a common classification system across the countries used in this analysis.

In this analysis, we used 22 main groups and sub-groups with the purpose of covering the entire components of the diet, except for two relatively minor and very heterogeneous groups – soup and miscellaneous.

To further standardise the mode of expression of the food consumption across countries, the food quantity was systematically expressed as edible parts, concerning particularly fish, meat and vegetables and fruit, and as finally consumed.

In order to make a full comparison of the consumption of alcoholic beverages across countries, alcohol was systematically estimated as ethanol in this analysis.

Crude and adjusted food group means were systematically calculated using a multiple linear regression model. Furthermore, different models were tested, first of all using some basic dependant variables, like age, weight and season, and further by adding total energy intake into the model, anthropometrical measurements and socio-economic status. Whichever model was used, the overall pattern remained about the same in most of the centres.

To compare dietary patterns across countries using the same reference scale, multidimensional graphic representation of the consumption of the 22 food groups considered in the analysis was obtained by rescaling the mean of each individual food group relative to the overall EPIC set and food group specific mean. In other words, this relative consumption was expressed as a percentage deviation of the mean food group intake relative to the total EPIC mean.

All the analyses were stratified by gender and centre and again, the overall patterns are comparable, even though we have some specific correctives for gender and specific consumption, like alcohol or fish. However, for this presentation, I decided to present you only the women, knowing that they are represented in all the centres, in contrast to men.

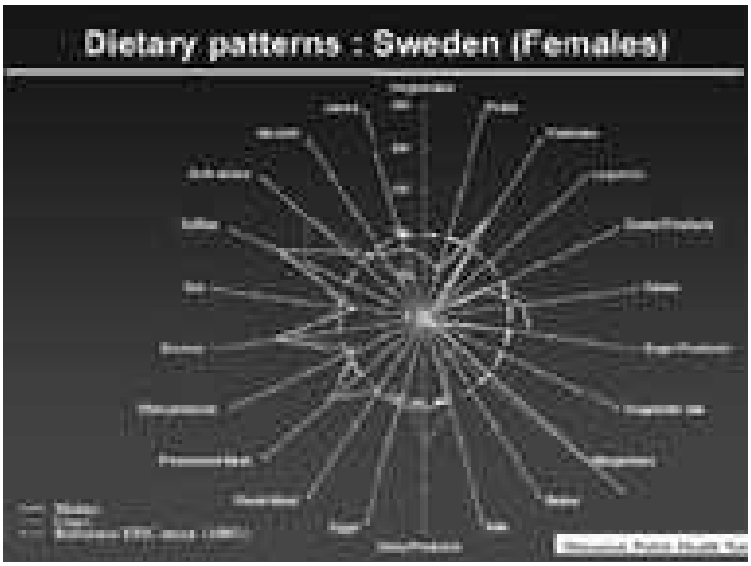
Before presenting you the results in more detail, I think it will be easier and helpful for the later presentation if I summarise them first. If we put aside the dietary pattern of the health-conscious in the UK, which will be detailed afterwards, we can identify three main broad categories of dietary pattern in EPIC.

The first kind of dietary pattern we observed is a consumption characterised by high consumption of plant foods, like vegetables, fruit, legumes and vegetable oils (where potatoes were not included among this group), with a relatively low consumption of animal and processed foods. This first category of dietary pattern concerns a whole series of possible variants of what we traditionally call the Mediterranean type of diet, and in EPIC, that appears particularly in Greece and Italy

In contrast, we have other different sorts of dietary patterns, which are characterised by a high consumption of potatoes, animal foods and all processed and sweetened foods, with a relatively low consumption of fruit, vegetables – particularly legumes – and vegetable oils. That again may be seen as a kind of different variant of what we call the Western diet which occurs particularly in the Nordic countries, the UK general population, the Netherlands and Germany.

In addition to these two categories, there is a third very interesting one, which combines the characteristics of the two previous ones. In other words, this mixed pattern combines both the characteristics of a high consumption of plant foods, which is traditionally associated with the Mediterranean diet, as well as a relatively high consumption of animal products, which is traditionally associated with the Western diet. That is in Europe in France, and much more strongly in Spain, as we will see soon.

Before going further into the explanation of the different dietary patterns, I would like to give you some guidance on how to interpret these graphs. There is a dotted circle with a radius of 100: this is the EPIC mean reference for all the different food groups. For each individual food group, the mean consumption in grams has been re-expressed relative to the EPIC mean, so the consumption of individual food groups is expressed as a percentage deviation relative to the EPIC mean. The higher the deviation of the spike above the EPIC mean, as you see for legumes in Greece, for example, the more the given food is a strong characteristic of the given centre, and inversely when it is below the circle, as is the case for butter.



I am going to present the relative consumption, so the notional absolute will be there implicitly, but not expressly shown on the screen. On this first slide only, what is indicated in brackets is the EPIC mean of each individual food group considered for the calculation.

Starting with the diet in Greece, as you can see this diet might be seen as a dietary pattern closer to what we call the traditional Mediterranean diet. It is characterised by a high consumption of plant foods, particularly vegetable oils, legumes and vegetables. In contrast to Spain and Italy, the consumption of vegetables is relatively higher than that of fruit, which is slightly below the mean. The consumption of animal products is always below the mean, even though there is a slightly higher consumption of fish. However, fish, fresh meat, eggs, dairy products and milk are 6 points below the EPIC mean, and processed meat is not at all a characteristic of the Greek diet, neither is butter nor, to a lesser extent, margarine, coffee and tea.

Another dietary pattern from the Mediterranean area is the dietary pattern for Italy.

Different centres were involved here but despite differences across centres, which can be seen by the magnitude of deviation of the different spikes, it is interesting to see that they have a comparable dietary pattern shape overall. This diet is also characterised by a high consumption of plant foods. However, a very country-specific characteristic, in comparison to the other EPIC centres and countries, is the high consumption of cereal products, which is very specific to Italy. The consumption of fruit and vegetables, however, is also high in most of the country – the consumption of legumes is particularly high in the Southern centres. The consumption of animal products is always below the mean, except that Turin and Ragusa, which are also Southern centres, are characterised by a high consumption of fresh meat. Although the overall consumption is below the mean for milk, butter and, to a lesser extent, processed meat, we can see a kind of geographical gradient with a higher overall consumption of those groups in the northern centres compared to the Southern centres. As you can see, margarine consumption is very low, as is the consumption of tea, which is not a characteristic of this kind of diet.

Spain probably has the most interesting dietary pattern because it has the characteristic of combining a high consumption of plant foods, particularly vegetable oils, legumes, fruit and vegetables. We also see a great difference in terms of magnitude across centres, but overall, as was the case with Italy, we can see a quite comparable pattern. However, the consumption of animal products also includes fish, particularly fish, eggs, milk and, to a lesser extent, fresh meat (Spain is among the countries with the highest consumption of this group). This group also has a quite high geographical gradient, usually with a systematically higher consumption in the northern centres compared to the Southern centres. It is also interesting to note in that sense that the highest consumption of legumes is in the northern centres and not in the Southern ones, even though the consumption is much higher than the EPIC mean.

Forty French geographical regions were involved in France, covering inland regions and the Atlantic and Mediterranean areas. Although we see differences across centres, in the end the overall pattern again remains quite similar. A characteristic of the diet in France is the high consumption of butter, dairy products, fresh meat as a whole, and vegetables. We see that the overall consumption of dairy products or, to a lesser extent, fresh meat, is quite comparable even though for other groups, like vegetable oils, legumes and vegetables, there are differences across the country. There are some regional characteristics, such as a high consumption of fish and potatoes in the North West of France, and also tea. This shows that there are still regional differences but they are not as large as we might have expected.

As I said before, the UK population, is composed of two different … [laughter from the audience] … Is it the tea that is making you laugh? We did not complete the spike for tea – as you understand, it is one of the unique characteristics of the UK in comparison with the other centres. There are some others, however, because they are also high consumers of soft drinks, potatoes, cakes, and sugars. You will note that there is a higher consumption compared to the EPIC mean of some of the more processed foods and refined sugars. The consumption of animal products is below the EPIC mean, particularly for fish, processed meat, fresh meat and eggs, but higher for milk and dairy products, particularly butter. The consumption of margarine is also quite high. I do not know whether you noticed, but you will see something very interesting concerning added fat: you will see that there is a very nice trend from the different patterns of the countries that consume only olive oil or vegetable oils, and from those that combine two kinds of sources of fat, like butter and margarine (which we will see later in the dietary patterns of the Nordic countries and the Netherlands). Therefore, there is an almost complete substitution of margarine for butter. Pay attention also to sauce, which is also a relatively large source of added fat in dressings.

We now look at the health-conscious cohort, which is a quite heterogeneous group. The strongest characteristic of that group is a very low consumption of animal products, particularly fresh meat and processed meat – about 5 grams. However, the consumption of milk and particularly butter is much higher, even though it is much lower than the general population. What is also interesting is that they have some characteristics making use of substitution, or the health effect, having a high consumption of plant foods like legumes and fruit, which are around the mean. However, the consumption of vegetables, which is above the EPIC mean, shared the characteristics of a vegetarian diet to a certain extent, although the absolute consumption is much lower, but still much higher than the general population. It is also important to know that, although that group has this healthy kind of diet, it has still kept very strong British Western characteristics, like a relatively high consumption of cakes, sugars, margarine, soft drinks, coffee, and tea of course. Another strong characteristic of that group, which is not reported (again because of the unique aspect of that diet), is the high consumption of soya products, particularly soya milk – about 90 grams on average in these women.

The last slide is an example of dietary patterns from the Nordic countries. In Sweden, the characteristics, as I said before, made use of the extremely high consumption of margarine compared with the other EPIC centres. There is also, however, a high consumption of potatoes, coffee, sauce and processed meat. There are some differences across the two centres – one in the south (Malmo) and the other in the north (Umea) – with a higher consumption of dairy products and soft drinks in the north.

Another way to give an idea about the range of heterogeneity dietary consumption in EPIC is to give you some rates between the lowest and the highest sex-specific centre mean intake.

You can see in this table that the ratio varies from 2 to 5 for groups like vegetables, fruit, potatoes, milk, milk products and eggs, to a more than 100% rate variation for the highest groups, like legumes and soy products. There is a series of intermediate groups of major etiological interest, like alcohol, processed meat, fresh meat and coffee, which are from between 5 to 9 and 15 to 20 for all differences across the 27 centres considered in this analysis.

For concluding the presentation of results, here I also wanted to show you that what I have just presented is for the analyses which were done mainly at the aggregated level, so the main groups. But as soon as you try to introduce more reference groups taking into account the type of vegetable consumed – here we have different categories, like leafy vegetables, fruiting vegetables, roots, cabbages and onions – this adds further heterogeneity in dietary patterns.

Concerning vegetable consumption, the main contributors to the total consumption of vegetables in most of the countries are fruiting vegetables – tomatoes, egg-plants, cucumber and so on – which in most of the countries might represent 1/3 to 2/3 of total intake.

Roots, cabbages and leafy vegetables represented 10% to 15% of the overall total intake, but we can see quite interesting variations across countries. To summarise, taking into account both the quantitative and the qualitative aspects, the Mediterranean diet tends to have a high proportion of fruiting and leafy vegetables, whereas roots are relatively more frequently consumed in Nordic countries and the UK, and cabbages in the Netherlands, Germany and the UK. Onion and

garlic, which are appropriate to estimate with a 24-hour recall, appear to be particularly consumed in Spain and Greece. Bear in mind that when we talk about dietary patterns we also have to take into account the possible different levels of investigation.

For the main conclusion, I would like to answer the question I addressed at the beginning of the presentation and confirm that we actually do have a very large heterogeneity in this middle-aged population in EPIC. In addition to that, we can see that there are quite strong country-specific patterns. That means that although the patterns really differed from one country to another and there were also differences across centres from the same country, the overall shapes seem quite comparable. I think it is probably something related to the association of food groups more than the quantitative issue.

In addition, we see that we have different variants of the Mediterranean diet and two more or less pronounced Western diets. We can also see a quite strong north to south gradient within and between countries, particularly in Southern countries where the consumption of different animal products varies according to a geographical gradient.

If you put this data into perspective, it tends to support some general trends that have been reported elsewhere. The dietary patterns in Western European countries have changed, especially over the last 30 or 40 years. Sometimes they tend to keep some of the strong characteristics of their initial diet but they also adopt new ones, which may tend to go more towards a Western diet, while still keeping these two components. That is true both in Nordic countries and in Southern European Countries.

As a conclusion, these results support a number of needs, which particularly suggest a better identification, and probably redefinition, of the different variants of what we call the traditional Mediterranean diet (which now refers to a definition given 40 years ago), and also the different kinds of Western diet (because there seem to be different ones). I think we all admit that there are quite drastic changes in dietary patterns, which seem to be changing faster and faster, so it may become important to monitor these different dietary changes over time and identify the determinants. That will be particularly important in the younger generation where this phenomenon seems to be accelerated.

As a last comment, I would like to say something of the importance of certain methodological aspects. We probably need to use and further develop the multi-variant statistical model approach to dietary patterns and investigate the association with disease, both as the main measure of exposure and also as a possible co-variable.

Thank you.

Questions

Chairman
Thank you very much, Nadia, for such a nice presentation. The data was really amazing.
We have five minutes for discussion.

Member of the Audience
Thank you, Ms Slimani, for a very interesting presentation.
I would like to know why you chose to compare each country to the EPIC mean instead of comparing to an ideal Mediterranean diet, because the EPIC mean is just the mean of the countries you have chosen, so it is not a reference.

Ms Nadia SLIMANI
In this kind of presentation the purpose was to compare within EPIC. It is something we are thinking about but because this is the first kind of descriptive analysis from EPIC, the first purpose was to answer the question I addressed at the beginning of the presentation. The reference can be of any kind because in this sort of comparison within and between countries, whatever reference you use as a denominator, it will not change the differences across centres.
We think we will be faced with more methodological problems of comparing the EPIC data, for example, with the representative samples in different countries. Then we will be faced with the problem of data comparability, so that is something which needs further investment. I take the point, however.

Member of the Audience
I have a question about terminology.
In Spain, you said that they have a diet high in animal foods, but if you look at the Spanish diet and you look at the animal foods, then fish plays a very important role in their diet. Is it correct to use the terminology 'animal foods', because it adds negative components like dairy products, that are very rich in saturated fat, and also includes foods like fish, which have a high amount of 16.57 and polyunsaturated fatty acids? Can we use 'animal foods'as a general term anymore? Should we not separate them into animal foods that have adverse effects in relation to health and those with a positive effect, and not put them together? Is that not the problem in relation to the Spanish situation?

Ms Nadia SLIMANI
I see the point, but I think it is correct to use the term 'animal products'when it is purely descriptive. However, I agree with you that as soon as you want to associate diet to a possible effect, it would be important to make this distinction, even though for some of them it is still unclear whether they should be in one group or in another, I think it depends on what you are looking for.
I was a bit neutral here because this was purely descriptive and maybe (as we see in countries going through a transition in terms of dietary consumption) reflects that people tend to substitute their basic foods, usually plant foods, with animal products, and they actually change fish and meat. In terms of a general increase in consumption, it is a general trend that you observe in societies changing their diet because they are improving their quality of life and socio-economic conditions. They tend overall to increase the consumption of animal products, but of course the qualitative type is very important as soon as you are concerned with the dietary association.

Member of the Audience
Thank you very much for a very nice presentation Nadia.
I have a question on the presentation and the way of presenting the results and also relating a little to what Daniel was asking about the food patterns.
Because you presented this as stars – this is radius and so on – I noticed that the food groups are clustered according to grains, sources of fat, sources of protein, plant foods and so on. You often see a lot of replacement within those food categories, so there are negative correlations between food groups within one sector of the whole circle. I wondered whether the interpretation of the whole figure might be more natural if you could order these radii in a different way so that you have those with a positive correlation close to one another, and those with a negative correlation on the other side (because the way you do it is arbitrary, in fact). You would then see a whole shift from one side to the other side. Maybe this way, you could improve the ease of interpretation and presentation. I am eager to know what is behind this way of presenting the data and whether you considered other possibilities.

Ms Nadia SLIMANI
I forgot to explain how I arranged the food groups. Again keeping a neutral approach, it was because that is how the first kind of descriptive data had been published. However, the food groups as they are presented in the graphs are in order according to their plant foods and the degree of processing. If you remember, I put the different plant foods and cereals over to the right, then the cakes, then the sugar, and then you move on to the vegetable oils, margarine, butter, animal products and so on.
I agree with you that there are probably other ways of presenting data and I would be happy to think about it.

***Denis LAIRON** – INSERM Unit 476 – Lipids and Human Nutrition, France*
Thank you for your talk. I have a short comment and then a question.
The comment is that in the 1950s and in the Seven Countries Study, there is a huge difference in Europe, where the trend is to have a big homogenisation of diet, but your data supports the concept that there are still very big differences within Europe. My point of view is that the general concept of what you are saying now points to a true faith of thinking and doing something.
My question is about the selection of the subjects, because to my knowledge the EPIC study cohort is a very big cohort with the most alpha-medium subjects studied and all you selected was a small number of women within the large cohort.

Ms Nadia SLIMANI
The sub-samples used for this analysis have been selected for a sub-calibration study for the purpose of correcting systematic errors between the questionnaires used in the different EPIC countries. These sub-samples are representative of each of the EPIC cohorts. They are representative of 5% to 12% of each individual cohort, which themselves represent 30,000 to 70,000 subjects.

Member of the Audience
I am Marie Francis - journalist.
I found your presentation of great interest, with the differences in consumption between women in different countries and the latest graphics. Just one food sub-group surprises me, that of the sauces, because between those consumed in Italy and the UK, on the nutritional side can we find similarities?
In the same way between margarine and vegetable oils, are there not in some instances groupings of these?

Ms Nadia SLIMANI
That is also a good question. The question was about the fact that I used sauces, which combines dressing sauce and tomato sauce, as a quite heterogeneous group. The question is whether we can actually fully interpret that, knowing the heterogeneity.
There are two points here. This is an inevitable problem as soon as you are concerned with making comparisons. The problem is that as soon as you want to compare two groups, you are concerned about a certain heterogeneity within the groups as was shown. That was the point I made by showing the sub classification of kinds of vegetables – as you can see, it varies across countries. However, there is also some interest in maintaining a global pattern because it is easier to compare at the aggregated level for a first analysis.
For sauces, the problem would be to break them down into ingredients. We did not do that for all of the food groups because it was a bit complicated, but you are right. If you remember the graph, Ragusa has a particularly high consumption of sauce and it is mainly tomato sauce, about 50 grams of tomato sauce a day. Of course, they consume ketchup in the Northern countries too but they also have other different kinds of dressings. That will be better covered when we look at nutrient levels, so we will hopefully get rid of these problems.

Member of the Audience
Why did you not include potatoes in your vegetable classification, because potatoes are seen to be competition with vegetables for potassium, for vitamin C, fibre and poly flavanols? It seems they are not frightened of butter, so it is an important question for me.

Ms Nadia SLIMANI
First of all, potatoes are consumed differently and they are on the opposite side. Potatoes are mainly consumed in Nordic and central countries, compared with fruit and vegetables which are mainly consumed in the south, so there is already an implicit need to separate them. In addition, the nutritional content – energy, protein and so on – is quite different from that of vegetables. That has become a more classic way to present data in relation to diseases; they would prefer to have potatoes separate from vegetables, knowing that they can always be aggregated. The problem in many studies where they are all aggregated is that you can no longer make a scientific analysis by saying how you obtained results associated to disease by including potatoes or not. It is therefore easier to have them separate and maybe recombine them if needed.

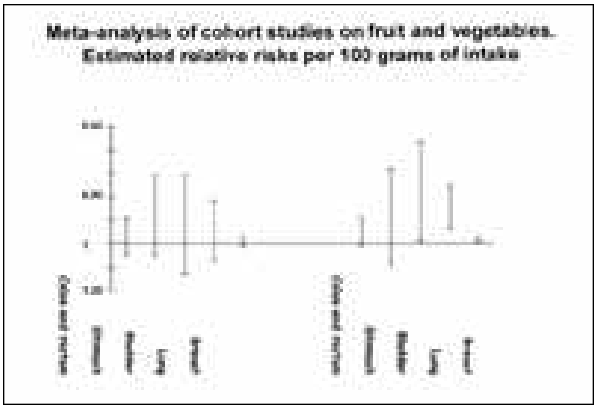
Consumption of fruits and vegetables and prevention of cancers of the digestive tract : result of EPIC study

Elio RIBOLI
International Agency for Research on Cancer (IARC-WHO), Unit of Nutrition and Cancer,
150 cours Albert Thomas, 69372 Lyon Cedex 08, France

First of all I would like to thank our colleagues from APRIFEL for having organised this conference: without the personal engagement of Dr Barnat, this would have never have taken place.

I will present you two types of results.

Firstly I will present the results of what, in epidemiology, is called meta-analyses. It is a reanalysis of previous studies which was carried out at IARC in collaboration with Teresa Norat. This was conducted with the aim of summarising the results from previous case control and cohort studies. I will then present some first results from the EPIC study; the reason why I am doing this is because of the size of EPIC, which includes 1/2 million people and therefore is almost as large as most of the previous studies combined.



These graphics presents the relative risk (i.e. the relative probability of developing a disease) for those with a high consumption of fruits or vegetables compared to those with a low consumption of these. Each point estimate of the relative risk represent the average of several studies.

The studies of cancer of the mouth, pharynx, larynx and oesophagus, which also include one prospective cohort study and several case control studies, indicate that high consumption of fruits is associated with a substantial reduction in risk, which goes from a 24% to 47%. These results were adjusted for consumption of alcohol and tobacco the main risk factors for these cancers of the upper aerodigestive tracts. The protective effect is less clear for vegetables. There is a protection, but it is not statistically significant.

Several cohort studies have been conducted on cancer of the colon and rectum, more than two thirds of which in North America. We found that case-control studies reported stronger evidence of protection than cohort studies. Cohort studies are generally considered of a higher quality than case controls because the baseline measurement of diet is made on healthy people, years before cancer diagnoses, which avoid differential recall bias associated with disease status. These results raise the possibility that the protection observed in case-control studies may partially be due to some uncontrolled confounding, recall and/or selection bias.

The results are even more disappointing regarding fruits neither cohort nor case-control studies show any clear protection against colon or rectum cancers. This analysis includes the two major cohorts from the United States which were recently published and which found no protection from fruits and vegetables.

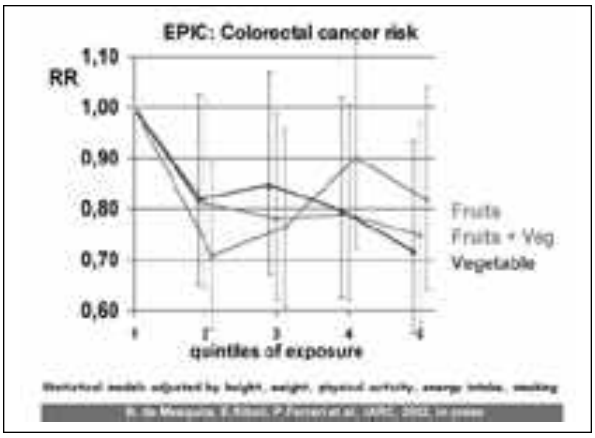
For cancers of the lung, which is strongly related to tobacco consumption, the cohort studies found no significant protection, while the case-control studies found only some protection from vegetables. For fruits, there is some evidence of protection from both case-control and cohort studies
Therefore, all together, considering the recent results from cohort studies conducted in North America and in Europe we can tentatively conclude that there is epidemiological evidence supporting the protection from fruit and vegetables, however the effect does not appear to be as strong as previously thought.
From a public health point of view, these results can be translated into how much we would gain if people increased their consumption of fruit by a given amount. To use a round figure we considered 100g per day. How much would the incidence of these cancers be reduced if the entire population, and particularly those with a low consumption, would increased their consumption of fruit by 100g per day?

Our estimates suggest that there would be a reduction in the order of 14% to 20% based on cohort studies, but there would be a stronger protection based on case-control studies.

This discrepancy is an important methodological issue which currently limits in the interpretability of the epidemiological studies on the relationship between diet and cancer. These methodological issues had a central role in the design of the EPIC study.
EPIC is a large prospective cohort study, based in 10 European Countries.
Between 1993 and 1999, 521,000 subjects volunteered to participate in the study. Questionnaire data on diet and life style and blood samples were collected and will be used to investigate cancer risk factors.
In the design of EPIC we considered three possible ways for increasing the power of identifying dietary factors related to cancer risk. The first is to select populations with a very wide variation in dietary habits. Ideally, one would like to compare cancer risk between people who have either a very high or a very low consumption of a given food in the same population.

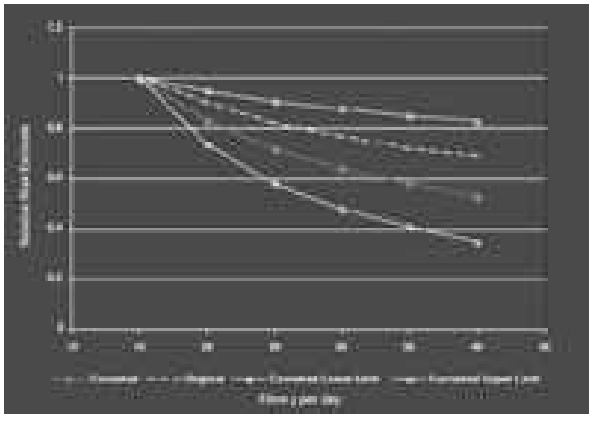
Secondly, to design a better and innovative method of measuring diet.
As previously presented by Dr. Slimani, we implemented for the first time in EPIC a large sub-study aimed at calibrating dietary intake measurements. The study was conducted on a sample of 38,000 subjects out of the 521,000 EPIC participants. Diet was measured with two independent methods : a detailed questionnaire on usual diet and a highly standardised 24-hour recall, as reference method. Thirdly, we designed the study with the aim of taking advantage of the large variations in cancer incidence existing across Europe. For example the incidence of cancer in Murcia and Granada is about 1/3 lower than in England or Sweden. Variations are even larger variations for specific cancers and the variations in diet are very large as well as shown by Dr. Slimani in her presentation. Therefore, despite a tendency towards more uniform lifestyles, diet has remained substantially heterogeneous.
In addition to diet, detailed data on life style were collected. The collection and storage of blood samples will provide the possibility of studying the role of nutrition in cancer aetiology, using both biomarkers of diet and individual genetic markers. During the first 5 years of follow-up over 20,000 cancer cases occurred among the EPIC subjects

The following slides report the preliminary results for colorectal cancer risk and consumption of fruit and vegetables



These results suggest a modest reduction in risk associated with fruit consumption, which is statistically significant. The results from vegetables are less clear and for the moment do not show any statistical association with colorectal cancer risk.

The following slides present the results on consumption of fibre and colorectal cancer risk (Bingham et al., Lancet May 21^{s}, 2003). These results indicate that consumption of more than 30 grams of fibre per day compared to 15 grams or less is associated with a significant reduction of colorectal cancer risk of about 40%. The estimate of the RR corrected for random measurement error using the calibration study data indicate that the protection could be even stronger in the order of 50 to 60% risk reduction.



Almost 30 years ago, Burkitt proposed the fibre hypothesis based on the observation that in South Africa the African population had very low colorectal cancer incidence and very high fibre intake, while the population of European origin had high colorectal cancer incidence and low fibre intake. It was estimated that fibre intake in the rural African population could be as high as 70 to 80 grams per day. So, the high consumption level within Europe are still 50% or so lower than the level that could be found in populations with a very low colorectal cancer incidence.

Regarding stomach cancer, the study is still on going. A preliminary analysis suggest a 37% reduction, in stomach cancer risk among subjects consuming more than 500 grams per day of fruit and vegetables. On the other hand, stomach cancer risk seems to be increased in relation to processed meat consumption. The association of processed meat with stomach cancer has been reported several times in the past.
It has been suggested in the past that these contrasting effects could be explained by the putative carcinogenic effect of nitroso compounds from processed meat, which could be antagonised by antioxidants from fruit and vegetables. More studies are needed, however, to confirm or disprove this hypothesis.

Finally, I shall present some preliminary results from EPIC regarding cancer of the upper GI tract: mouth, pharynx, larynx, and oesophagus. These cancers are very strongly related to alcohol and tobacco. Therefore great attention must be paid to carefully adjust the study on diet for tobacco and alcohol consumption.

In EPIC, after having carefully stratified for lifetime's history of alcohol and tobacco consumption, we found strong evidence of protection from fruit and vegetable consumption : for example, a consumption of more than 450 grams of fruit and vegetables per day, compared with those who eat less than 180 grams per day, is associated with a 50% reduction in risk of developing these cancers. However, this should be interpreted within the general context, as the risk from alcohol and tobacco is much stronger than any protection which could be expected by fruit and vegetables. The risk associated with smoking one pack of cigarette per day and drinking the equivalent of one bottle of wine per day is 50 times higher compared to the risk for non-smokers and non-drinkers. Clearly the way to prevent these cancers is to eliminate tobacco and to moderate alcohol consumption.

Conclusions: the overall evidence from these first results from EPIC as well as from previous epidemiological studies do support the recommendations that consumption of fruit, vegetables and whole cereals in large amounts in the daily diet can only be good for health.

The current data suggests that the protection may be on the level of a 20% to 40% reduction in cancer risk. This is more of a guess estimate than an accurate estimate, but it is my personal conclusion at this point in time. It may not be as strong as was thought 10 years ago, but it is there.

The evidence on the protective effect of fibre in food on colorectal cancer is, in my opinion, between moderate and strong. However, there are still many unknowns and the current research ongoing within EPIC and other major cohort studies should bring us some more information within the next five years.

Questions

Dr Arthur SHATZKIN
Thank you very much Dr Riboli for giving us a little hint of some things to come from one of the most important epidemiological studies of nutrition in cancer ever conducted.

Member of the Audience
Thank you Elio for this excellent presentation
You consider fruit and vegetables separately. Is there any stronger effect if you add fruit and vegetables together in the EPIC study?

Dr Elio RIBOLI
This is a very important point. In this analysis, we started by analysing specific foods as well as major food groups.
There are instances, as for the upper GI tract, where when we add up the total consumption of vegetable products – with the exception of potatoes, which do not seem to be related to cancer risk in our data– we apparently get a stronger protective effect than when we look at one fruit or vegetable at a time. My interpretation is that the total amount is probably a better indicator of the global diet. It may well be that it is simply because in taking the whole, there is less measurement error than taking one specific subcategory. However, your point is well taken and it is an issue we are trying to find out about and sort out.

Member of the Audience
Thank you for your presentation.
A lot of people this morning have told us that we should eat more fish. We all know that fish is contaminated with a lot of PCBs and things like that.
Has anybody done a risk analysis on the effects of eating the recommended fish intake and the effects of PCB intake?

Dr Elio RIBOLI
This is a good point and there are two ways to answer.
Firstly, we share your concern. We are setting up a study on mercury and we hope to set up a study on PCBs soon within EPIC to look at mercury, which mainly comes from fish, and PCBs, which come from plenty of things. Our major concern with seafood is mercury.

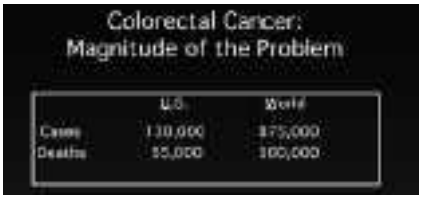
Now, when we look at fish and we find a protection, it means that fish is protective as it is – whether it contains mercury or whatever other contaminants. It is like vegetables: when we find the protection, it means that the vegetables are as they are in real life. This is not an experiment; we do not give pure fish: this is what people buy in the market. Therefore, as people buy vegetables in the market that have a residual content of agricultural chemicals, so they buy fish, which has a residual content of whatever. A lot of the mercury, by the way, comes from natural sources; it is not industrial pollution at the seaside. PCBs are obviously not natural.
We are also planning to look at mercury in details, particularly for cardiovascular diseases. The issue is how to separate the consumption of foods which may be protective from the detrimental effects of chemical or physical contaminants.

Fibers and cancer prevention

Arthur SCHATZKIN
National Cancer Institute, Division of Cancer Epidemiology and Genetics, Nutritional Epidemiology Branch, 6120 Executive Boulevard, EPS Room 3040, Beteshda, MD 20892-7232

I am going to put some of the remarks that Elio Riboli just gave us about fibre into a larger context and try and deal with what has become one of the most controversial questions involving diet and cancer.

You can see, both in the US and around the world, the very large number of people who are diagnosed with colorectal cancer, and then die from the disease. Around the world, half a million people will die from colorectal cancer each year.



It is true that there have been some major advances in treatment that have improved the prospects for the disease, but prevention clearly remains a critical strategy if you consider that case fatality is still somewhere in the neighbourhood of 50%.

Diet has long been regarded as one of the most important environmental influences on colorectal cancer. This is hardly a new idea. Take the following quote, which comes from Hippocrates: "To the human body it makes a big difference whether the bread is processed or 'coarse'and whether or not the grain retains its outer coat. If you don't pay attention to these things, or fail to comprehend them, how can you understand human disease?"

A few centuries later, in the 16th century, one observer wrote: "Doe we not see the poore man that eateth browne bread health fuller, stronger, fayrer complectioned and longer living than the other that fare daintelie every day?"

A little more recently, in the early part of the last century, Metchnikov stated that the products of anaerobic putrefaction from bacterial metabolism of protein and other nitrogenous compounds was harmful; fermentation of carbohydrate in colon by lactic acid bacteria was beneficial.

Now we turn to the modern version of the dietary fibre colorectal cancer hypothesis. Denis Burkitt based his formulation of the hypothesis on his observations of a diet that was very high in fibre coupled with the very low incidence of colorectal cancer in Africa. He argued that fibre provided protection against colorectal carcinogenesis.

A lot of people have seized on this: this is one of the more popular cereals in the US, and you will notice "Wheaties may reduce the risk of some cancers."

Now, it is probably worth it to say a bit about what dietary fibre is. This has been an involving and complex area for a long time.

The following definition was recently published. It is based on the work of chemists and physiologists over a long period of time: "Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances." The whole area of definition and measurement of dietary fibre is currently in a state of flux.

Now, the objective of this talk is to look at the evidence that fibre really is causally related to colorectal cancer. What kinds of evidence are there? I will briefly talk about animal studies, briefly about human metabolic studies, and a little bit more on observational epidemiology, case control studies and cohort studies (Elio Riboli has already introduced you to those concepts), and then, finally, a little bit on intervention studies or clinical trials.

Animal studies. There have been enough studies conducted, although the evidence is far from consistent, to conclude that fibre can alter colorectal tumoregenesis in animal models. But – rodents are not people.

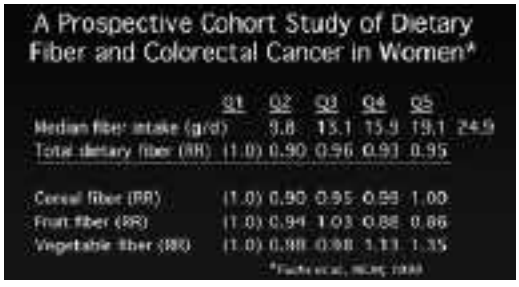
Human metabolic studies: there have been a number of studies to suggest that dietary fibre can have a variety of physiologic effects that could be related to colorectal carcinogenesis. Dietary fibre has been shown to affect faecal weight and transit time; faecal bile acid concentration (and bile acids, particularly secondary bile acids, have been implicated in colorectal carcinogenesis); faecal pH; short-chain fatty acid production (notably butyrate, which may have anti-cancer properties); and finally, proliferation and apoptosis in colorectal epithelium.

However, a key problem with these intermediate endpoint studies, as interesting and suggestive as they are, is that none of these intermediate endpoints are cancer. So the fact that, for example, dietary fibre might reduce colorectal proliferation does not prove that dietary fibre reduces colon cancer.

That brings us to epidemiology, because among the virtues of the epidemiological investigation is that it has cancer, or at least colorectal neoplasia, as the explicit endpoint in the study.

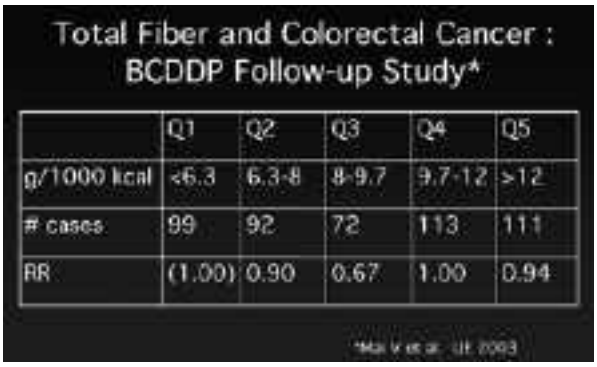
Elio already mentioned case-control studies and the discrepancy, at least for some diet and cancer, between case-control study results and prospective cohort study results, and we have that situation again with dietary fibre.

What you see here are two systematic reviews, or meta-analyses, of case-control studies of dietary fibre versus colorectal cancer. The first one showed about a 35% reduction for those in the highest versus the lowest category of dietary fibre intake; and still another meta-analysis showed that there was again an inverse relationship between dietary fibre and colon cancer.

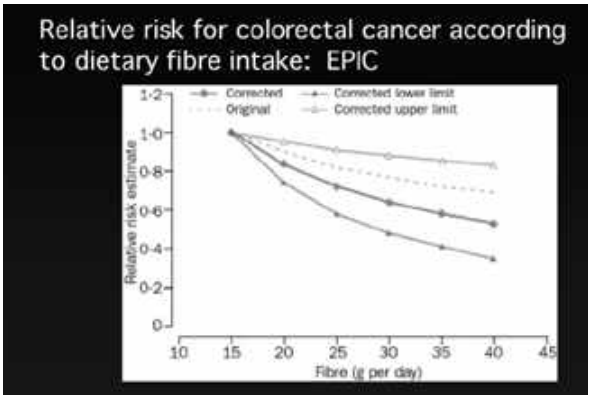


Then we come to the prospective cohort studies. This sums up a study from the Harvard cohorts that came out about four years ago, and this study had an enormous impact on the field. If you look at medium fibre intake across the five increasing categories, and these are the relative risks, this is essentially a null study. These relative risks are almost one and the reduction is by no means statistically significant. This study, along with a couple of others I will show you, had an enormous impact a few years ago and suggested to people that maybe the dietary fibre-colon cancer hypothesis just does not hold water.

This was a study that we just published from the Breast Cancer Detection Demonstration Project. This was originally a breast cancer screening programme that the National Cancer Institute turned into a prospective cohort study with about 60,000 women. The results are summarised below – absolutely no relationship between dietary fibre and colorectal cancer in this cohort of older women.



However, we then have these two Lancet articles from large studies – different countries, different types of studies. One was EPIC, a pooling of multiple cohorts going to colon cancer as the endpoint. You have already seen these results so I do not have to say any more, but there was an inverse association between fibre and colon cancer in EPIC.



The second study was the PLCO (Prostate, Lung, Colon, Ovary) study. It was originally set up as a screening trial to see whether the screening modalities for those sites really reduce mortality, but the original trial has been transformed into an observational cohort and we have the opportunity to look at adenomas (polyps: precursor regions for colon cancer) as the primary endpoint. It was quite large, with some 3,000 individuals diagnosed with colon adenomas. We found an inverse relationship – as fibre goes up, the risk of disease goes down – with about a 30% reduction for those in the highest quintile versus those in the lowest quintile of dietary fibre.

Now, what is going on with these observational studies? Why do we have these inconsistencies and how much credibility is there to observational epidemiological studies?

There are some methodological concerns to worry about. One is the area alluded to as the exposure assessment or measurement area problem, and there are at least two dimensions to this. One is what you could call 'specificity'– do we want to focus on total fibre or perhaps on just some of the sub-components of fibre. People in these observational studies have usually looked at soluble versus insoluble, or looked in particular at fibre from different food groups. The studies that showed something generally showed a protective association for fibre from a number of different food groups.

There is this nagging question that has become extremely controversial in the field about whether the primary tool that we use to assess what people eat–the food frequency questionnaire – is sufficiently accurate to detect modest, but still extremely important, relative risks. This is a topic of intensive debate. However, if the true reduction in risk is, say, 30% for those with a high intake versus those with a low intake, then is the observational epidemiologic study based on the food frequency questionnaire sufficiently accurate to pick that up? Is that possibly why we are getting some null results even in our otherwise well-designed studies?

There is also the issue of intake range or homogeneity (and that is something that Elio just alluded to). This is a complicated slide. The essence of it is that the lines reflect the point estimates, the relative risks and the confidence intervals for a series of prospective studies. The main point is that the intake range is rather narrow in a number of these studies – people in the upper category of intake are just not eating that much fibre. Some of the more recent studies are getting up there more – this is the PLCO one. This is EPIC, where the median intake for the lowest category is higher than that for the upper category in some of these other studies. However, look at the intake that spawned this hypothesis in the first place. This (around 70 grams per day) reflects the range of intake in Africa; so therefore, none of the studies even begin to approach that. We would hope that we can have studies where we are capturing sufficiently high intake, with people getting people up into 40 grams a day, where you can begin to investigate a meaningful biological comparison.

Finally, the last issue is what is called confounding. That is an epidemiologic term for the idea that people who eat more fibre, compared to people who eat less fibre, differ on a whole bunch of characteristics – social, demographic, biological characteristics – that can be truly related to colon cancer, even if the dietary factor is not. One of the biggest challenges that epidemiologists have is to control for these confounding factors. Elio referred to smoking in some of the smoking-related cancers, like oral upper area digestive cancers. Controlling for smoking, which is correlated with how we eat, is one of the most difficult problems in all of epidemiology.

One of the ways of getting around that is to take the experimental epidemiologic approach – namely clinical trials. One of the great virtues of the clinical trial is that it avoids confounding bias because randomisation makes the intervention and control groups similar with respect to both known and unknown factors related to the disease outcome. Clinical trials are very hard to do, they are very expensive and they do have their own limitations, but they have the great strength that when you find something – even a modest reduction, a modest protection – it is highly unlikely to be due to confounding.

I want to just briefly run through some aspects of a trial that the National Cancer Institute conducted, called the Polyp Prevention Trial (which was designed in part to look at the fibre colorectal

cancer hypothesis), just to give you a flavour for this methodology – the clinical trial.

This was a study with adenomatous polyps as an endpoint. The rationale for the adenoma endpoint is that first, the recurrence of adenomas, that means the development of a new one after one or more has been removed, is nearly a hundred times greater than the incidence of colorectal cancer itself. That means you can do these polyp trials a lot more inexpensively, a lot quicker, and a lot faster than you can do a trial where you would have to go to colon cancer as your primary endpoint.

Second, because there have been standards of clinical practice with regard to surveillance of adenomatous polyps once they are found, it is possible to piggyback or integrate a trial onto the standard of practice.

Finally, the main reason for doing these polyp trials is what is called the adenoma-carcinoma sequence. This is the idea that most colorectal carcinomas go through a polyp stage as a necessary precursor lesion for the cancer itself, which means that an intervention that reduces adenoma occurrence in the adenoma recurrence or polyp trials, will likely reduce colorectal cancer incidence.

This trial was set up in 8 centres around the United States. At the time that we set up this study, and this was 13 years ago, a study had just come out from the Harvard Group. Interestingly (keep in mind what the current study of fibre is), this was a study on adenomatous polyps and what it showed was that fat increased risk, red meat increased risk, vegetables were protective and fibre was protective. It was on the basis of this study and a couple of other studies that we set up this trial. What happened subsequently was that most of these things, except maybe for red meat, went away in these Harvard cohorts over time.

However, we set up the trial based on the evidence at hand and the hypothesis was that the adoption of a low-fat, high-fibre, fruit and vegetable-enriched eating plan or diet reduces the recurrence of colorectal adenomas. The targets were fat: 20% of total energy; fibre: 18 grams per 1000 kilocalories a day, so if you are eating 2,000 calories that means your target is about 35 grams of fibre a day; and for fruits and vegetables, the goal was 3.5 servings per 1,000 calories, or a target of approximately 5 to 8 servings per day.

This is not wholly a Mediterranean diet, particularly around the fat issue; this was in the biomedical tradition of the low-fat intervention at the time and there was epidemiologic evidence that total fat intake was a risk factor for colorectal cancer.

The Polyp Prevention Trial really involved an intervention with a dietary pattern. What is the rationale for this? First, as some speakers indicated, there can be the joint effects of nutrients and foods that you can capture by intervening with this multifactorial eating plan. There may be unknown nutrients or non-nutritive food constituents that you could capture by intervening with a multifactorial intervention but that you would miss if you were intervening with a single food or nutrient intervention. Finally, because each of those three hypotheses at the time was a strong hypothesis for colorectal cancer prevention, we wanted to maximise the likelihood of observing a clinical trial effect by using all three interventions at once.

Briefly, we randomised some 2,000 people to either the low-fat, high-fibre, high fruit and vegetable intervention, or usual diet. They were followed for four years. To get into the trial you had to have had at least one adenomatous polyp removed. Participants were colonoscoped at one year, which was thought to eliminate missed lesions and 'clean out'the colon. Then they were followed for another three years, this three year period being the main analytic period for adenoma recurrence, and we compared the adenoma recurrence rate in the two groups.

A lot of work from a lot of different people goes into an intervention study of this magnitude. Materials had to be presented, all kinds of notebooks for both the nutritionists and the participants involved. I should add that to get the 2,000 participants in the polyp prevention trial, we had to screen nearly 40,000 people at those 8 clinical centres.

There were nutritionists involved in the assessment and nutritionists involved in the intensive dietary counselling, which began on an individual basis and later transformed into group counselling. This is an example of participants with some of the nutritionists at one of our centres in Chicago. All kinds of social events were set up for both the intervention participants and the control participants, separate newsletters for the participants and so on.

What happened? Well, the trial worked really well in one sense – logistically. We randomised over 2,000 people. Over 90% of the participants showed up for their final colonoscopy. That is critical for a trial because if you have a large loss to follow-up as the potential for bias increases, but our loss to follow-up was minimal. Follow-up was about the same in the groups and the number of procedures was the same in the two groups.

What happened with the diet? Well, there were substantial changes made by the intervention group, as reported on food frequency questionnaires, 4-day records and spot 24-hour recalls. There was about a 33% reduction in dietary fat intake, fibre went up about two thirds, and fruits and vegetables increased about 60%.

Other things change when you make changes on those three factors. For example, red meat consumption went down 20%, whole grains went up about 40%, and cruciferous vegetables went up about 50%.

We did have some biological markers, or what we thought were biological markers. We looked at serum cholesterol, blood carotenoid and weight. It turns out that cholesterol did not really budge, and that is probably a function of the fact that we did not change the P/S ratio. We, focused (for better or for worse) on reducing total fat. Carotenoids did change. There was a statistically significant increase in carotenoids in the intervention group, and there was a statistically significant reduction in weight in the intervention group compared to the control group – although this was not a weight-loss study and we made a big point when we were recruiting people that this was not such a study.

So, what was the end result of 30 million dollars and ten years' work? Well, this is the relative risk of any adenoma recurrence— the main endpoint--for the intervention versus the control group, and the relative risk was 'one'– absolutely no difference.

We also looked at whether there was an effect on large adenomas. This is one study that suggested something there – just a smudge – but by far not statistically significant. Nor was there an effect on advanced adenomas, those that are either large or have other histological features, such as dysplasia or villous features, that make them potentially dangerous.

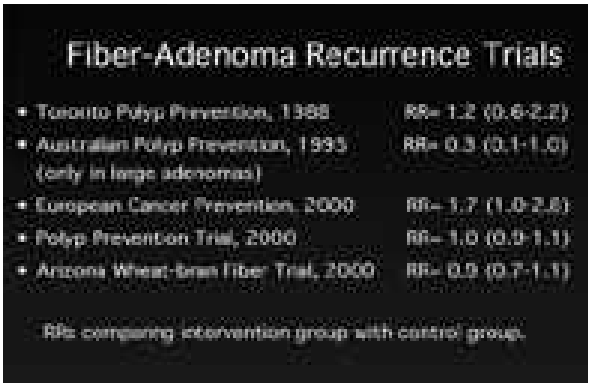
Now, what is the interpretation of all this? Well, the straightforward interpretation is that dietary change has no effect on colorectal neoplasia and therefore, by inference, no effect on colorectal cancer. However, we need to consider the limitations of these polyp trials, and this is important.

First, the trial encompassed a relatively short observation time – people were only followed for about four years. We are continuing to follow people passively, but no longer with the intensive intervention. If the dietary intervention affects very early events in the neoplastic process, it is possible that we could see an intervention control group difference emerge after four years, or as long as six, seven or eight years down the line.

This was really a limited snapshot of the process of neoplasia in carcinogenesis. People had to have had a polyp to get into the trial, so people were already different in that regard. If diet affects early events before the development of the first polyp, we could not evaluate that. Most of the recurrence lesions are small lesions; if diet affects the growth of small lesions to large lesions or the transformation of large lesions into cancer, we could not evaluate that either.

Perhaps we did not intervene with the optimum diet. Maybe we needed more extreme changes in fat and fibre, for instance vegetables. Maybe we needed changes in other factors, like red meat – not just a 20% reduction but an 80% reduction in red meat. Finally, we cannot be absolutely certain that our intervention participants ate as they reported – our biological markers were soft, and although we did have fairly consistent results across our dietary instruments, there is a possibility that our participants told us what we wanted to hear. Therefore, due to the limitations of the polyp trial model that I just outlined, it is still possible, even given the results of the polyp prevention trial, that dietary change reduces the risk of colorectal cancer.

At the same time that the results from this polyp prevention trial came out, the results from the Arizona Wheat-bran Supplement trial were published. This was bran supplements, not fibre from food. This was also a very large study, with some 1,400 polyp participants. Their study was also null no effect of wheat-bran. This slide sums up the fibre adenoma recurrence trials, and it is largely a summary of null results.



These are some smaller pioneering studies – one in Toronto, one in Australia. The Australian study, with about 400 participants, was overall null. They did see a hint of something for large adenomas, which was not their primary endpoint. That was one of the reasons we wanted to investigate large adenomas in the polyp trial and in the Arizona trial, but the results were null.

In this cartoon, we see a figure carrying a tablet down from the top of a mountain ; the people want to know what it says about fibre and calcium. (As I said, there has been a long history in this business). I wanted to share a word about calcium, just to show that the polyp trial is not necessarily a fatally flawed tool for investigating. These data are from a study that came out a few years ago around the same time as that negative fibre study hit the New England Journal of Medicine (this was also in the New England Journal of Medicine).

What is remarkable about this study of calcium supplements in relation to polyp recurrence, which had the same design as the Polyp Prevention Trial, is that they got a significant positive result, a reduction of about 20% in the recurrence rate of adenomas for those on calcium supplements. This is a positive result, and although it is easier to dismiss negative results on the grounds that I showed earlier, it is hard to dismiss a positive result, except, maybe, on the grounds of chance.

There has been at least one other large trial of calcium, the European Cancer Prevention Study that showed, as in the previous slide, that fibre actually increased risk. However, that trial actually showed that calcium did reduce the risk of adenoma recurrence. Unfortunately, the trial was too small to get a statistically significant result at that level, so at the moment we only have one positive statistically significant trial. It does suggest, however, that the polyp trial can yield positive (that is, non-null) results.

On balance, though, although trials theoretically provide important data, it remains to be seen how informative intervention studies will be in resolving the colorectal cancer prevention potential of dietary fibre.

Where do we go from here in this murky business of fibre? Well, there are now newer animal models, some of the genetically engineered models, such as the MIN mice. I think it will be useful to see what the fibre effect is in spontaneous cancer models, rather than models that have been fed massive doses of carcinogens.

Some people have suggested further studies of the ecology and physiology of the proximal colon, better assays of complex carbohydrates reaching the colon and perhaps revision of our databases that are used in our various dietary assessment instruments. Again, going back to this measurement area business, can we get a handle on the measurement error structure for fibre intake? This would require something of a gold-standard biomarker, perhaps stool fibre. This is work to be done: looking at not just fibre pre se, but fibre-rich dietary patterns and their associated measurement error structure; large cohorts with wide fibre intake range and perhaps bio specimens in diverse geographic areas. My own view, and the view of some people in the field, is that doing more case-control studies just is not worth it at this point.

Then finally, there may be some value in trials in persons with hereditary cancer predisposition. We should await the results of the massive Women's Health Initiative in the United States, which will give us some information on a diet that has increased servings of cereals among its components. Finally, we should perhaps consider new fibre trials in diverse geographic areas. Again – just reminding you – two positive trials with similar results would have enormous inferential strength.

So, to conclude, with regard to fibre, this cartoon says, "Yesterday in this space, I predicted that cancer would come to an end. It did not, however, and I regret any inconvenience this may have caused."

Thank you very much.

Questions

Member of the Audience
You mentioned these polyp intervention studies. These are people who had already had an adenomatous polyp and if I am right, I think that the recurrence rate after three years was something like 30% to 40% in general. In the PLCO study that was recently reported in the Lancet, you have about a 10% prevalence (for a general screened population), so that may indeed be a big difference. How do you place this in the context of your findings? Do you have any specific ideas on this?

Arthur SCHATZKIN
Well, you said it yourself. When you are dealing with the PLCO type study, you are dealing with, for many people, new adenomas. These adenomas may reflect adenoma development over many years. In some ways, you could argue, that is more reflective of the important biology of colorectal cancer. The polyp trial has this limitation that you have got people who have already had an adenoma. They may have a 'field defect'throughout large pieces of their intestine, and perhaps you are just not modelling the development of sporadic colorectal neoplasia in cancer as you might be with EPIC, PLCO and so on.
Now, that is not to say that you cannot show something, because there was something with that calcium trial. However, we went into those trials, as many people have gone into trials, with the idea of going with a high-risk population. A high-risk population means that they are going to tend to get more events, have higher rates, so you can do a cheaper, faster trial but it has the downside of maybe getting people who are not adequately reflective of normal biology.

Member of the Audience
In just having the negative results, have you looked at the genotype of these patients in relation to colon cancer?

Arthur SCHATZKIN
No, we have not done that yet, but we have finally extracted DNA from everyone so it is something that we will probably be looking at in the future.
If what you are suggesting is that if we stratified on a particular variant for a gene that might be related to fibre metabolism or fruit and vegetable foliate metabolism and so on, might we see something? It is possible. I do not know if we have sufficient power to do that, but that remains to be seen and it is something that is important. Not only are we going to do that type of analysis where we genotype individuals in the trial and perhaps stratify on genes encoding variants involved in metabolising enzymes, but we are also going to look at mutations in the polyps themselves to see whether perhaps we might see an effect for particular polyps that have specific mutations, but as yet we have not done that.

Member of the Audience
In relation to research and fibre, what is your suggestion: should we look to total fibre or to the different components of fibre, and what is your favourite component in fibre in relation to future studies?

Arthur SCHATZKIN
If you had asked me some years ago, I would have probably said it is vegetable fibre. In PLCO, the association with vegetable fibre was null ; there was an inverse association for the fibre from legumes and cereals, but vegetables was null.
My sense is that probably we should be looking at everything. We do not know enough to be able to say we are going to mount a study and just look at cereal fibre or fibre from legumes. I would look at total fibre, I would look at the fibre from key food groups, I would look at soluble and insoluble. Generally, you have the opportunity; any limitation is the limitation of the databases that we use. We have these food frequency instruments that ask 125 to 150 questions and only a certain number of them really are relevant to fibre. We are dependent on the values for fibre that are out there in databases, some of which are rather old. There are efforts going on in NIH, for example, to update. There was a move to update the fibre database about ten years ago and there are efforts now to upgrade some of these databases for foods in general, including fibre.

Member of the Audience
Please correct me if I am wrong, but I think there are some studies suggesting a protective effect of physical activity to colorectal cancer. I do not know if the situation is similar for adenomas. Did you consider physical activity in your study?

Arthur SCHATZKIN
That is a very important point that gets at this issue of confounding again. Actually, one of the strongest pieces of colorectal cancer epidemiology is the relationship between physical activity and colon cancer. There are some 50 studies in the literature that have suggested that physical activity is protective and there was a recent monograph put out by IARC that showed that. However, the relationship is modest, somewhere in the neighbourhood of a relative risk of 1.5 to 2 for people in the lowest activity level versus the highest activity level.
We had no formal physical activity component in our study. There was an effort to get the people in the intervention group to do a little walking on the grounds that that would help them. It was part of the whole intervention programme. We monitored physical activity as best as we could and there were no major differences in activity in the two groups in the long run. However, we measure physical activity pretty poorly, as you may know, with questionnaires, so it is possible there were some group differences that we just didn't pick up. However, what you would have to argue is that the control group was much more active than the intervention group and that we did not detect it, and that that was offsetting a true effect of diet, and that is probably not especially likely.

Folate and cancer

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Our knowledge of folate and cancer stem from animal experiments, observational and experimental studies in humans. Here we will review two lines of evidence from observational studies in humans : (1) cohort studies on folate and cancer 1-4, and (2) case-control studies of cancer and the 677C .T single nucleotide polymorphism (SNP) of the methylenetetrahydrofolate reductase (MTHFR)gene (ref.5).

In cohort studies the participants were characterised with respect to folate status (blood measurements or intake estimated from information on dietary habits and use of vitamin supplements). The participants were then followed for many years and occurrence of cancer monitored. Most cohort studies of folate and cancer have studied breast cancer or colorectal neoplasia. In none of six cohort studies a direct and significant association between breast cancer and folate intake or folate measured in blood was observed. In four of five studies, however, where an interaction with alcohol consumption was assessed, there was evidence of an inverse association between folate and breast cancer in women with high intake of alcohol.

We identified 14 reports from cohort studies on colorectal neoplasia and folate or multivitamins with folic acid. The inverse relationship between folate and colorectal cancer or adenomas that was reported from the U.S. Nurses Health and Health Professionals studies was not consistently reproduced in later cohort studies. As with breast cancer there was evidence that risk is particularly elevated when low folate status is combined with moderate to high alcohol consumption. The largest study on dietary folate and colorectal cancer from the Netherlands demonstrated an inverse relationship between folate intake and colon cancer in both men and women, and for rectal cancer in men (ref.3). In some studies the inverse relationship was only observed in subgroups defined by sex, alcohol consumption or anatomical localization of the tumor. One study found no evidence of an association between folate and colorectal cancer. Both measurement of folate in blood (plasma, serum, full blood or erythrocytes)and estimation of folate intake from dietary questionnaires is problematic and measurement error likely to weaken any true relationship between folate and cancer. Therefore associations between cancer risk and single nucleotide polymorphisms (SNPs) with functional effects on folate metabolism may yield valuable new insight into this area.

Of particular interest is the 677 C/.T SNP of the methylenetetrahydrofolate reductase (MTHFR) gene that has been extensively studied in relation to birth defects, cardiovascular disease and cancer, in particular colorectal neoplasia (ref.5). This gene codes the enzyme responsible for the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate.These folate species serve different biologic functions. The 677C/.T SNPaffects the conversion rate and hence the availability of folate for RNAand DNAsynthesis, on one hand, versus the availability of methyl groups for DNA methylation and protein synthesis on the other. We identified 27 case-control studies of cancer occurrence in relation to the MTHFR 677C/.TSNP. Seven studies in more than 3,000 patients assessed the relationship between colorectal or colon cancer and this SNP. Overall, the TT genotype had a highly significant and moderately protective effect against colorectal cancer that contrasts with its weak and non-significant association with increased risk for colorectal adenomas or polyps. Strongly increased risk with the TT genotype was observed in three case-control studies of gastric cancer,one study of esophageal cancer, and one study of endometrial carcinoma. No clear patterns of association were observed with breast cancer (three studies), lung cancer (one study), cervical dysplasia (one study), prostate cancer (one study), bladder cancer (one study) or cancers of the oral cavity (one study).

For acute leukemias (three studies) there was a tendency towards decreased risk associated with the TT genotype. There is extensive evidence that the relationship between the 677C/.T SNP and cancer is modified by folate status, methionine and alcohol intake and indications that it may differ among subtypes of neoplasias.

In conclusion, the current evidence points towards a role of folate in carcinogenesis and neoplastic development that is complex and interacting with genetic background, diet, and types and subtypes of neoplasia and stages of carcinogenesis.

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The use of biomarkers to validate reported dietary intake

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This morning we have heard a lot about dietary patterns, food and nutrient intake etc. and, between the lines, we have also heard certain remarks: we need to do further studies on calibration, we don't know about measurement errors exactly and maybe some of the studies were undertaken in populations which were too homogenous as compared to the measurement errors etc.
This is exactly the area which I will cover in more detail, according to the following schedule: firstly, I will say a few words about biomarkers in epidemiological research in general, then I will go into the concepts of validity and reproducibility of dietary intake. I will then move to the topic of correlated errors, because this is relevant to the interpretation of results from epidemiological studies and validation studies. I will present some selected results from the literature, in order to illustrate how nutritional epidemiologists present their results on validation studies nowadays and what the role is that biomarkers play in the validation of dietary assessment. Finally, I will say a few words about biomarker development and I will draw conclusions at the end.

Biomarkers in epidemiological studies

Biomarkers of exposure play a crucial role in validation studies. This is because the measurement errors in the assessment of biomarkers are independent of the errors in the assessment of dietary intake. My presentation is focused on the use of biomarkers for validation of dietary assessment by questionnaires. In his book 'Nutritional Epidemiology' Walter Willett (1998) enumerates seven approaches to evaluate dietary questionnaires, three of which I consider essential. Firstly, the simple approach is to look at reliability (also denoted as reproducibility), then we have validity – comparing the abbreviated questionnaire with another method assumed to measure the same concept more validly and, as a third option, we may want to compare the dietary intake with biological markers of exposure.

Before going into further details, I want to present a schematic overview of the issues that are relevant to studying diet in relation to risk of disease, and the different role biomarkers may play in this. Research in the field of nutritional epidemiology usually starts with the nutrient exposure resulting from a given dietary pattern and the primary interest is in its direct association with the risk of disease, expressed in measures of (relative) risk. We have seen a number of examples of this morning.

Biomarkers may also reflect dietary exposure, so adequate biomarkers of exposure can replace dietary intake data as more proximate markers of internal dose in the causal pathway; but even if exposure markers are not causally related to disease at all, they will be associated with disease provided that the dietary pattern and risk of disease are causally related. It should be realised that causality of the association between biomarker and diseases is an interpretation, based on the biological model underlying the observations. It is a matter of interpretation as to whether the biomarker is an intermediate or whether it is just a bystander reflecting the diet.

When using biomarkers for the evaluation of the validity of dietary methods, you have two remember two things : first, biomarkers integrate exposure to nutrients or other dietary components over a number of food sources. The dietary pattern consists of a number of foods with different contents of several compounds and this is all ingested in the gastro-intestinal tract. Then, the body starts to digest, absorb, distribute, metabolise and incorporate the food components in a time-dependent manner, resulting in accumulation and time integration until a steady state is reached with similar input and output in the medium of the biomarker. Some components are excreted in the urine within a few hours, while others are integrated in other components of the body, for instance in the fat tissue, and their distribution and metabolism is completely different with respect to the time frame. In both cases, the marker integrates aspects of metabolism, food composition, and dietary pattern.

In the steady state, the biomarker is measured in a medium, usually the plasma. However, extraneous determinants of the biomarker level may be present as well. For instance, if you consider the plasma carotenoids, other determinants of plasma carotenoids may be non-dietary factors, such as smoking or body size, which may influence the steady state level of the biomarker. Because of these extraneous determinants, the biomarker will be less specific for the intake of the dietary constituent of interest, and its diminished ability to distinguish high and low exposed subjects within a population renders the marker less suitable for application in an epidemiological investigation. On the other hand, if a biomarker is strongly related to the dietary intake, with only a few or weak extraneous determinants, this will result in a more specific and sensitive marker of individual intake.

Error in assessment of individual diet.

In epidemiological studies we aim to assess individual food and nutrient intake, so we need to define validity and precision at the individual level. Figure 1, taken from Livingstone (2003), provides four theoretical possibilities of intake of food or specific nutrients. In the first panel, intake is assessed both validly and precise; all the dots are closely around the true intake. In the second panel, assessment is valid, the individual average is around the true intake, but it is not very precise, as the dots are widely dispersed. The third panel is very inaccurate because the observed dots are systematically below the true intake, which is the thick line, but they are very precise because each time we measure about the same value, it si highly precise at the individuel level. In the last panel, both problems are present.

Now, if in an epidemiological study, the validity and precision of all subjects behave like the upper two panels, the individual average will approach the true individual time-integrated intake, provided sufficient replicates are taken. However, if all subjects behave like the lower two panels, this will still result in a precise individual average, but it will lack validity at the individual level. If all subjects behave in the same way, i.e. with the same degree of under-reporting, this does not affect the strength of the association in the epidemiological study, provided sufficient individual replicates are taken. However, this view is too simplistic, because we know that the reporting bias may be person-specific, i.e. the degree of reporting bias may differ from

person to person: Some subjects systematically over-report food intake or specific nutrients, and we have systematic under-reporters. In the field of biomarkers, there may be systematic hypo-responders to certain exposure and systematic hyper-responders to certain exposures; the latter is, of course, becoming more important because of the increasing use of biomarkers that have sources of genetic variation in common.

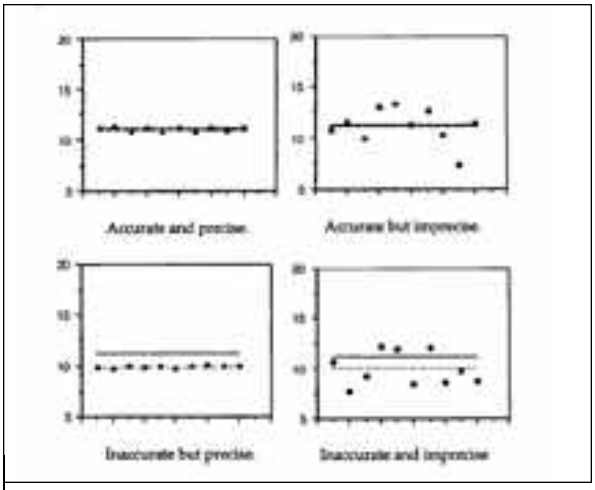
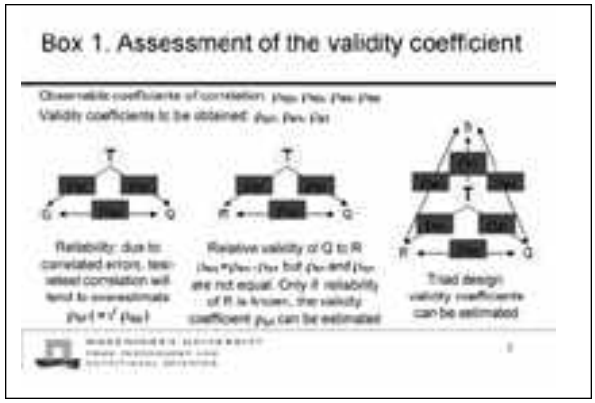


Figure 1. Validity and precision at individual level (from Livingstone, 2003)

Reliability studies to assess validity.

For epidemiological studies, the relevant issue is the relation between the true long-term intake, denoted T, and the observed intake, obtained by e.g., independently replicated diet records, denoted by R. Unfortunately, we cannot measure this association because we don't know T. However, we can assess the reliability by means of the test-retest correlation between k=2 replicates. Now, under the assumptions of the parallel test model, the test-retest correlation is the product of the validity coefficient of the initial measurement and T on the one hand, and the validity coefficient of the second measurement and T on the other hand. Thus, under the parallel test model the square root of the test-retest correlation yields the validity coefficient. If the number of replicates k>2, the intraclass-correlation, or the ratio of within- and between-person variance (lambda) of independent replicates can be used to derive the validity coefficient under this model. Of course, the same holds if you have replicated questionnaires (Q), e.g., frequency questionnaires, or independent assessments of a biomarker (B).



However, the assumptions of the parallel test model are often not very realistic. This is because one of the model assumptions is that the errors in the replicate assessments are independent. However, we know that if diet is assessed in duplicate you may run into trouble because both observations share the similar sources of error. So, apart from T, there are other determinants of the observed exposure. These include the memory of the subject who is reporting, his/her perceived intake and it is influenced by the use of the same food tables which contain the same errors and it is also influenced by the usually assumed standard portion sizes. Thus, there are a number of correlated errors in replicate assessments. Thus, the test-retest correlation, and therefore the validity coefficient may be overestimated because of the shared determinants of reported food intake. Because of perception and memory-based errors, this problem is considered especially relevant to food questionnaires in epidemiological studies. Therefore, the validity of such methods may be considerably less then one might conclude from a reliability coefficient.

Relative validity.

In the ideal case, validation studies use two independent methods to assess the true intake. This is possible for, e.g., energy intake by using deuterated water, and protein intake by urinary nitrogen. Unfortunately, such methods are available for a limited number of dietary factors only. Therefore, validation studies have often relied on so-called relative validity, i.e., the comparison of two different methods of dietary assessment.

Relative validation of dietary assessment, however, may exhibit similar problems with person-specific correlated errors as the reliability studies mentioned before. For instance, a dietary history with a check list on the one hand and a food frequency questionnaire on the other, does not take away the correlated errors because both methods largely share similar sources of error: the same food table and portion sizes (instrument-related), and memory or perception issues (person-related). In addition, extraneous determinants of reported food intake will not only add person-specific biases and inflate the test-retest correlation, but they will also increase the apparent correlation between different methods. For instance, we know that subjects with a high BMI underreport energy intake and vice versa. As an example, I refer to the paper by Livingstone (2003). So, both the measurement instruments as well as extraneous factors contribute to person-specific biases in reported food consumption.

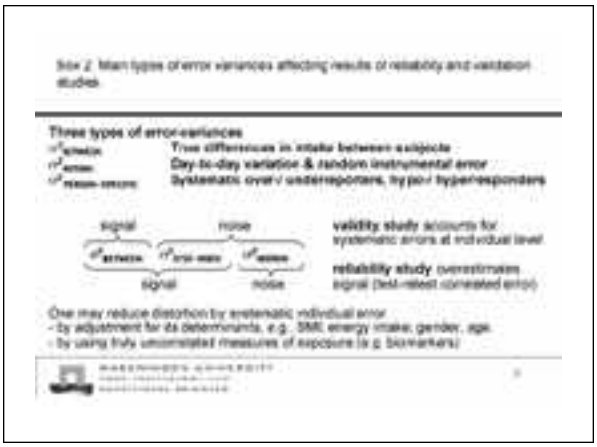
The above can be improved considerably if multiple independent recordings on random days are available, e.g., averaged 24-hour recalls or averaged diet records, that are compared with either a dietary history with cross check or a food frequency questionnaire. The correlated errors will be minimised because they do not depend so much on memory and cognitive patterns anymore, although some degree of correlated error cannot be excluded with certainty (food tables, portion sizes).

So, as depicted in Box 1, we distinguish within-person variation, between-person variation, and person-specific errors originating from shared instrumental or extraneous factors. With respect to validity, only the variation in T is the signal of interest, whereas the independent random errors, correlated instrumental errors and person-specific errors are considered noise. At the same time, the latter two types of errors provide us an overoptimistic idea of the reliability of dietary assessment. What can be done to reduce the consequences of such errors? Insofar we know their determinants, e.g. BMI, gender or age, we can either use reasonably uncorrelated measures of exposure such as repeated recalls/records or biomarkers, or we take the determinants of over/underreporting into account. So to account for these errors in nutritional epidemiology, the method of dietary assessment is an important issue in study design, whereas known extraneous factors can be accounted for in data analysis.

Validation using three instruments.

What is interesting about biomarkers is that the true – but unknown – intake and the observed intake have conceptually completely different errors. For the biomarkers, the errors are related to laboratory errors, absorption and distribution of substances in the body and the metabolism etc. Therefore, association between intake and biomarker does not share the same sources of error and can be considered as having truly independent. Of course, there should be sufficient evidence that the biomarker is indeed related to the intake.

We can now use this third independent biomarker of exposure to assess the relationship between our food frequency questionnaire and the true intake. This is depicted in Box 2. If two different methods of assessing intake are being compared, the validity coefficient cannot be derived as the square root from the correlation between the two methods, as was done in reliability studies, because the validity coefficients for both methods cannot be considered identical. To solve this problem, three independent measures of intake are required. This will yield three observable correlations and using the so-called method of triads the three validity coefficients can be estimated (Kaaks, 1997). In the ideal case, we have repeated 24hr recalls/records (R), a biomarker (B) of exposure and the (food frequency) questionnaire to be validated (Q). Among others, this approach assumes that these records and questionnaires have independent errors and that they are linearly related to T.



Illustrations for macronutrients

For total energy intake, we have the ratio of total energy intake and energy expenditure which is around 0.85 on average in the Western population. This ratio decreases as BMI increases so this provides one example of person-specific bias, that need to be taken into account in epidemiological studies (Livingstone, 2003).

For total fat, Walter Willett has shown an interesting association with fasting triglycerides but that is not very practical in epidemiological studies (Willett, 1998).

Slimani (2003) analysed the EPIC calibration study. She observed that urinary nitrogen excretion and protein intake were associated on the cohort level, suggesting validity at the group level. In case of complete agreement the cohort-means would have been on the line Y=X. However, it is systematically lower but there is quite a good (gender-adjusted) correlation of 0.84 of urinary nitrogen and nitrogen according to 24h recalls. For the memory-based diet questionnaires the (gender adjusted) correlation is 0.72 and the systematic error increases when true nitrogen intake goes up, illustrating the need for calibration between cohorts. Note that the between-cohort variation in energy intake has not been accounted for here.

There is still discussion ongoing on validation with biomarkers. One of the EPIC validation studies showed that diet diaries have variances that are very much correlated within the method and between methods, if seven-day-diaries and food frequency questionnaires are compared. It was concluded that regression dilution was greater with a food frequency questionnaire than with a seven-day diary (Day & Bingham, 2000). Willett (2000) advocates analyses that such analyses need to account for (part of the) correlated error between both methods – e.g. originating from age, gender and BMI as indicated before (Kipnis, 2001).

Illustrations for minor dietary constituents

Other reports in the literature address minor constituents, such as fatty acids from dairy, polyunsaturated fatty acids and fish fatty acids (Wolk, 1998; Baylin, 2002; Tjonneland, 1993). For these fatty acids, correlations were observed with repeated diet records / 24 hour recalls or food frequency questionnaires in the range 0.4, 0.5 to 0.6; they tended to be a bit lower for the food frequency questionnaires, but not in all instances (Tjonneland, 1993). For B-vitamins, the correlations between markers and FFQ were around 0 for B2 and B6, and almost 0.4 for B12 and folate. Correlations between FFQ and replicated records were much higher, i.e. ranging from 0.6 to 0.9 (Mason, 2003).

Other studies have "reversed" the triad design: two biomarkers and one/two method of dietary assessment were used. This provides the opportunity to derive validity coefficients for these biomarkers (provided they have independent errors). One study addressed fat-soluble components (Kabagambe, 2001). Contrary to expectations, validity coefficients for plasma carotenoids were quite high (around 0.5-0.7), as compared to the validity coefficient for adipose tissue carotenoids (0.05 to 0.5). As this was done in Costa Rica, it is difficult to know whether this can be translated to European populations.

Many presentations this morning addressed dietary fibre. It would be useful if we had a biomarker for dietary fibre intake. We are currently working on the mammalian lignans enterolacton and enterodiol, as potential markers of fibre intake, which is derived from many different dietary sources. Most studies done until now have only looked at grain products, fruits and vegetables or total dietary fibre intake. Apart from having biologically activity themselves, they may also be useful independent markers of the intake of dietary fibre or plant foods. Based on the literature so far, the reliability of the assessment of mammalian lignans enterodiol and enterolacton is quite reasonable. One paper reported a correlation of 0.7 – 0.8 between repeated measurements (Stumpf, 2003), whereas the correlation with dietary sources/fiber is considerably less i.e., 0.3 – 0.4 (Kilkkinen, 2001; Lampe, 1999; Homer, 2002), comparable to many other dietary factors.

Conclusion

You may have noticed that in the literature I only mentioned validity coefficients once. This is because results from reliability and validity studies tend to use very different designs and measures to express validity. There is currently no agreement on how to present the results from validation studies. Thus, in contrast to the central role of measures of (relative) risk in epidemiological studies, there is no tradition in using standard measures of reliability and validity yet. Thus, still a lot of work needs to be done to come to an agreement here on design, analysis and reporting of validation studies.

Based on the discussion between Day and Willett and others, It also seems important to reach consensus on how to handle person-specific individual errors in the analysis of validation studies and how to account for them in the analysis of epidemiological studies. Therefore, we need to improve our knowledge on determinants of under-/over-reporting and, in case of biomarkers of exposure, determinants of hypo/hyper-responders to nutrient intake.

Finally, it should be noted that assessment of validity for one single compound does not inform us about the validity for other compounds or of the method as a whole. In order to say something about validity of methods as such, a number of compounds need to be measured. So far, however, most people are validating dietary compounds individually and independently, ignoring the fact that the nutrients in the diet pattern and biomarkers have clearly intricate interrelationships both at the level of intake patterns, and presumably also on the level of biomarkers patterns.

In conclusion, nutritional epidemiologists need to develop a shared view on the design, analysis and interpretation, of validation studies, including the determinants and the control of person-specific bias. Both the EPIC study and the american cohorts have a wealth of data that can help us to make progress in this area.

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CONCLUSION

Arthur SCHATKIN

I have some very brief concluding remarks.
First, you have this cartoon stating that 'fruit and vegetables prevent cancer'. We have had some interesting remarks from the EPIC study on this. The conclusion is, frankly, 'maybe' with respect to whether fruits and vegetables truly prevent cancer. The EPIC findings, at present, could also be reasonably summarised as indicating 'maybe', with respect to both total fruits and vegetables as well as components. I think that the issue for folate and colorectal cancer is also probably a 'maybe', certainly promising, but I think that we have hardly proven that folic acid will prevent colorectal cancer.

We have to be aware of not making excessive claims for things that turn out not to be the case. Another example is lycopene and prostate cancer. Scientists in the field would acknowledge that the data on this question are far from definitive, but that does not stop advertisers.

Concerning fibre, as you know, the picture there is certainly not so clear but it clearly lends itself to cartoons, 'After I've ruined a perfectly good set of patio furniture!' and this one came out when the trials came out, there was a series of cartoons, 'That means that you are feeding him too much fibre'.

This is my favourite, 'Wellness update - 30 year old man starting on the 25,000 pound bran muffin that he must consume over 40 years in order to significantly reduce his risk of death from large bowel cancer!'. The numbers there are a little uncertain but … !

On biomarkers I think that Pieter raised a number of important points suggesting why this is such a difficult area. Dietary intake biomarkers can provide important information on underlying biochemistry and physiology, but I think that the reality at this point is that we really don't have that many good, unbiased markers, and even for some like carotenoids or triglycerides, they have the problem of a lot of variability person to person in dose response, what Pieter called hypo hyper-responders. When you have that kind of variability, and it is related to characteristics like smoking or weight or a variety of things that we may not even know of, those characteristics themselves can be correlated with the error in the instruments and that compromises the validity of those biomarkers. This is a tough area.

Biomarkers as intermediate end points – we didn't talk very much about that, but it is a potential part of this whole nutrition and disease business. They can tell us a lot about mechanisms, but these intermediate end points are not cancer.

As part of a concluding summary, I am now going to reiterate what Antonia and Nadia and several people have said about the importance of a multi-factorial approach to looking at what we eat – patterns, indexes, cuisines, whatever you want to call it.

Here, in this slide, Greece was 'Voted [as having] best food in the world'. What you can't read is 'by 10,000 olive trees' which might be as good an assessment as some of the ones which we come up with!

We used these two pictures in the polyp trial. You can imagine the difference between this kind of a cuisine or dietary pattern [first slide], which you might call the 'Dunkin Doughnuts / McDonald's' cuisine, versus this [second slide] , which is not not bad for a Mediterranean diet, although you probably also need a jar of olive oil there.

Again, why are these patterns important and why will we be talking more about it? They model real life, we eat mixtures of food, substitution is inherent; to capture the complex biochemistry and physiology they can reflect international differences in diet which are clearly associated with differences in disease rates.

Thus far we have heard some suggestive evidence that the Mediterranean diet prevents chronic disease, including cancer, but we should be hearing more particularly with non-cancer with non-communicable diseases over the next day and a half so stay tuned !

Thank you very much !

INTRODUCTION

Daan KROMHOUT
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I will present an introduction on the Mediterranean diet. Maybe this is superfluous because, yesterday, we had a very nice presentation on the Mediterranean diet and its development by Dr Naska; she emphasised the situation in Crete and in Greece in general.

I will show the data from the Seven Countries Study and point to some of the characteristics of the traditional Mediterranean diet as it was in the 1960s. Thereafter I will summarise the results on the Mediterranean diet in relation to coronary heart disease mortality.

Within the context of the Seven Countries Study, Professor Flaminio Fidanza was one of the collaborators that he did a lot of work on defining the Mediterranean diet. In order to recognise his contribution, I took his description of the traditional Mediterranean diet - he said that this diet was usual for people from countries whose coasts are washed by the Mediterranean sea and is moderate in cereal products, fish, legumes, olive oil, fruit, vegetables, in combination with little meat and wine. When you ask him where you can find that diet, he always answers that it can be found in the Southern part of Italy. The pilot study for the Seven Countries Study in 1957 was carried out in Nicotera in the Southern part of Italy and there a traditional Mediterranean diet was consumed. This diet is quite different from the type that you find in the northern part of Italy.

Also in other Mediterranean countries, this is the general feature of the diet, but no community sticks to this prototype. There is always some component in the diet that is more widely available; from that point of view, you can differentiate between different types of Mediterranean diets; so, in my opinion the Mediterranean diet does not exist.

I will focus on the diet of the four cohorts in the Seven Countries Study that have what we may call a traditional Mediterranean diet and, of course, the cohorts of Crete and Corfu are included. Also the coastal cohort of Dalmatia, in Croatia, had a typical Mediterranean diet. In Italy, the cohort that is closest to the traditional Mediterranean diet is the cohort of Montegiorgio. I like to focus my presentation on those four cohorts to show you the relation between the dietary patterns in the Seven Countries Study in the early 1960s and coronary heart disease mortality.

All those four cohorts – Crete, Corfu, Dalmatia and Montegiorgio, were cohorts that consisted of farmers that were quite physically active at that time and had a relatively high energy intake. For most of the cohorts the energy intake was in the order of about 3,000 calories per day. Those men were really physically active, because their average Body Mass Index at that time was only about 22 kg/m2.

You should take this into account; the reference is the 1960s, physically active farmers who were on a traditional Mediterranean diet.

In the Seven Countries Study there have been several rounds of examinations, but in relation to diet there has only been one round of examination that was the baseline survey around 1960. In that survey, dietary data was collected in all 16 cohorts. At that time, there was no money available to carry out dietary surveys in all 13,000 middle-aged men. Therefore, the investigators decided to do surveys in small samples of only 20 – 50 persons, depending on the cohort. The seven days record method was used as survey method. In 1960, for instance, in the rural cohorts several men were illiterate, so dieticians had to stay with the family for a whole week in order to get the correct information.

At that time they also collected duplicate portions of all the food that was eaten. When the men had eaten a certain dish then the same amount of what they had eaten was put into a container and after a week all the food in the container was either sent to the laboratory of Professor Fidanza in Naples or to Minneapolis to the laboratory of Professor Ancel Keys. The major analyses that were done at that time were fatty acids and protein. That was all, no more was done.

In the 1980s we decided to code all the dietary data, of the 16 cohorts again. We composed 18 different food groups and calculated the average intake of all those food groups for the 16 cohorts. In 1987 we went back to the 16 cohorts and collected locally the foods that represent the average intake of each cohort in the 1960s. We really tried to get the food consumption pattern as it was in the 1960s for the 16 cohorts. We brought the foods to the laboratory of the Division of Human Nutrition at Wageningen University and there chemical analyses were done, not only on the major classes of fatty acids, because we already had that information, but, also on the subclass of omega-3 fatty acids. We also measured all nutrients and bioactive compounds– in total more than 60, in those food composites – so that we could get a better picture of the nutrient content of the different diets.

What I will show you first is a couple of slides in relation to the dietary pattern thereafter the results of the chemical analysis and finally I will say a few words about the mortality pattern in relation to the Mediterranean diet.

All the slides have the same structure and I would like to draw your attention especially to the cohorts of Montegiorgio, Dalmatia, Corfu and Crete.

The average bread consumption in the 1960s , was in the order of about 400g per day for the cohort of Montegiorgio. In Dalmatia and in the two Crete cohorts it was a similar order of magnitude. So the average consumption of bread in those middle-aged Mediterranean farmers was about 400g per day.
There were large differences between the other cohorts, for instance, the American railroad workers only had an average consumption of about 100g of bread per day but in Velika Krsna about 800g of bread per day, so there was quite a diversity in bread consumption.

For the cereal consumption, you must take into account the consumption of pasta in Italy, and the consumption of rice in Japan. In the Italian cohorts and especially in Montegiorgio, there was quite a high consumption of cereals that was lower in Dalmatia and it was also quite low in the two cohorts from Greece and, of course, very high in Japan.

In most Mediterranean cohorts, there is a relatively high consumption of legumes . The average intake was about 30g of legumes per day in the cohort of Montegiorgio. That was higher

than in Dalmatia, which had a very low level of consumption. In the two Greek cohorts, the average consumption was also in the order of 30g-40g per day around that time. The highest consumption was in Japan, because of the high level of consumption of soybeans.

The vegetable consumption does not differ very much, especially in the Mediterranean cohorts. In Montegiorgio, Dalmatia, and the two cohorts from Greece the average consumption is about 200g per day. In the 1960s, for instance, the consumption of vegetables was fairly low in Finland, the average was only about 100g per day and there was no cohort with a really extremely high intake of vegetables.

Fruit consumption gives a totally different picture compared to vegetables. The fruit consumption was low in Montegiorgio in Italy and very low in Dalmatia, but in Greece the fruit consumption was extremely high – the average was about 450g per day.
These results show that within the Mediterranean cohorts there was a very large variation in the level of fruit consumption.

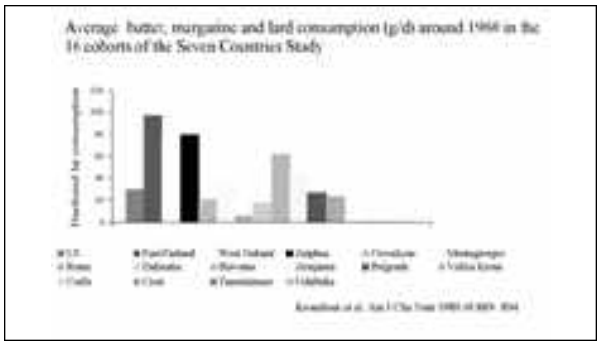
In the traditional Mediterranean diet, meat consumption was quite low. This was the case in Montegiorgio where the meat consumption was in the order of about 80g per day, in Dalmatia it was a little bit higher, in the order of about 120g per day; but in the two cohorts from Greece the average consumption of meat was only 30g per day. So, especially in the Grecian cohorts at that time, meat consumption was very low.
Of course, the Americans were the champions in relation to meat consumption; the average intake in the United States at that time for the railroad workers was 270g per day.

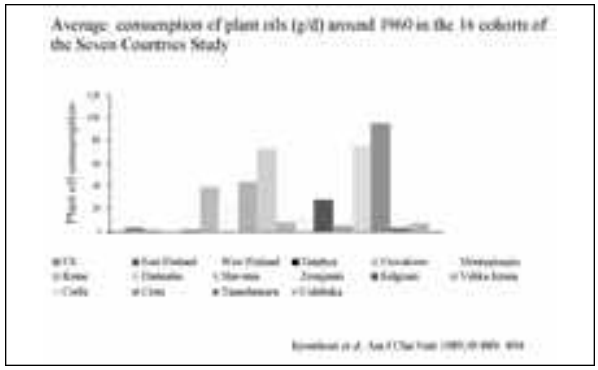
There was quite some variation in fish consumption in the Mediterranean cohorts. Fish consumption was moderate in Montegiorgio and in the two cohorts from Greece with a higher level in Corfu compared with Crete. The highest level of fish consumption in the Mediterranean cohorts was observed in the cohort of Dalmatia, with an average of about 100g per day. Of course, the highest level of fish consumption was found in the cohort of fishermen in Ushibuka from Japan and they had an average intake of about 200g per day.

Milk consumption in middle-aged men is generally low in the Mediterranean cohorts. That can be illustrated with the cohort of Montegiorgio with almost no milk consumption. In Dalmatia there was quite a high consumption of milk of about 400g per day, and in the cohorts from Greece it was lowest in Corfu but also in Crete the average level was not higher than 200g per day. The champions on milk consumption were the Finns. They drink more than a litre of milk per day on average. In contrast to Southern Europe where milk is viewed as food only for babies and children.

Edible fat consumption also shows some variation. In the Mediterranean cohorts the total edible fat consumption was on average about 60g per day in Montegiorgio and about 100g per day in Dalmatia and the two cohorts from Greece. The total edible fat consumption in the cohorts from Greece was similar compared to that of the cohorts from Finland.

The type of edible fat used is however, totally different.
The consumption of butter, margarine and lard were taken together because those types of fat are high in saturated and in trans fatty acids. In the Mediterranean cohorts, the highest level of consumption was in Montegiorgio, about 40g per day. It was much lower in Dalmatia, only on average about 20g per day and it was 0 in the two cohorts from Greece.
Therefore, if we consider butter and margarine the top level of consumption was in Finland and in the Netherlands. In Finland, at that time, it was mostly butter, because it was the 1960s and in the Netherlands that high level was due to hard margarines that were rich sources of saturated and trans fatty acids.





The situation for the consumption of plant oils, is completely different. The level of consumption in the Italian cohorts is, on average, around 40g per day - the lowest level, about 30g per day, was found in Montegiorgio. The intake of olive oil was much higher in Dalmatia, with an average of about 80g per day and the highest level was found here on the island of Crete and that was almost 100g per day on average.

Opposite results were observed for the consumption of butter, margarine and lard on the one hand and for different plant oils on the other hand. The intake of plant oils and of oils in general was extremely low in Japan at that time.

Finally, alcohol consumption was expressed as ‘absolute alcohol’ in order to make different alcoholic drinks as comparable as possible. This is needed because in Southern Europe, especially at that time, mostly wine was drunken and in Northern Europe mostly beer and spirits. In the 1960s, the farmers from Italy and Dalmatia were the champion wine drinkers. The average intake was in the order of 60 to 90g of absolute alcohol per day. This means about seven glasses of wine on average per day.

Professor Fidanza once told me of a story about one of his dieticians who was interviewing a participant in the Seven Countries Study: she went through the dietary pattern of a farmer and asked him, “Do you drink ?” the farmer said “No”. She went through the whole dietary pattern and she questioned him about breakfast and asked if he had taken anything else then the foods mentioned. He answered “Of course, I drink two glasses of wine at breakfast”, and she said “You told me first that you did not drink!” to which the farmer replied, “That’s not drinking, that’s just a part of my breakfast !”

The alcohol intake in the 1960s was much lower in the cohorts from Greece and it was in the order of 20g to 40g of alcohol per day on average. The big difference between the Italian cohorts and the Dalmatian cohort on the one hand and the cohorts from Greece on the other hand is the large difference in alcohol consumption which should also be taken into account when judging the mortality patterns.

As I said earlier, we collected in 1987 the foods locally, sent them to the laboratory in Wageningen, and homogenised the foods for chemical analysis of fatty acids and antioxidants.

The total fat intake in the Mediterranean cohorts: Crete, Dalmatia, Montegiorgio varied between 30% and 40% of energy. As a reference the Zutphen cohort from The Netherlands was taken with an average intake of fat in the order of about 44% is taken.

Population average dietary fatty acid intake in three Mediterranean cohorts and Zutphen around 1960

Cohort	Total (%E)	SFA (g)	TFA (g)	MUFA (g)	PUFA (g)
Crete	40.1	28.0	0.4	84.1	13
Dalmatia	33.7	39.9	1.1	77.6	24.0
Montegiorgio	30.1	31.4	0.5	49.5	15.0
Zutphen	43.7	60.9	8.1	49.5	20.7

SFA = Saturated Fatty Acids; MUFA = Mono Unsaturated Fatty Acids
TFA = Trans Fatty Acids; PUFA = Poly Unsaturated Fatty Acids

The pattern of fatty acids is different. In Crete it was about 30g of saturated fatty acids per day and that corresponds to about 7% or 8% of energy, about 40g/day in Dalmatia and about 30g/day in Montegiorgio. However, in the cohort of Zutphen in 1960 the saturated fatty acids intake was about double, it was about 60g per day.

A very interesting difference was seen in trans fatty acids. In the Mediterranean cohorts there were almost no trans fatty acids in the diet, 1g/day in Dalmatia 0.5g in the other cohorts. In the cohort of Zutphen it was almost 10g per day. This is one of the big differences between the cohorts in the beginning of the Seven Countries Study. In the past most emphasis has been on the large difference in saturated fatty acid intake because there was no interest in trans fatty acids. However, besides the large difference in saturated fatty acids, there is also a very large difference in the intake of trans fatty acids – and they are even more detrimental for coronary heart disease than saturated fatty acids - so they must be taken into account.

The highest intake of monounsaturated fatty acids was found in Crete and Dalmatia. The average intake was about 80g per day. In Montegiorgio it was about 50g per day and this was similar to the amount consumed in Zutphen.

The total intake of polyunsaturated fatty acids varied between 15g and 25g per day and this did not differ between the Mediterranean cohorts and the Zutphen cohort.

Currently, there is a great interest in the health effects of the antioxidants. We also determined the average intake of beta-carotene, vitamin E, vitamin C and flavonoids. The average intake of beta-carotene in the Mediterranean cohorts varied between 3 and 4mg per day and was in the same order of magnitude as that in the cohort from Zutphen. So, in terms of beta-carotene intake there is not much difference between the Mediterranean cohorts and the cohort from Zutphen.

Crete had the highest average intake of vitamin E with 23mg per day. It was 18mg/day in Dalmatia, 15mg/day in Montegiorgio and only 12mg per day in Zutphen.
In the Mediterranean diet, the intake of vitamin E is on average, 50% to 100% higher compared to the Northern European cohorts like Zutphen. This is, of course, due to the high intake of olive oil in the Mediterranean cohorts.

Vitamin C gives a different picture: the highest intake was found in Crete because of the high consumption of fruit and much lower levels were found in Dalmatia and Montegiorgio and also quite a high level was found in the cohort of Zutphen. One of the reasons for the high intake of vitamin C in The Netherlands was the very high intake of potatoes.

Crete had with 16mg of flavonoids per day the lowest intake. In the other Mediterranean cohorts it was about 35mg per day. A similar level was also found in Zutphen.

I have doubts as to whether the low level of flavonoids in Crete was correct. In the 1960s, we did not have detailed information concerning the intake of different plant foods as the study was really focused on fatty acids. Together with Professor Kafatos, we are doing a study where we also get information on all kinds of vegetables consumed in Crete that are rich sources of flavonoids to see whether this low level is correct. My guess is that the intake in Crete is in the same order of magnitude as that of the other cohorts, when more accurate data on the fruit and vegetable content of the Cretan Mediterranean diet are used.

Population average 25-year mortality rates (%) in three Mediterranean cohorts and Zutphen around 1960

Cohorts	CHD	Cancer	All-causes
Crete	4.6	8.8	31.4
Dalmatia	8.1	10.8	43.3
Montegiorgio	11.5	12.2	46.2
Zutphen	19.7	17.1	48.0

How do these foods, nutrients and bioactive compounds relate to mortality patterns? During 25-years of follow-up there is almost a four-fold difference in mortality from coronary heart disease between Crete and Zutphen. Mortality from coronary heart disease in Dalmatia was almost twice as high as in Crete, and in Montegiorgio it was more than 2.5 times higher than in Crete.

The lowest total cancer mortality rate was observed in Crete and amounted about 9%. It was higher in Dalmatia and even higher in Montegiorgio, and the level in Zutphen was twice as high compared to Crete. There is thus not only a difference in mortality from coronary heart disease, but also from cancer.

The lowest level of all-causes mortality was found in Crete. It was in the order of about 30%, in Dalmatia it was 43%, in Montegiorgio 46% and that was almost comparable with the level that we found in Zutphen of 48%.

The Mediterranean cohorts are not at all homogenous; there is quite some variation in mortality patterns. One of the reasons that mortality was much higher in Dalmatia and Montegiorgio, is probably the high level of alcohol intake. My colleagues from Italy wrote several papers about this and showed that the alcohol-related mortality was quite high in Montegiorgio and this is probably also true for Dalmatia that had a very high level of alcohol consumption.

These results show that the Cretan diet can be viewed as a prototype of the traditional Mediterranean diet. During 25 years only 30% of the Cretan farmers died compared with 60% of the farmers in Eastern Finland and Slavonia. If we adjust for the high prevalence of cigarette smokers in Crete (57% in 1960) the mortality rate in non-smoking Cretan farmers would have been approximately 20%.

With a traditional Mediterranean lifestyle, which means a Mediterranean diet, being physically active, with moderate alcohol consumption and no smoking, the all-causes mortality rate is very low.

To conclude, the traditional Mediterranean diet associated with the lowest coronary heart disease and all-causes mortality rate is plant food based. Olive oil was the principle source of fat of the farmers, wine was consumed in moderation and they were physically activite - the general recommendation is about 30 minutes per day of moderate physical activie - and together with non-smoking this gives a long life expectancy.

Questions

Question from the Audience
You mean flavonols or flavonoids? Remember that a glass of wine represents about 100 mg of flavonoids. So flavonols are part of the larger family of flavonoids.

Daan KROMHOUT – Bilthoven, The Netherlands
Yes, it depends on the definition - In the early analysis that we did, we only looked at quercetine and a couple of other flavonoids and that is only a small part of all flavonoids. What we analysed in those days were mostly flavonols, you are correct from that point of view. We later found that catechins also play an important role and they are not included here in what we call flavonoids, but that was the way we published it originally and I therefore kept it as it was, but thank you for clarifying that.

Cretan diet to prevent clinical manifestation of coronary heart disease

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Ladies and Gentlemen, good morning.
I would first like to express my gratitude to the organizing committee for inviting me to this excellent meeting in such a beautiful place.

I will talk about the Cretan diet and its role in preventing clinical manifestation of coronary heart disease.

You have already heard that saturated fats have been considered as one of the main factors for coronary heart disease.
One example is the studies comparing the Japanese in Japan to those who moved to Hawaii and California, they are all from Japanese ancestors, and so there is no question of genetic. However, if you look at the mortality rate in these three groups, 160, 280, 360 - there is a huge difference. In other words, moving to Hawaii and California resulted in a marked increase in the mortality rate from coronary heart disease. We might say that it is because they have been smoking more, but it is the opposite, they lowered their smoking; so perhaps it is because they are drinking too much alcohol, but this, again, is just the opposite - in California they have been drinking much less.

So what about fat, in general? Fat intake has increased in general for those in California and especially saturated fat. So this is one study that suggests that really the intake of saturated fat could be the villain, the main cause of coronary heart disease under different conditions.

Once you have that hypothesis, however, as was said yesterday, if you really want to demonstrate this, what you must do is an intervention trial.

In the last 50 years or so, there have been 27 randomized intervention trials with the following results:
Two groups were compared - one well fed, which had a normal diet and the other which had a high intake of linoleic acid (which lowers cholesterol but there have been different considerations in these 27) but this is about the basis of most of these studies - cardiovascular mortality was not significantly reduced. Only the cardiovascular events were reduced by 16% and it is significant, but is really far removed from the difference that I will show you concerning the difference between the Japanese or the other conditions as well.

Consequently, if it is diet, it is not that diet that has been used in these studies that can really prevent satisfactorily the mortality rate from coronary heart disease.

One good example of this is the Minnesota study that was carried out in psychiatric hospitals. There were around 9,000 subjects followed for more than 4 years, and you can see that there was not much change in total fat; the saturated fats were decreased by 50%, but the P/S ratio means a marked increase due to the higher intake of linoleic acid. Serum cholesterol was decreased by 15%. However, when we look at coronary events and all cause mortality instead of being decreased, they were slightly increased (8%).
So what do we do with that? It is supposed to be the best trial ever done, double blind, and, of course, we were certain that the diet was changed and so the cholesterol was decreased but what could we do about that?
In my opinion, you could conclude that diet has nothing to do with coronary heart disease; I really thought that it was not the proper diet.

Let us now consider what is important in fat - it is the fatty acids. The fatty acids are what make the difference between oil and fat; therefore each fat has a different composition in fatty acids.
Firstly you have the saturated fatty acids and the monounsaturated, mostly oil, basis of olive oil and those two families have something in common, they can be synthesized by the human body, in other words they are not essential. If you supply them then, fine, but if you don't supply them, then the human body can manage without them.

It is completely different with the polyunsaturated fats.
In the polyunsaturated, there are two families one is linoleic acid or omega 6 or n-6 fatty acids and the other alphalinolenic, or omega 3, or n-3 fatty acids. Previously, in general, only linoleic acids were considered - why? What was the reason for mainly working with or investigating this fatty acid? Well, because it lowers cholesterol, while alphalinolenic acid does not do this.
So, these two families are essential, in other words they are not synthesized by the human body. Consequently, they have to be supplied everyday. These two families also compete for the same enzymatic system; and so, consequently, they have to be supplied at a certain level - if you have too much linoleic, you have no more synthesized fatty acids that are normally produced from alphalinolenic acid such as EPA and DHA.

Thirdly, these two families compete for the same enzymatic system, but most of the time they have opposite effects. For example, linoleic acid is pro-inflammatory and alphalinolenic acid is anti-inflammatory; we could think that we don't need linoleic acid in case of inflammation but, yes, it is also essential and you need a competition between the two.
Linoleic acid on platelet for example, linoleic acid increased platelet activity, alphalinolenic acid decreased platelet activity.
In many other systems it is the same, they have opposite effect, consequently it is not only one polyunsaturated fatty acid, but also two families that we have.

Instead of trying to innovate, as my colleagues have done in the past, we thought that it really was very simple, but it is in fact much more complicated.
Instead of innovating, I thought that it was easier to try tocopy the Cretan longevity that, as you heard, the Cretan cohorts had the lowest mortality rate from coronary heart disease and all causes. Their longevity could be due to the exceptional climate, the absence of stress and pollution, the after lunch siesta or other factors yet to be discovered, rather than due to the poor people diet that they used.

For forty centuries, the Cretan people have been using a diet, which has not markedly changed over the years; consequently, it is a type of diet that is apparently associated with their longevity.

Nevertheless, as I have said, it could be due to a completely different factor and not diet. Therefore, to be able to demonstrate that it is the diet, as I said at the beginning, we have to do what we call a randomized intervention trial.

A few moment ago you heard about the real difference in the Cretan cohort - , the mortality rate from coronary heart disease were 184 in the Mediterranean cohorts while it was 420 in the Netherlands and 574 in the USA, but if you look at Crete it was 9 after ten years - 9 ! My goodness! What a huge difference! Even with mortality from all causes the figure from Crete is much lower.
However, if you look at cholesterol it is slightly higher than the other Mediterranean groups, so this could suggest that this may be the reason why not much attention was paid to Crete, when the Mediterranean countries were being considered in general.

You have heard about the consumption of food in Crete, so I will summarize :
They consume a lot of bread, but not much more than the other populations, much more than the USA of course; legumes, a much higher amount of legumes, and the same amount of vegetables as in the other Mediterranean countries. Finally with vegetables, we do not find such a big difference as we could have expected. For fruit, by contrast, a huge amount of fruit is consumed in Crete, much less meat, and fish - a very moderate intake of fish. As for edible fat, this is mostly olive oil, of course, very large amounts of olive oil and alcohol - a moderate intake of alcohol mostly in the form of wine.

The point is that in what I have shown you up until now, is there anything that can really explain such a huge difference from mortality in coronary heart disease ?
My colleague from the Netherlands, Martin Katan determined plasma cholesterol levels from Crete as compared to the Netherlands, fatty acid composition, and here you look at 18-2, so the linoleic acid is the one that decreases cholesterol; in Crete it is much lower than in the Netherlands. By contrast if you look at 18-3, which is alphalinolenic acid, it is about three folds higher in Crete.

As I have been working in the past with alphalinolenic acid, I was interested in that, and so let's say that the effect of alphalinolenic acid on platelets was discovered for the first time and published in 1964 by Paul Owren from Norway. He said that the increased adhesiveness of platelets can be reduced to normal not by fatty acid EPA or DHA, but by alphalinolenic acid.

That was the first starting point, but this is a very difficult technique to evaluate platelets adhesiveness, and 10 years later we published, together with another of my colleagues from Norway, Arne Nordoy, that alphalinolenic acid is elongated and desaturated to EPA in man, even if the absolute concentration of alphalinolenic is lower in membranes, it is associated with striking changes in platelet behaviour.

That is why there is the basis for trying to investigate the effect of diet, copied over the data of Crete. In 1984, I designed a secondary prevention trial, organised the finance and main aspects of this trial, here are the first two publications that I wrote and put the name of my colleague in first.
The basics, in order to make these diets similar or comparable to the Crete diet - more bread, more vegetables and legumes, more fish, less meat replaced by poultry, no days without fruit, no more butter and cream, but replaced by something else : rapeseed margarine and sunflower oil replaced by rapeseed oil.

The point of fact most difficult to answer is - Where does the alphalinolenic acid come from in the Cretan population? It does not come from olive oil, as I will demonstrate later, because olive oil does not contain much alphalinolenic acid. It comes from different substances, wild-green and snails and many things that already exist in this country.

However, if we wish to give alphalinolenic acid to a population, let's say in Europe or elsewhere, this can be done, but it is much more difficult than if we want to replace the cream and butter by something else which we must supply. In that case it was margarine, but in Crete they don't have margarine. The reason why we selected margarine is because if it works with that hypothesis, then it demonstrates that the mechanism really worked as expected otherwise.

Here is an important demonstration of the fatty acid composition of different fats. In red you see the separated fatty acid, you see that the butter and cream is the richest in saturated fatty acid, which is why we wanted to replace that with something else. Here is olive oil, very little saturated fat, but mostly oleic acid here, and the amount of alphalinolenic acid is very small, olive oil does not contain enough of this. This is the rapeseed oil, which has a somewhat similar composition to olive oil, except it contains much more alphalinolenic acid. This is what we used - the margarine that we used in this study, as compared here to olive oil, there is a slight difference but it brings much more alphalinolenic acid.

This is the experimental group and what they have been eating, you see, is a little more bread which is significant but which is not a big difference.
Fruit intake increased markedly, meat was decreased, poultry and cheese remained about the same, but the most marked changes were with butter and cream, which were highly decreased in the experimental group and replaced with the margarine, which we supplied.
Fish consumption was not changed and oil consumption remained the same as well, although it was a different oil.

Of course, when you work with fatty acid, when you work with fat, in my opinion, you cannot do all this without analysing the fatty acid composition of plasma and in all our studies this is what we are doing.
We have the direct demonstration as to whether or not these people in the experimental group have really been eating different types of fat.

Here, we emphasized that linoleic acid has been decreased, this is significant, and partly replaced by alphalinolenic acid passing from point 39 to point 65, almost double. Also, the EPA has been significantly increased, but apparently coming from elongation starting from alphalinolenic acid and not by a higher intake of fish.

To give a rapid overview, in this study we did not want to increase cholesterol but, finally, what we obtained after 8 weeks or 22 weeks, is that everything was similar in the two groups concerning cholesterol HDL etc. Consequently, if there is a difference in the experimental group - it is obviously not due to the changes in the cholesterol.

Important to note, up until recently, the Lyon study was unique; this was not reproduced for several reasons. However, finally, last November Singh and collaborators published a study in India and Israel in which they reproduced the Lyon study and here sudden death was reduced by almost 70%, and non-fatal myocardial infarction was reduced by more than 50% and total coronary events were reduced by 52%.
As you will see, this is very similar to the results that we obtained in the Lyon study, consequently it is almost too good to be true, but this is what can be obtained when you do that type of study, by increasing the intake of alphalinolenic acid in particular.

In the Lyon study, what we observe is that here is the control, this is the cardiovascular death and you see that it was 3 in the experimental group as compared to 16, a reduction of 76%, and when you see that in a study, you have to stop the trial because it is impossible to continue with such a big difference in mortality. When you add the non-fatal myocardial infarction, the total of the two, there was a reduction of 73% and even overall mortality was reduced by 70%; for the different total cardiovascular events there was a reduction of 76%, of course this result was exceptional.

Another very important point to note - these are the life survival curves.
This is the control, this is 1 year, 2 years, and this is the experimental group. Within the first month we already obtained a big difference between the experimental and the control groups. So this means that this type of diet, surprisingly enough, worked very rapidly and we have recently had some other studies demonstrating this fact; even in just a question of weeks, this type of diet works and prevented the two manifestations of coronary heart disease, which are, as I said, death and non-fatal myocardial infarction.

You could now say that these studies are not used anywhere.
However, the Japanese are known to have the best life expectancy at the present time. If you study the fatty acid composition in the Japanese, who have the greatest life expectancy in the world, as compared to our experimental group, you are surprised to see that the fatty acid composition in the Lyon study and the Japanese study is very similar. Including alphalinolenic acid, you see that this is even slightly higher than in Lyon and we believe that the Japanese owe their protection partly to the quantities of fish consumed, but as for alphalinolenic acid, in fact, the Japanese consume a lot of rapeseed oil. This is a surprise, for sure.
Even for the Inuits, the Eskimos, the same is also thought to be true, because they consume large quantities of fish; but if you look at alphalinolenic acid in the Eskimo, as compared to the people of Vancouver, you see that it is also a much higher level. In other terms, we find high levels of alphalinolenic in different places, countries and different populations in connection with coronary heart disease.

Now we try to determine, in our study in Lyon, to what extent the protection was due to the Cretan diet, this explains the 73%, when we compare this to the level of alphalinolenic, as determined by plasma level, 80% of the explanation comes from this calculation.

In addition to our interventional trial, do we have other evidence that really this alphalinolenic acid could be so important for health?

These are large prospective studies, there are several of them, you see that this is the first one published in 1992 and which showed that alphalinolenic acid was associated with the lower rate of death.
Here, in this study on 40,000 men in Boston, non-fatal myocardial infarction is lower, also here - 70,000 nurses - cardiac death - so these studies, in general, showed that both non-fatal and fatal myocardial infarction are significantly and markedly reduced and this is associated with the higher level of alphalinolenic acid.
The demonstration, however, as I said, can only come from international trials.

In the last two months there have been papers appearing which confirm what I am saying here, it is a case control study done by Boston. In 482 cases, compared to 482 controls, an increased level of alphalinolenic was associated with a 20% to 60% lower risk of non-fatal myocardial infarction independently of long chain omega 3 fatty acids.
Another study, also from Boston, this is dietary alphalinolenic acid and carotid atherosclerosis and this is in the 1,575 healthy subjects of the National Heart, Lung and Blood Institute - alphalinolenic acid, but not fish consumption, was associated with a 50% lower prevalence of carotid artery plaques and sickness.

The further we move in this direction, the greater is the proof that alphalinolenic is very important for health.

To conclude, you heard yesterday about Finland and the high mortality rate from coronary heart disease and why, because of this, they began to change their diet more than 20-30 years ago. Low fat is now used by 80% of men, soft vegetable margarins are the most popular products, rapeseed oil is widely used in the margarine industry, this is an important point and you will see why. Meat consumed is mostly lean pork and fish, cheese consumption has continually increased, the consumption of fruit has doubled and vegetables consumption almost tripled in 20 years.

However, are we certain that alphalinolenic acid has really been increased in that population? Here is a study that was published not too long ago, in 1999, showing the trans fatty acid investigation and, for example, here we have several countries. In Finland, the intake of C18:3 and n-3, is the highest as compared to the different countries, Greece, Italy and even France.
Now if you study adipose tissue, it is the same - in Finland it is the highest - which was not the case, of course, 20 years ago, and this is recent.
Therefore, this means that in Finland at the present time, they markedly increased their intake of alphalinolenic acid.
What happened during that period of time, I have just emphasized, for people in the 33-44 age bracket ? This is the mortality rate from coronary heart disease and you can see for men that there is a reduction here of 75%. It is different depending on age, but still you can appreciate that it is a tremendous difference. A population such as Finland, has been able to change and have such an impact by using a high intake of alphalinolenic acid.

Here you see the mortality rate from coronary heart disease, the way it started in North Karelia, the place where they started the diet, but the rest of Finland also continued - this is the decrease that they have in the mortality rate from coronary heart disease.

In our study in Lyon, at the end of the study there were 17 deaths from cancer in the control and 7 in the experimental. However, if you exclude the subjects that developed cancer in the first two years and probably already had cancer when they were here, the figure is 12 to 2, so that does not mean, of course, that alphalinolenic acid prevents cancer, because this data is too small, but it is still interesting to note this.

Therefore, to conclude, more cereals, vegetables and fruit, less saturated fat, a P/S ratio of 0.4 to 0.7, much less than was used before, a balance between alphalinolenic acid and linoleic acid, a moderate intake of wine at mealtimes - this is the lesson from Mother Nature or the wisdom of privileged population such as Crete - the cradle of our civilization.

Thank you very much for your attention.

Questions

Daan KROMHOUT - Nutrition and Consumer Safety Division, Netherlands
Thank you very much for an interesting overview of the role of alphalinolenic acid in the context of the Cretan Mediterranean diet.
There is time for questions. Are there any questions?

Member of the Audience
What do you think, Professor Renaud, about the possible adverse effect of alphalinolenic acid intake on prostate cancer? How do you see that?

Serge RENAUD - University Bordeaux II Epidemiology & Public Health, France
Unfortunately, we have not yet worked on prostate.
I think that on cancer in general, yesterday's team has been working on this and I am sure that they will study that in detail.

Daan KROMHOUT - Nutrition and Consumer Safety Division, Netherlands
Are there any other questions ? May I ask you a question about the effect of alphalinolenic acid in the context of the Cretan Mediterranean diet?
You showed a lot of evidence of the importance of alphalinolenic acid, but would you like to draw the conclusion that in your diet alphalinolenic acid was the major component responsible for the effect?
Or, could it be that alphalinolenic acid in the context of the Cretan Mediterranean diet that has several other advantages has played a major role?
So, is it only alphalinolenic acid or is it alphalinolenic acid in the context of the Cretan Mediterranean diet?

Serge RENAUD - University Bordeaux II Epidemiology & Public Health, France
I cannot say, of course, because the reason we did such a trial and used higher amounts of fruit and so forth, is because I was not certain.
I wanted to see the extent when including alphalinolenic acid. The results were obtained, especially those of India, this study was carried out on vegetarians who consequently have quite a different diet and still you see that alphalinolenic acid has the same role as in our studies.
Therefore, really, I don't know and I think that we will require several other studies to be able to answer that question. Obviously, I am not ready to say that it is just alphalinolenic acid, I am saying that alphalinolenic acid plays a dramatic role in protection.
However, if you study the role of alphalinolenic acid, you see that it enters all membranes and stabilizes them and is, consequently, very important. I don't know if this is the only importance - it is a balance, as you said, but alphalinolenic acid is probably playing a tremendous role in protection, not only of coronary heart disease, but also of other diseases as well, which I have never really investigated.
Although, I do not mean by this that the rest of the food that one consumes every day is not important.

Elio RIBOLI - (IARC - International Agency for Research on Cancer - WHO)
The Lyon trial was very, very important to show that with dietary changes you can have a dramatic, very important and major effect on mortality amongst people who already had a cardiac problem; so, people who already had atheromas; just to remind the audience, in order to die from a medical infarction you generally first need a very long process which may take decades for the formation of the atheromas, the lesion and then you need the acute event, which is the formation of the thrombosis, the occlusion.
What has been shown here is a very important result, that if you prevent the formation of the thrombosis, you have an immediate benefit.
Now, the question, for me, is that one should also consider how to prevent the formation of the atheromas, which are important for atherosclerosis processed globally.
I would not share the view that the only important factor is alphalinolenic acid, because all other dietary and lifestyle factors are important for preventing the formation of the atheromas, also important for cerebral vascular events etc.
I would like to have Serge Renaud's view on a lifetime diet rather than on a post atheroma diet.

Serge RENAUD - University Bordeaux II Epidemiology & Public Health, France
You are quite right, but the title was 'Clinical Manifestation of Coronary Heart Disease', the two main clinical manifestations being non-fatal myocardial infarction and cardiac death.
Those two events are really protected by alphalinolenic acid and they are independent of cholesterol. What is dependent of cholesterol is atherosclerosis, you also have to work with that, but the essential part is really the clinical manifestation.
Now, you have seen that I have presented the recent data indicating that carotid artery atherosclerosis seems to be also prevented by alphalinolenic acids, so we have plenty of work still to do.

Atrial fibrillation and cretan diet

Dominique LANZMANN-PETITHORY
Centre Hospitalier Emile Roux, 1, avenue de Verdun, 94456 Limeil-Brévannes Cedex

Ladies and Gentlemen, first of all, I would like to thank the organising committee for having invited me to this important meeting.

Sudden deaths from cardiac causes is estimated to account for 50%, probably more, of all cardiovascular deaths, even in people who don't know that they have coronary problems. The chief mechanism of cardiac sudden death is ventricular tachycardia or fibrillation, often triggered by coronary disease or acute coronary events, in 80% of cases, or cardiomyopathy.

We have some indicators of an increased risk of life threatening arrhythmia, such as, for example
- frequency of premature ventricular depolarisation, but they have a low predictive power if they are not combined with other variables,
- heart rate variability, the exact predictive value is unknown,
- for ECG variable - the presence of electric abnormalities, but they have a low power to predict death from arrhythmia,
- coronary artery or structural disease : how discriminate who will suffer from an arrhythmia. Low specificity
- Specific genetic pattern to an arrhythmic response to ischaemia ?

The prevention of life threatening arrhythmic events remains problematic because patients at risk are difficult to identify as the drugs are incompletely effective and impose serious toxicity.
For the primary prevention only beta-blocker therapy is accepted but is non-specific. In secondary prevention there is only the implantation of a cardioverter defibrillator, but this can be heavy.

Therefore, the nutritional approach could be of interest in this case of a big problem of public health. Often now in science we try to make the demonstrations, the studies shorter, we work on the markers before working on the end point, and some teams try to show the effect of Omega 3 on these markers of risk of sudden death like, for example, ventricular complex, and they show a slight reduction of this with fish oil with 2.4 grams of fish oil.
Other teams looked for effects on heart rate variability.

For example, this found a slight association between the level of DHA in cholesteryl esters, (as explained by Professor Renaud, DHA is derived from alphalinolenic Omega 3) and heart variability; it is a standard deviation of the length of heartbeat during the whole day. There is a slight association here, but as explained to us by Professor Schatzkin, markers are not end points - in fact this approach is not sufficient.

I would like to remind you the story of Omega 3 and sudden death.
For the first time in the 1980s, there was an observation made in Eskimos that the rate of heart diseases in this population was very low and they linked this relationship with the consumption of EPA and DHAof fish. Later Charnock who was an Australian in Canada worked on the influence of dietary lipids on arrhythmia in animals.
At a later date and for the first time the first human intervention trial was carried out in Wales, the Diet and Re-Infarction Trial, which showed that fish consumption prevents cardiac death but not non-fatal myocardial infarction.
In 1983, Serge Renaud showed that alphalinolenic acid prevents both sudden death and non-fatal myocardial infarction.

In 1984 Alexander Leaf developed the electrophysiology of the phenomena on animals, on cells, myocytes of neonatal rats. In 1999, the Gissy Trial confirmed the Dart Trial with fish oil and recently Singh confirmed the results of Renaud.

This allows us to now consider in a different way the physiopathology of the clinical manifestations of coronary heart disease. There is not only atherosclerosis but there is also thrombosis and ventricular fibrillation, three parallel distinctive phenomena influenced by nutritional factors.

In the Seven Countries Study, you remember that the rate of coronary mortality was the lowest despite the fact that fish consumption, source of Omega 3, was quite reasonable, or less than in the other Mediterranean countries.

In the case-control studies: there is the Willett Group you see that the risk ratio is 0.19, between highest and lowest quartile of blood level of long chain n-3 fatty acids, which is very significant in relation to sudden death.
Recently, a study in elderly people, a case-control study, shows that the odd ratio of plasma phospholipids were 0.3 for EPA, DHAand 0.48 for alphalinolenic acid, with a significant p, and 2.42 for linoleic acid, omega 6, also with a significant p for trend. The risk increases with omega 6. This means that maybe the quantity of omega 6 also plays a role, and the ratio between the two families - omega 3 and omega 6.
As Elio Riboli explained to us, case-control studies are less significant than prospective studies.

What do the prospective studies say for alphalinolenic acid in the MRFit Study?
The relative risks were 0.58 for alphalinolenic, for 1% of the calories under the form of alphalinolenic, and 0.5 for fish oil fatty acids, 0.2 % of the calories, with a significant p.
The study carried out by Willett's team, showed no real effect of fish consumption, no-dose effect - only a risk ratio of 0.74 between fish and non-fish consumers.
The Nurses Study showed a risk ratio of 0.55 in the high quintile of alphalinolenic consumption versus low quintile with a significant p.

Now, the animal studies. McLennan's Group in Australia carried out a lot of studies on arrhythmia. For 12 weeks, they fed animals with different types of fat supplements, sheep fat, olive oil, sunflower oil, fish oil and they provoked infarction by ligaturing a coronary artery for 15 minutes and after made a reperfusion and observed what happened.
During this reperfusion, you see that the best protection from arrhythmia came from the fish oil with a 10% incidence rate, versus 25% for the sunflower oil and 40% for olive oil. You can see that the olive oil does not protect and the time of normal rhythm was longer for fish oil.
After this, they compared fish oil with rapeseed oil (canola oil), another source of omega 3, which has 9% of alphalinolenic. You can see that rapeseed oil is still more efficient than fish oil, with a significant difference.
The incidence of ventricular fibrillation is 15%, compared with 60% in the control and the duration of fibrillation is shortest for the rapeseed oil.
Then they, compared different oils rich in alphalinolenic :

rapeseed oil and soybean oil. They demonstrated that despite the fact that soybean oil also brings alphalinolenic acid, it is not as efficient as rapeseed oil. It supposes that the presence of omega 6, 50% of soybean oil, plays a negative role, but the conclusion cannot yet be drawn because it is an animal study.
In the team of Alexander Leaf, they worked on dogs and they tried to compare the different omega 3 fatty acids: EPA, DHA and alphalinolenic fatty acids, almost pure, and then, the three mixed together
You see that on a group of 13 dogs, 10 were protected against ventricular arrhythmia by the fish concentrate; all the omega 3 seem to be efficient; however, the number of dogs was not big enough to show a difference, between these 3 kinds of Omega 3 fatty acids.

The omega 3 fatty acids act at the level of the ion channels by a reduction of electrical excitability caused by partitioning of the free fatty acids into the phospholipid myocytes membranes, which modulates ion channels, ion specific or not, voltage gated or not, because there are many kinds of different channels, it is very complicated and we don't exactly know how it works.
The mechanism of the anti-arrhythmic effect of the n-3 fatty acid in normal and calcium overload myocytes appears to be primarily by reducing the calcium entry via calcium channels.
There is also an action on junction channels (connexins), which are the channels between the myocytes, which communicate by these channels to transmit the action potential. Arachidonic acid, omega 6, accumulated after an infarction, blocks the junction channel and then, the propagation of action potential and favors ectopics.

I remind you that the two families of essential fatty acids are transformed into superior derived fatty acid and in eicosanoids, prostanoids, (prostraglandins, thromboxans), very active substances, and these two families compete for the same enzyme system : desaturase, elongase, cyclo-oxygenase etc. Probably, when there is too much of one family, for example, too much n-6, it has an effect on the elongation of the n-3. For example, the prostanoids derived from n-6 are pro-arrhythmic; whereas, the prostanoids derived from n-3 are anti-arrhythmic : Prostaglandin 2 or Prostaglandin 3.
To summarise the mechanism of anti-arrhythmic effects, there is probably an anti-inflammatory effect because an inflammatory state promotes arrhythmia. We saw that the prostaglandins derived from n-3 are anti-arrhythmic. There is also a mechanical action on the cyto-skeleton when the fatty acid is integrated into the membrane; it remodels the channels and the junctions (connexons).
There is also an activation of transcription factors and gene expression of the channels themselves. Some studies show a blocking effect of sodium channels. In fact, it is very complicated and not yet understood very well.

Only the intervention trial demonstrates - the Dart Trial -you see the curve of survival, compared with no fish and it can be seen that at a very early point the curves separate.
The Gissy Trial has a decrease of 14% to 20% of cardiac deaths and 45% of sudden death with fish oil.
In the Lyon study, there were 16 cardiac deaths, versus 3 in the experimental group, with 1.2 grams of alphalinolenic in the experimental group versus the control.
The control had 0.6 grams of alphalinolenic and the experimental 1,8 grams.
You can see the sudden death : 10 versus 0 in the experimental group.

Even if this endpoint were not the primary endpoint of the study, it was designed to show a difference in the non-fatal myocardial infarction.
You also remember the curve of mortality in Finland; also the recent study of Singh with a diet rich in alphalinolenic acid +1.33 grams per day, in the form of mustard oil, which is very similar to rapeseed oil - even in vegetarian subjects who eat no fish and a lot of vegetables, the risk ratio of sudden death was 0.33 and for non-fatal myocardial infarction, I remind you that it was 0.47.
To summarise these four studies, considering the relative risks of cardiac deaths, we can think that alphalinolenic acid is more efficient than EPA - DHA, even for sudden death, where in the Renaud study the relative risk is 0, in Singh study 0.37 and in Gissi, 0.55. In the Dart Trial, they did not distinguish cardiac death and sudden death by ventricular fibrillation.

Singh also observed the frequency of ectopics, which have a low predictive power except in this context of coronary heart disease. He observed 8 ectopics in the intervention group, versus 30 in the control group. The relative risk is very significant. Ectopics are defined as more than 8 premature ventricular complexes per minute, requiring treatment.

There is another kind of arrhythmia, less severe than ventricular fibrillation, but an increasing health problem at the moment, as demonstrated by Professor Kromhout : 6% of the over 65 years old, 15% of the over 90 years old, incidence 35 per 1000 per year over 85 years, there is a huge increase in the age of the population and, as for other arrhythmia, there is no satisfactory treatment and it is necessary to administer anti-coagulant drugs.
The recurrences are 25 % in the month and more than 50 % in the year, and the risk of stroke is x 4, dementia x 2, and death from all causes x 1.5 in men and x 1.9 in women.

Here is the curve of the recurrences with Amiodaron, you see that in one-year there is about 40% recurrences and without Amiodaron 75%, but not everyone can take Amiodaron, there are many side effects.

Atrial fibrillation could be a possible model to confirm the effect of n-3 on arrhythmia because atrium fibrillation concerns atrium instead of ventricle. It is an arrhythmia resulting from myocytes fibrillation in the atrium with the same electrophysiological phenomena.
Intervention trials are easier than for ventricular fibrillation because the recurrences are very early, frequent, no lethal and there is no satisfactory treatment.
The question raised is whether n-3 will also protect from atrial fibrillation.
Clinical studies are presently ongoing to determine whether EPA DHA and alphalinolenic acid would also protect from atrial fibrillation.
An interesting additional approach could be within the framework of the Seven Countries Study, to evaluate whether the Cretan cohort was also protected from atrial fibrillation.

Conclusion :
Cardiac death, sudden death, following an infarction, is generally due to a ventricular fibrillation.

All n-3 (omega 3) fatty acids can prevent myocytes fibrillation.

Alphalinolenic acid seems to be more efficient.

The Mediterranean diet includes the use of olive oil, which makes very little contribution to n-3.
Cretan people with traditional diets ate moderate quantities of fish. Their high level of plasma alphalinolenic came from the Cretan traditional food chain.

Occidental people lack of n-3 and alphalinolenic ; the effect of alphalinolenic is maybe due to a relative low status in n-3 and an excess of n-6, owing to the large use of linoleic

acid rich / alphalinolenic poor oils - this ratio is from 9 to more than 10,000. For example, in sunflower oils the ratio is more than 10,000, because there is 70% of omega 6 (linoleic) and almost zero omega 3.

How to make sure to get daily 2 grams of alphalinolenic acid ?
You can do the same as in the Lyon or the India study, with two spoons daily - 20g - of rapeseed oil for cooking and dressing, possibly mixed with olive oil.
Or you can do the same as the traditional Cretan dwellers and eat 300g of wild greens daily, which provide 750mg of alphalinolenic acid (ALA).

In France, or elsewhere, you can eat 300g of green leafy vegetables, which provide 600mg of ALA, for example dandelion, corn salad, spinach or watercress or cauliflower.
Or, twice weekly, you could eat the meat of animals that have been fed with wild greens. In fact, two years ago with Professor Kafatos, I discovered that Cretan people traditionally ate quite a lot of pork during certain times of the year, between Christmas and Easter and they cooked with pork fat from pork which had been very well fed with wild greens etc; as well as goats and chickens.
There is also the milk, cheese and eggs of these animals - Serge Renaud analysed Cretan eggs and milk in comparison to French milk and eggs and the quantity of ALA was half in the French product.

Also, Cretan snails are richer in alphalinolenic than French snails and, traditionally, Cretan people consume it three times a week and have been doing so, for the last 40 centuries.

Thank you very much.

Questions

Daan KROMHOUT
Thank you for this overview of the anti-arrhythmic effects of different n-3 fatty acids.
There is time for some questions.

Member of the Audience
I have two points - I do not agree with you that the implantable cardioverter defibrillator does not work very well; the only problem is that it does not prevent anything. If people have an event then the therapy goes well, but you cannot prevent the event.
I would also like to say that there are currently three trials going on investigating the effects of fatty acids on ventricular fibrillation in patients with an implantable cardioverter defibrillator - 2 in the US and we are doing a trial, ourselves, in several countries in Europe.
We have included just over 200 patients now and we hope to have around 500.

Dominique LANZMANN-PETITHORY *- Centre Hospitalier Emile Roux, France*
I don't understand - did you make a poster?

Member of the Audience (same)
I also made a poster, yes, but that was on a different subject, alphalinolenic acid and heart disease and prostrate cancer.

Dominique LANZMANN-PETITHORY *- Centre Hospitalier Emile Roux, France*
(To answer your question)
Yes, I wanted only to say that these implantable cardioverter defibrillator can be triggered all the time and it provokes too many electrical shocks, too often. It is heavy. But, I agree, it is the only current equipment that proves its curative effect on repetition.

Member of the Audience (same)
Yes, that's what I said. It can't prevent anything. It provides good therapy and the nice thing is that it has a storage function so that you can actually see what has happened, so that it is possible, afterwards, to say whether the person really had an event, or not.
Otherwise, even with atrial fibrillation, if people come to the hospital, it is very hard to distinguish whether they have atrial fibrillation, or not.

Daan KROMHOUT
Thank you. There is another question ...

Member of the Audience
Thank you. I found your presentation very interesting.
I have one question about the composition of the diet that would prevent atrial fibrillation. Have you studied the composition in salt / sodium chloride?

Dominique LANZMANN-PETITHORY *- Centre Hospitalier Emile Roux, France*
You mean what is the role of salt on arrhythmia?

Member of the Audience *(same)*
Yes, what was the composition of salt studied in your diet because some studies say that salt is very effective in provoking atrial fibrillation?

Dominique LANZMANN-PETITHORY *- Centre Hospitalier Emile Roux, France*
Not to my knowledge, but I know that one factor of atrial fibrillation is high blood pressure. If salt acts on blood pressure, then possibly this is true. I never said that salt provokes atrial fibrillation.

Daan KROMHOUT
Are you saying that you have evidence that salt provokes atrial fibrillation, is that what you are saying?

***Dominique LANZMANN-PETITHORY** - Centre Hospitalier Emile Roux, France*
High blood pressure is one of the most important risk factors of atrial fibrillation.

Daan KROMHOUT
We will continue with final questions ...

***Member of the Audience** (same member as above)*
I have one remark and one question:
One of the mechanisms of the anti-arrhythmic effect of Omega 3 fatty acid is that increase of free fatty acid. This is very important because in the Parisian Prospective Study, it was shown that the n-3 fatty acid was like in the Seven Countries Study and Omega 3 may decrease in metabolic syndrome, for example.
In your opinion, what is the main mechanism to explain the effect of the Mediterranean diet, or the Lyon diet? Is it the anti-coagulate aggregation effect, or is it the anti-ar rhythmic effect that may explain the very quick effect of these kinds of diets?

***Dominique LANZMANN-PETITHORY** - Centre Hospitalier Emile Roux, France*
I think that nutrition is a question of balancing and that most of the people in developed countries lack of alphalinolenic or omega 3; this is not a normal situation when taking into account the theory of the Paleolithic diet and gene evolution with a ratio of omega 6 and 3 around 1, and also that these fatty acids are essential, we cannot synthesize them.
We are completely dependent on what we eat; if someone lack of alphalinolenic and you give him alphalinolenic, this has an immediate effect on many kinds of metabolic ways that I cannot explain now. Between thrombosis and the effect on rhythm, I think that this depends on the person, whether they are old or young, the main risk etc. Maybe Professor Renaud could answer this question better than I can, but it acts within days on the platelet aggregation and it is also quite similar for the rhythm.

Serge RENAUD
I would like to make a comment.
You see, the level of alphalinolenic acid is really very small and in different foods you obtain very small amounts of this. In other words, in all those people we are currently studying, they are all relatively deficient; consequently, this is why it is so remarkable that as soon as you administer alphalinolenic acid, you markedly increase in a very rapid way, the response of these people - for atrial fibrillation prevention, for example.
Therefore, you have to realise that alphalinolenic acid, in most of us here is almost deficient and this fatty acid is essential for life! Consequently, it is almost like Vitamin C in the scurvy. As soon as you give a small amount, you correct the syndrome.

Fruit and vegetables and fatal myocardial infarction

Rodolfo SARACCI
(Italy & IARC-WHO) - IFC-National Research Council, Pisa, Italy - International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon cedex 08, France

The relationship between intake of fruits and vegetables and ischaemic heart disease (IHD) has been investigated in several epidemiological studies. The evidence for a protective role has been recently reviewed within the IARC programme for the evaluation of preventive interventions on cancer, within which outcomes other than anticancer effects are taken into consideration [1]. The available studies have several limitations, potentially capable of introducing biases in the ascertainment of a relationships between intake of fruits and vegetables and IHD. In cohort studies the more easily obtainable endpoint of death from IHD has been often used rather than the more directly relevant endpoint of a first major clinical event (myocardial infarction and revascularisation procedure) : this has the disadvantage that associations may appear with nutritional factors influencing disease evolution and survival after a first event rather than with the occurrence of the first event ; a further complication may derive from changes in diet subsequent to the occurrence of a first event. Case-control studies have been conducted on hospitalised survivors, hence missing the sizeable number of cases dying suddenly and/or unexpectedly outside hospital : again any relation between exposure and endpoint may in this situation reflect an association with survival after the occurrence of the event (acute IHD), rather than with the aetiology of the event itself. Moreover, both in cohort and case-control studies the control of cofounders, in particular tobacco smoking and energy intake, may have been incomplete. The IARC review left out the numerous clinically based or epidemiological studies that have examined IHD and a single compound or class of compounds related to fruits and vegetables, for example a vitamin or antioxidants , and considered only those studies aimed at investigating primarily the actual intake of fruits and vegetables : three case control studies and twenty cohort studies were available according to this criterion. Taken together they provide irregular results, inconsistent in respect to the presence or absence of a detectable association of fatal IHD with fruits and vegetables as a whole or separately, or with particular items within fruits and vegetables. Although a firm conclusion did not appear supported by the available evidence, the inverse association found in the larger cohort studies, better controlled for cofounders, favours a likely protective role of fruits and vegetables on IHD.
Given this still not firm conclusion it is of interest to investigate the relationship between fruits and vegetables consumption and IHD within large prospective studies based on different populations, like EPIC.

Methods

EPIC-Heart is the cardiovascular component of EPIC (European Prospective Investigation into Cancer and Nutrition), an ongoing prospective study conducted in 21 centres in 10 European countries (Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden, United Kingdom). As described in details, elsewhere, [2] subjects were administered extensive dietary and lifestyle questionnaires at the time of recruitment ; anthropometry measurements were taken and blood samples collected for long term storage. The ongoing follow-up is implemented through regional or national mortality registries in seven countries and via active follow-up procedures (including health insurance records and contact with the subjects, next-of-kin and health professionals) in France, Germany and Greece. For the present analysis cases are subjects with underlying cause of death (or the only cause mentioned) in the death certificate as either ICD-9 codes 410-414 or ICD-10 codes I20-I25.
Cox's proportional hazard regression with age as the underlying time dimension was used. The analyses were stratified by centre to control for differences in follow-up procedures, questionnaire design and other possible centre effects and were adjusted by sex, energy intake and main risk factors (smoking , hypertension, physical activity, alcohol intake and antecedents of diabetes and hyperlipidaemia at baseline). Variables were categorised by quintiles or, in alternative analyses, used as continuous log-transformed values. Subjects who at baseline reported previous myocardial infarction were excluded from the analyses.

Results

457311 participants without previous heart attack and mostly aged 35 to 69 at baseline (141 233 men and 316 078 women) were followed since 1992 for 4.8 years on average. Ascertainment of the cause of death was possible for 90% of the deaths. The distribution of fatal IHD by countries is given in fig.1 : of the 744 deaths the dominant proportion is from Sweden and UK, the latter being a cohort with an older age distribution than the other cohorts (data from Norway, which joined the study in the late 90's were not yet available for this analysis). Body mass index, tobacco smoking, hypertension and antecedents of hyperlipedemia significantly increased the risk of fatal IHD and job physical activity, alcohol intake (up to 40 g/day) and being a woman decreased the risk.

Fig.1. Distribution of fatal IHD in nine European countries

Distribution of fatal IHD by country

	Women	Men	Both
Denmark	13	33	46
France	11	-	11
Germany	8	51	59
Greece	15	38	53
Italy	7	19	26
Netherlands	21	21	42
Spain	6	36	42
Sweden	20	115	135
UK	121	209	330
TOTAL	222	522	744

As shown in fig.2 when the intake of fruits ad vegetables taken together was categorised using quintiles, no statistically, significant decreasing trend appeared with the hazard rates for fatal IHD ; the same applied (fig.3) for fruits, either total or citrus ; also, no trend was present for total vegetables (fig.4) ; while a statistical significant trend was found for leafy vegetables .

Fig.2. Hazard rates of fatal IHD by fifths of the distribution of fruits and vegetables intake

Fruits and vegetables and fatal IHD

Q1	1
Q2	0.83
Q3	0.82
Q4	0.81
Q5	0.88
trend p =	*0.80*

Fig.3.
Hazard rate of fatal IHD by fifths of the distribution of intake of total fruits and of citrus fruits

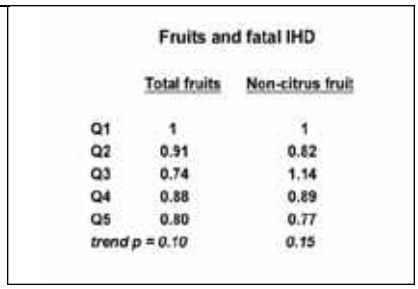


Fig.4
Hazard rate of fatal IHD by fifths of the distribution of intake of total vegetables and of leafy vegetables

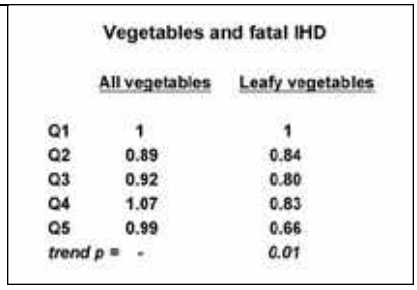
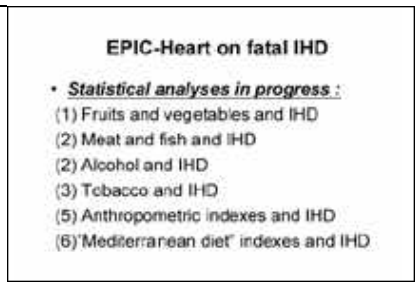


Fig.5
Some ongoing analyses of questionnaire data in the EPIC-Heart prospective study



Discussion and conclusion

Except for leafy vegetables, no inverse association appeared in this preliminary analysis of EPIC-Heart data between consumption of fruits and/or vegetables and fatal IHD. The statistically significant trend for leafy vegetables might be a chance finding arising from the multiplicity of tests performed or may in fact reflect, also in light of the previously discussed results from the literature, a real protective effect : compounds like folic acid could be hypothesised to induce such an effect. Further analyses on an increased number of cases (deaths) are in progress to investigate the consistency of this finding between different subsets of data, in particular by centres. These analyses are one aspect of current work within EPIC-Heart along three main lines : (a) statistical analyses of the relation between a number of exposures (in particular those in fig.5) and fatal IHD ; (b) planning of nested-case control studies within the cohort to investigate on stored plasma and DNA samples the relations of fatal IHD and selected variables (lipid profile, fatty acid profile, genetic polymorphisms related to lipid , coagulation and inflammation biomarkers) ; (c) strengthening the EPIC-Heart study design by acquiring information on non-fatal events.

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Questions

Kafatos.
How do you interpret the lack of inverse association of fruits and vegetables consumption and certain cancers ? Could this be due to the presence of carcinogenic contaminants ?

Saracci.
I do not know, but in the first place the protective effect itself does not apply to cancers at several sites.

Kromhout.
It would also depend on the concentration of contaminants : they are not that high, hence it would be unlikely that an absence of protective effect could be explained along that line.

Member of the audience.
Did you look at non-fatal cases of IHD ? I think it important to distinguish between fatal and non-fatal IHD as fruits and vegetables may have different effects on atherosclerosis and thrombosis , perhaps because of their content of some fatty acids like alphalinolenic acid.

Saracci.
I agree on the need of looking at non-fatal cases as well and this is the reason why we are currently working towards acquiring information on non-fatal cases, a not light task as shown by the experience of such projects as the WHO "Monica" investigation and the fact that diagnostic criteria for the acute ischaemic syndromes are evolving.

Meat and fish and fatal myocardial infarction.

Teresa NORAT
IARC/WHO on behalf of EPIC, International Agency for Research on Cancer, Unit of Nutrition and Cancer,
150 cours Albert Thomas, 69372 Lyon Cedex 08, France

Preventing coronary heart disease (CHD) mortality and morbidity is a leading public health priority. The preventive approach to CHD depends on the modification of the main recognised determinants of the disease, namely tobacco smoking and physical inactivity, but also hypertension, high blood cholesterol, obesity and diabetes, which are substantially influenced by dietary factors. The classic diet-heart hypothesis postulated a primary role of dietary saturated fat and cholesterol in the cause of CHD in humans (1) and dominated most epidemiological and clinical investigations of diet and CHD. More recent studies have investigated the relationship between consumption of specific foods or overall dietary patterns and risk of CHD. Several prospective studies have reported a beneficial effect of fruits and vegetables and an inverse association between nut consumption and risk of CHD (2). Replacement of red meat with chicken and fish has been associated with reduced risk of CHD (3).

Atherosclerotic effects have generally been attributed to the relatively concentrated content in red meat of dietary cholesterol and saturated fatty acids, factors that have been clearly demonstrated to influence blood cholesterol concentrations, a known risk factor for CHD and atherosclerosis. Regular consumption of beef, when added to a vegetarian diet, has been demonstrated to increase blood cholesterol. Especially low-density-lipoprotein (LDL) cholesterol, and to increase systolic blood pressure

Fish consumption has been inversely associated with fatal ischaemic heart disease (IHD), consistent with the prevention of arrhythmia by long-chain n-3 PUFA's in fish.

In the present study, we examined the relationship between high intake of meat and fish, and the risk of fatal myocardial infarction, as an expression of CHD, in a prospective cohort of European mean and women with very diverse dietary habits (EPIC-HEART project).

The European Prospective Investigation into Cancer and Nutrition (EPIC) is an ongoing prospective cohort in 23 centres located in 11 European countries and is the parent study of the EPIC-HEART project: France, Germany (Heidelberg, Postdam), Greece, Italy (Florence, Turin, Ragusa, Varese), The Netherlands (Bilthoven, Utrecht), Spain (Granada, Murcia, Navarra, San Sebastian), UK (Cambridge, Oxford), Sweden (Malmo, Umea), Denmark (Aarhus, Copenhague) and Norway. The study includes extensive dietary and lifestyle questionnaires, anthropometry measurements and blood collection (4). The follow-up was done using mortality registries at the regional or national level in most centres. A combination of methods including health insurance records and active follow-up through study subjects and their next-of-kin was used in France, Germany and Greece. Cases are subjects with underlying cause of death (or only provided cause of death) in the death certificate as either ICD-9 codes 410-414 or ICD-10 codes I20-I25.

We used Cox proportional hazard regression with age as the underlying time metric. The analyses were stratified by centre to control for differences in follow-up procedures, questionnaire design, and other centre effects and adjusted by sex and energy, and main risk factors as smoking, hypertension, physical activity, alcohol intake and antecedents of diabetes and hyperlipidemia at baseline. The analyses were performed with the variables in both categorical and log-transformed continuous scale.

A number of 457 311 participants without previous heart attack at baseline (141 233 men and 316 078 women) mostly aged 35-70 years were followed for 4,8 years on average since 1992. 844 subjects, 592 men and 252 women, died for fatal myocardial infarction. The percentage of ascertainment of cause of death was 90%. Body mass index, smoking, hypertension and antecedents of hyperlypidemia significantly increased the risk of fatal myocardial infarction and job physical activity, alcohol intake and being a woman decreased the risk.

Total red meat intake (fresh plus processed meat) was positively associated to risk of death for myocardial infarction (p linear trend =0,0120). The hazard ratio associated to intakes above 90 g/day of fresh and processed red meat intake compared to less than 30 g/day was 1,37 (95% CI=1,00-1,89). The risk increase was explained by processed meat (p-linear trend=0,0037) with hazard ratio of 1,44 (95% CI=1,01-2,00) for intakes over 80 g/day compared to less than 10 g/day, while for fresh red meat no significant association was observed (Figure 1).

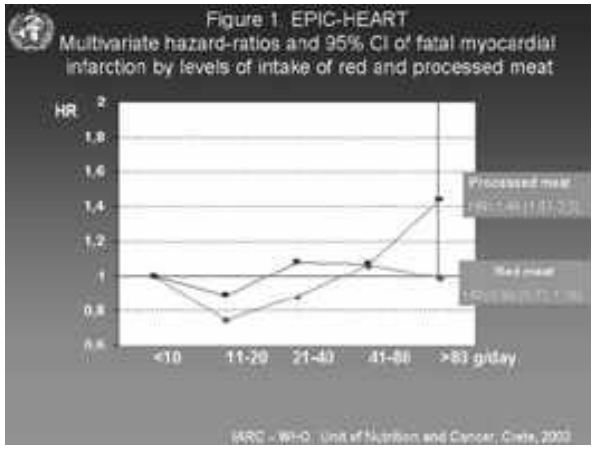

Figure 1

The data were suggestive of a protective effect of high intake of white meat (fish and poultry), but it was not statistically significant (p- linear trend=0,0833). The hazard ratio for intakes over 80 g/day compared to less than 10 g/day were 0,9 (95% CI=0,66-1,24). The association with fish and poultry was suggestive of a protective effect but the results were not statistically significant (Figure 2).

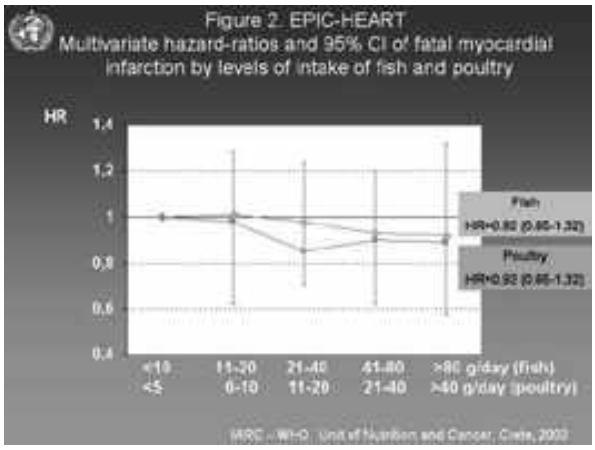

Figure 2

In this cohort of middle aged European men and women, high red meat intake, and in particular processed red meat, is associated with an increased risk of fatal myocardial infarction in multivariate models including main risk factors. High white meat intake was not associated to a risk increase of fatal myocardial infraction. The protective effect of fish intake, previously reported in other prospective studies was not corroborated in this study.

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Questions

Daan KROMHAUT
Thank you for a nice overview of this preliminary analysis.
This paper is open for discussion.
Maybe I can start with fish?
The reason that you did not find an association could be that the heterogeneity in fish consumption between the different cohorts in the EPIC study is very large.
In your introduction you referred to the paper where the different prospective studies were taken in relation to the association between fish consumption and coronary heart disease; it also shows that in cohorts, with a low absolute risk, there is no association. However, when there is a high absolute risk from coronary heart disease in the societies you consistently find the inverse association.
My question is: Did you do an analysis where you took into account the absolute level of risk? - In the EPIC study you could do that quite easily by comparing, for instance, Northern Europe with Southern Europe – because in Southern Europe the absolute risk for coronary heart disease is much lower than in Northern Europe, have you done this type of analysis yet?

Teresa NORAT SOTO
I also did the analysis grouping the countries in: Mediterranean countries (Italy, France, Spain and Greece), Central European (Germany and the Netherlands), North-European (Sweden and Denmark) and United Kingdom. There is a protective effect of fish in the Northern-European countries, but not in the other groups. We have to consider that creating sub-groups we reduce considerably the number of subjects in each analysis.

Daan KROMHAUT
The question between fish and coronary heart disease is extremely complicated, because it is not only a question of absolute risk but also the fact that several studies from Norway and Finland have been negative in the past and especially in populations where they had a high level of fish consumption.
The other problem is that there was no real control group who were not consuming any fish, so all those things have to be taken into account and it is very complex to do this type of analysis and this is only preliminary, so there is a lot of work to do.
Are there any other questions?

A Member of the Audience
Are you going to look at the effect of alcohol by type?

Teresa NORAT SOTO
I also did preliminary analyses of myocardial infraction risk by intake of ethanol from wine, beer and spirits in EPIC-Heart. We do not have the data of consumption of red and white wine separately. There is a protective effect associated to current alcohol consumption compared to no consumption independently of the source of alcohol: wine, beer or spirits. Energy-adjusted mean consumption of ethanol in men was about 20 grams per day and in women was about 8 grams per day. We do not have high consumers of ethanol in EPIC, which is a cohort of volunteers. As we know, very high consumption of ethanol has been associated with an increased risk of mortality.

Mediterranean dietary pattern and cardiovascular disease in Italy

Salvatore PANICO
Dipartimento di Medicina Clinica e Sperimentale, Università Federico II, Napoli, Italy

I would like to thank the organising committee for having invited me.

My task is to represent and report some of the Italian contributions to the relationship between Mediterranean diet and cardio vascular risk.

I grew up as a doctor and a researcher in a very special environment.
The department where I have been working for the last 27 years and my former chief, Professor Mario Mancini were greatly involved in the early steps of the organisation of the Seven Countries Study in the late 1950s.
So the diet-heart hypothesis for us working in this department was not only an historical cornerstone but also a sort of mythological concept.

It was in Naples in the early 1940s, where the Seven Countries Study was actually conceptualised when Ancel Keys and Paul D. White, the cardiologist of General Eisenhower, were with the troops at the end of World War II and they were very impressed by the fact that there were very few myocardial infarctions in the Neapolitan and surrounding hospitals.

They realised that all over Southern Italy there were much fewer myocardial infarction patients than in the Boston area where White used to work.

At that time they decided to implement this collaboration with other researcher groups both in the Mediterranean and other countries, including in the investigation Italy, Greece, Yugoslavia, The Netherlands, Finland, Japan and US.

The classical figure, presented in the Daan Kromhout paper published some years ago on the 24 years follow up of the Seven Countries Study, shows there is a contribution of Italian centres including the samples from Montegiorgio, Crevalcore and Rome that have filled the gap between the very low and very high mortality groups providing data for a better interpretable scattering of data and "continuity" to the regression line between saturated fatty acid intake and coronary heart disease mortality (figure 1).

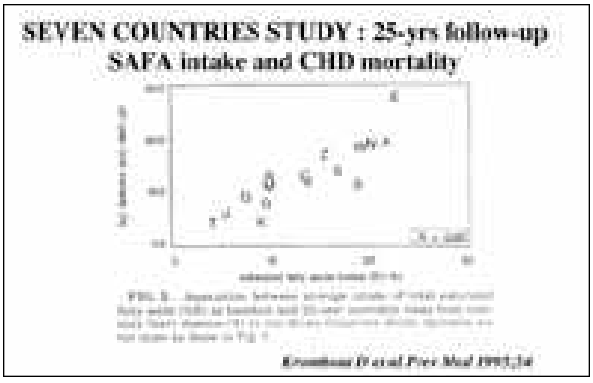

Figure 1

The core-idea of the research projects on Mediterranean diet in our department was essentially focused on the content of fat and these investigations have greatly influenced the Italian research activities in this field.

Another contribution of Italy to the Seven Countries Study was that this data convinced Ancel Keys to buy a house in a small village South of Naples; he has been living there for more than 30 years with his wife. Quite shortly he will 100 years old, the perfect living proof of how important it is to eat the Mediterranean diet and live in a Mediterranean country!

In that area, 15 years ago, a group of colleagues in my department together with a government nutritionist from Rome and in collaboration with scientists from Finland, decided to carry out an experiment. This experiment was based in supporting people to change their diet and to eat like Finnish people for six weeks – the Pollica Study. In Finland about 40 couples were asked to eat the Mediterranean diet for six weeks.
The results were quite impressive: Total-Cholesterol, LDL-cholesterol and blood pressure increased in the people living in Southern Italy during the experiment and returned to the original levels after the experiment stopped (Ferro-Luzzi et al, Am J Clin Nutr, 1984; Strazzullo Pet al, J Hypertens, 1986).

On the other side in the Finnish experiment, exactly the opposite happened, an impressive fall in blood cholesterol and blood pressure (Ehnholm C et al N Engl J Med 1982;307:850; Puska P et al. Prev Med 1985).

You could say that this is not ischaemic disease but only intermediate variables: blood cholesterol and LDL cholesterol, blood pressure; but they are on the way to ischaemic disease and cardiovascular risks in general.

During the 1980s we had the opportunity to analyse some interesting data that we collected together with many other colleagues in Italy. We measured the classical cardiovascular risk factors, including some specific dietary habits, in random samples of the Italian population aged 20-59 years old in nine communities. We decided to analyse some of the data, at a cross-sectional observation, just to look at some associations and, using data from the dietary food frequency questionnaire, we developed a dietary atherogenic index which was actually based on the content of saturated fat and cholesterol of some food items.
We got the interesting result that, according to consumption levels and this dietary atherogenic index - adjusting by age and body mass index and use of fats - cholesterol was strongly and positively associated with the index both in men and women (figure 2).
This was also the case for blood pressure and serum glucose (figure 3 and 4).

At that time we decided to explore also the association between the consumption of olive oil and other type of fats, including butter and margarine with total serum cholesterol, systolic blood pressure and serum glucose. This was in line with our education

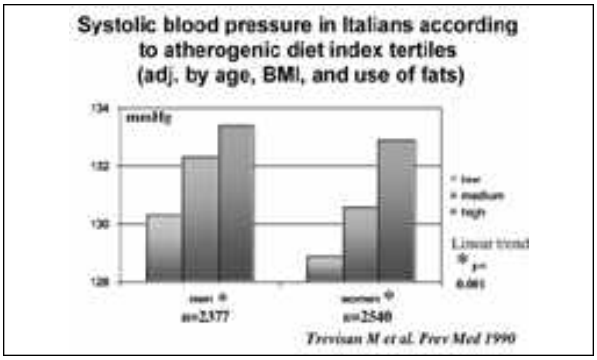

Figure 2

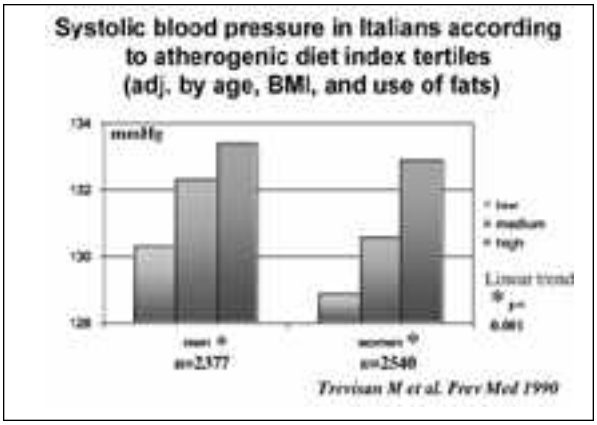

Figure 3

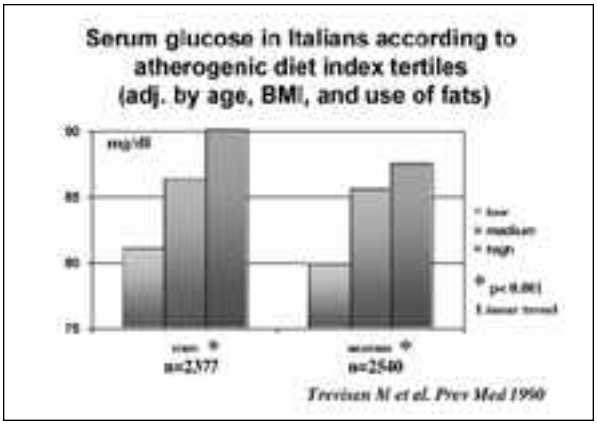

Figure 4

Figure 5

Figure 6

(laughs) so…. we were furtherly pushed to study the effects of olive oil (!) and probably with some advertisement influences during our infancy. The lady from the advertisement poster for olive oil is in figure 5 while the one from the poster advertising for the classic butter cake from Northern Italy is in figure 6!

The results, published at the end of the 1980s, indicated that at increasing level of consumption of butter and margarine serum cholesterol was higher in men and women. The case was similar for systolic blood pressure and glucose (Trevisan M et al. JAMA 1990;263:688) - this was actually one of the first studies to consider olive oil and serum glucose.

After a number of experimental studies looking at the effects of olive oil and monounsaturated consumption on insulin sensitivity, carried out in our department, a collaborative international study was organised in the late 90ies, which again involved our department. The study - named KANWU - found that individuals with a diet high in monounsaturated fatty acids have an higher insulin sensitivity, compared with those with a high level of dietary saturated fatty acid (Vessby B et al, Diabetologia, 2001).

In the beginning of the 1990s we started a prospective investigation to study the causes of heart disease in women, which had been studied much less in the past than for men. The Seven Country Study cohorts are only composed with men; and the Nine Communities Study was not continued to analyse incidence.
I was responsible for this new study, and I decided to name it 'PROGETTO ATENA'. In the Greek Mythology ATENA was the goddess of intelligence, so that was meant as a homage to the intelligence of women.
The study was then associated to the EPIC collaboration (European Investigation into Cancer and Nutrition), because the design is exactly the same as any other cohort study involved in the EPIC collaboration.

Following a specific grant I received by the Ministry of Health, we had the possibility of developing a cardiovascular collaboration within the EPIC-Italy cohorts. There are five different cohorts in Italy, associated with the EPIC study (figure 7); we decided to exploit this part of the EPIC collaboration looking at incident cases of cardiovascular disease, starting with ischaemic heart disease. For the five centres in Italy there was a nice distribution from North to South, providing a good variability of dietary habits.

Figure 7

For the women's cohort we had something like 32,578 individuals, a large number of women and we hope that this collaboration will continue in the future.
We actively looked for incident cases and performed an active follow-up in the five centres, using the results from vital statistics for the mortality and the hospital discharge registries to look for any suspected cases before validate the diagnosis using the medical documents.

There was also some active follow up on some of the groups, via telephonic interviews to cross-validate the completeness of information coming from the hospital discharge registries. Specifically in Naples we contacted almost all the 5,000 people in Naples directly, or their relatives, or medical doctors to see if there were any other possible in hospital admission outside the region. We were satisfied with the completeness of the follow up through the hospital registries.

We had 129 cases of myocardial infarction and/or coronary devascularisation with high specificity for the diagnosis of ischaemic heart disease.
We then developed a dietary index (the Mediterranean index based on nine dietary components, including fruit, vegetables, legumes, dairy products, cereals, fish, meat, alcohol and monounsaturated/saturated fat ratio). The construction of the index is based on the position of the individuals in the distribution of the nine predefined components. Finally each individual is characterised by a score. We distributed the Mediterranean index scores and divided the individuals into tertiles. The scores were nicely distributed all over the centres – the highest scores were found mainly in the Southern/central centres and the lowest in the Northern centres.
The results of a preliminary analysis are quite impressive: adjusting for age, education, body mass index, smoking, total energy and history of cardiovascular disease, we found about 50% reduction in the risk of ischaemic heart disease.
A few crucial remarks on these results: they indicated that Italian women following as much as possible the Mediterranean type dietary habits –as depicted by the nine components - are protected from ischaemic heart disease compared with those women who have less typical dietary habits.
According to these data, the protection appeared to be independent of the presence of known measured risk factors. The findings are also interesting and original since they derive from a prospective study on cohorts of women living in the Mediterranean countries. We hope that this study could be another supporter to the mythology of that dietary hypothesis.

Thank you.

Questions

Daan KROMHOUT
Thank you very much for a nice presentation and the nice results on the Mediterranean diet.
Are there any questions?
I heard a story concerning the first study you mentioned, the Pollica Study, when Finnish and Italian people were asked to swap diets for 6 weeks; it was not very difficult for the Finnish people to adopt the Mediterranean diet, but it was almost impossible for the Italians to adapt to the Finnish diet! Is this true or not?

Salvatore PANICO
Yes, it is true – it was terrible for them, but it was only 6 weeks!

Daan KROMHOUT
Did they really finish the study or not? Did they refuse?

Salvatore PANICO
The need was for all the people involved to stay and receive support to continue the experiment.

Daan KROMHOUT
In relation to the preliminary results that you presented from the EPIC-Italy study, did you have the possibility of stratifying each region because you had 5 different studies?

Salvatore PANICO
We stratified by centre in the analysis but, unfortunately, we have very few cases in some of them, so are waiting for the next follow up to increase the cases because there are very few in Southern Italy.

Daan KROMHOUT
The reason that I ask this is that it is very interesting to know whether the association is the same in the Northern part and Southern part of Italy, so that even if it works in the Southern part you can have people with a healthier diet than others.

Salvatore PANICO
We presume that this is the case but we still have to wait.

Daan KROMHOUT
Are there any other questions? – Nadia?

Nadia SLIMANI
I have one question about the Mediterranean diet index, because as it is applied here there are two components, a qualitative one that you have listed are the food groups and a quantitative one, is when you define the median and the different categories of individuals.
I am surprised that this quantitative index is based on the population distribution under investigation, because I can't understand how this can work in Italy but if you do exactly the same in Finland you cannot say that what you observe is the Mediterranean diet. Therefore, for me, the quantitative aspect of the index should, probably, be more greatly based on the Cretan distribution of dietary components; do you see what I mean?

Salvatore PANICO
I see what you mean. We decided upon this kind of process because, as with the median, it was easy for us. However, one can also try to build up a score using tertiles, quartiles and quintile and maybe this would be the easiest way for studies in other populations.

Daan KROMHOUT
Maybe I can add to that, we have a paper concerning these types of issues in the British Medical Journal from 1997.
In that paper, we tried to carry out this type of work in Finland, the Netherlands and Italy and it was extremely complex and difficult because in the Netherlands and especially in Finland, there are very few people who really adjust to that same type of Mediterranean diet.
We finally decided to dichotomise the situation, to say that when people were above a certain cut-off point, this was the healthy part, no risk, and the other was the unhealthy part and this was the only possible way to do it because the patterns are too diverse to use the Mediterranean diet in certain and Northern European cohorts.
That's the problem.

The RIVAGE intervention study on Mediterranean diet and risks for coronary heart disease

Mariette GERBER
Centre de Recherche en Cancérologie, INSERM-CRLC, Groupe d'Epidémiologie Métabolique, 34298 Montpellier Cedex 5

Ladies and Gentlemen, Mr Chairman; I would first like to thank Aprifel, and especially Saïda Barnat, for this opportunity to present the Med-Rivage Study together with my friend and colleague, Denis Lairon.

Introduction

The Rivage Study is an intervention study that was first designed as a potential ultimate evidence for the protective effect of the Mediterranean diet on cardiovascular risk, as it was previously shown by ecological and observational studies.

The Lyon diet study was an intervention study based on the Mediterranean diet principles, but with a special margarine in place of olive oil, and in a secondary prevention situation. We took up the challenge to approach the problem with a more traditional Mediterranean diet in a primary prevention situation.

We compared the specificity of Mediterranean diet versus the general AHA recommendation with the objective of studying their effects on risk factors for coronary vascular disease.
I will present the design and the nutritional strategy and Denis Lairon will present the effects of the diets on the risk factors.

Subjects

The participants were subjects visiting the Centre for Detection and Prevention of Arteriosclerosis (CDPA) in Marseille.
The exclusion criteria was treatment, therefore, we had to set up an upper limit for these variables –7.7 here for total cholesterol, 4.6 for the triglycerides and 6.9 for the glycaemia – and also for blood pressure: systolic up to 180 and diastolic 105.
Additional eligibility criteria were selected as a BMI ≥ 27, being smokers of 5 cigarettes or more, being sedentary and to have a family history of cardiovascular disease in at least one parent.
These participants were randomised into two groups and, after agreement, were offered two different types of diet and they ignored which one was the experimental one and which one was the control.
They were followed up at baseline, 3, 6 and 12 months using all the examinations that Denis Lairon will detail later.

Nutritional strategy

The main difference between the AHA type adapted by the CDPA and the Mediterranean diet was on the amount of lipids. We accept up to 38% of lipids in the Mediterranean diet, whereas the AHA asks for 30%. The difference is at the expense, of carbohydrates and proteins, and mainly made of monounsaturated fatty acid; we have an intake level of 8% to 10% more of monounsaturated fatty acids.
For the other nutrients, our aim was a little higher in fibre for the Mediterranean diet, we also aimed for 7mg of ß-carotene equivalents as an indicator of fruit and vegetable consumption and we aimed at 800mg of calcium as an indication of moderate dairy products intake.

All the participants in each group received the same attention and counselling by the dieticians.

Each participant also received a leaflet indicating the AHA, the usual recommendations for people at risk for cardiovascular disease: fish two or three times per week, skimmed dairy products, poultry rather than meat and never more than 150g per day, no fat, eggs no more than 3 per week, more fruit and vegetables, starch was allowed but there were a restriction on sweets, Energy intake was calculated according to the BMI.

In the Mediterranean diet, we were much more precise, not only on the frequency of the consumption, but also on the quality of the products. For example, here we recommend eating fish four times per week and meat, poultry and eggs only once per week.

Olive oil was the only added fat allowed, we accepted some Canola oil as a second choice. Fruit was to be consumed three to four times a day, vegetables two to three times a day. We recommended whole grain bread, pulses to be eaten three times in two weeks; dairy products – no more than twice a day, in the form of cottage cheese or yoghurt and preferably from sheep or goats. Again, the calories were calculated according to the BMI.

The very specific difference of the Mediterranean group was that we prepared two sets of menus, one for winter and spring and the other one for summer and fall in order to adapt the intake of vegetables to the seasonal availability because seasonally is important with regard to the nutrient content of many fruit and vegetables.
Each set was made up of 15 days with menus and each day had 3 menus so that the participants could select the menus according to taste or to cooking possibilities, we also gave them recipes and the participants in this group were given olive oil, tomato sauce, and oat-bran enriched pasta.

The compliance was assessed for both groups with a 3-day recall questionnaire at 3, 6 and 12 months and the measurement of plasma nutritional markers, essential fatty acids, usual carotenoids, folates and phenolics.

Subjects characteristics at baseline

At the baseline we had 212 subjects, 102 Mediterranean and 110 AHA. 169 of the subjects completed the follow-up at 3 months – 88 Mediterranean against 81 AHA. There was therefore a 14% drop out rate in the Mediterranean and 27% in the AHA – indicating, perhaps, that the Mediterranean diet was easier, or more pleasant, to follow.

There were no differences in the socio-demographic characteristics, neither at the baseline nor at the frame of sample. We also did not have any difference in the nutritional intake for both groups – the energy was around 8500 kilojoules, the lipids were around 39%, not so bad, but, saturated fatty acids represented 14.5% of total energy intake.
For the fibre carotene and folates we are in normal range, not completely satisfactory, but not too bad.

Also, for the nutritional markers, there was no difference in the two groups, and we have figures that are in the general range of what is observed in France, with a low level of alphalinolenic acid and a rather satisfactory level of beta-carotene and folates.

Results at 3 months

The energy intake and all macronutrients decreased significantly in both groups.

Therefore, there was no significant difference between the effect of the two diets, except for monounsaturated fatty acids.

The monounsaturated fatty acids total energy intake decreased for the AHA, indicating a decrease in meat consumption, but it was increased in the Mediterranean diet consumption, through the quasi exclusive use of olive oil, which compensated for the decrease in meat consumption. The inter-diet difference was significant

So far, in our database, we do not have the specific fatty acids, but we could measure the serum polyunsaturated fatty acids.
We observed an increase in alpha-linolenic acid in the Mediterranean diet and a decrease of the same in the AHA.
Both were non-significant, but because of the inverse direction, we have a borderline significant difference in the change between the two the diets.
The EPA was only significantly increased in the Mediterranean diet and, again, we have a borderline significance here, but the DHA was increased in both diets, although a little more in the Mediterranean diet.

With the nutrients, other than the fatty acids, the only one shown by the questionnaire to be modified by the diet was fibre. Fibre was increased in both groups, this only became significant in the AHA, only when expressed as nutrient density, but it was already significant in the Mediterranean diet group, when it was not relative to energy as an absolute figure. There is also a borderline difference here between the two diets for total fibre and this refers to the insoluble fibre, which suggests that the participants followed our recommendations to eat whole grain bread.

For our other nutritional markers, we did not have any other differences measured by the questionnaire.
With the plasma nutritional markers we could show that the beta-carotene, the lycopene and the folates were statistically increased in the Mediterranean diet and non significantly different in the AHAdiet.

As a conclusion, therefore, I would like to say that we achieved our essential goal, which was the improvement of the dietary pattern as a whole; we changed the fat, increased fibre and beta-carotene, which means that we also increased fruit and vegetables. This underlines the importance of the nutritional markers for assessment of data, because we could not have shown some increases without these, also that we had the greatest success in the diet change with fat, because that is probably the point where our population departs the most from the Mediterranean diet.

Thank you, I will now pass the floor to Dr Lairon.

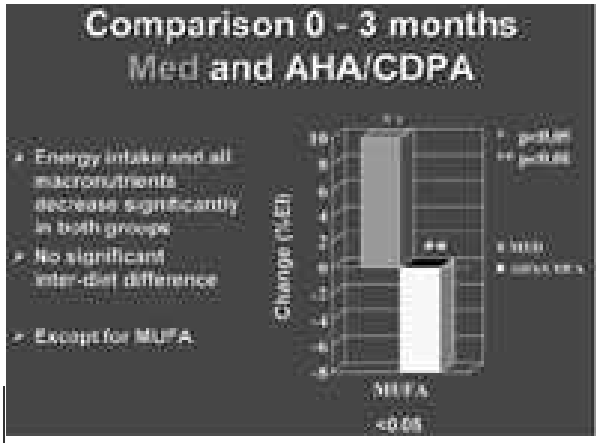

Figure 1 :
Comparison of the changes in the percentage of energy intake as monounsaturated fatty acids between the Med and the AHA diet.
Notes : The * refers to the significance observed in each group between baseline and 3 months. The p below the abscissa refers to the significance observed between Med and AHA.

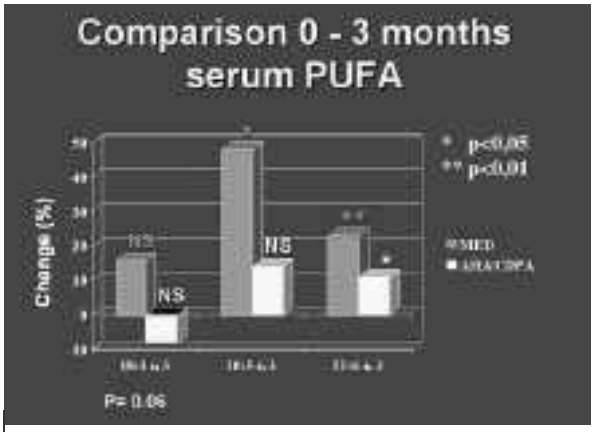

Figure 2 :
Comparison of the changes in the percentage of serum fatty acids between the Med and the AHA diet.
Notes : The * refers to the significance observed in each group between baseline and 3 months. The p below the abscissa refers to the significance observed between Med and AHA).

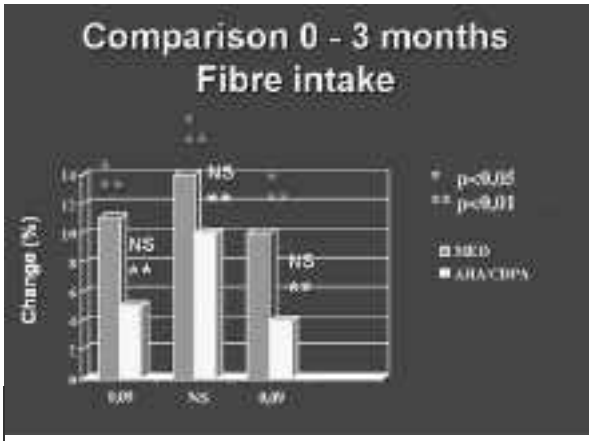

Figure 3 :
Comparison of the percentage of change in the total fibre, soluble and insoluble fibre intake between the Med and the AHA diet.
Notes : The upper * refers to the raw significance observed in each group between baseline and 3 months. The lower ones to the significance observed after energy adjustment.

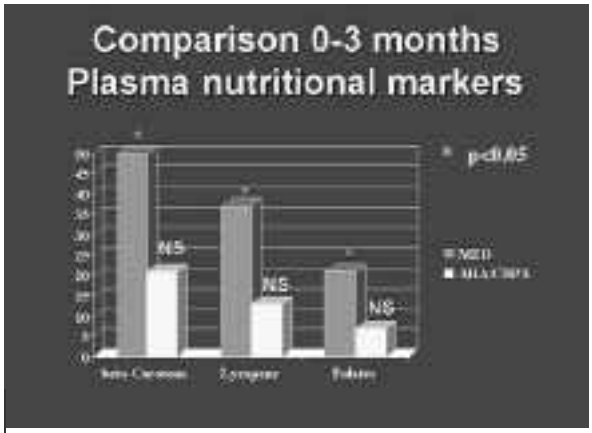

Figure 4 :
Comparison of the percentage of change in the plasma nutritional markers between the Med and the AHA diet.
Notes : The * refers to the significance observed in each group between baseline and 3 months.

Denis LAIRON
INSERM Unit 476, Lipids and Human Nutrition, Centre Viton, 18 avenue Mozart, 13009 Marseille, France

During this study we carried out a lot of measurements related to the risk factors for coronary heart disease.
These measurements were taken at baseline and at the end of 3 and 12 months.
For clinical parameters: we had BMI, blood pressure, intima-media thickness of carotid - many fasting parameters: glycemia, insulinaemia, triglyceride, triglyceride-rich lipoprotein triglycerides, LDL cholesterol, HDL-cholesterol and triglyceride-rich lipoprotein cholesterol. A number of Apoproteins – ApoA1, ApoB - ApoB48, which is a marker of intestinal lipoproteins and ApoC3, and also some thrombosis factors.

At the entry and at the end of the 3 and 12 months, the subjects had a standard test meal in the morning and we looked at several parameters postprandially after 2.5 hours and 5 hours and calculates the are-under-the curve, especially for triglycerides, triglyceride-rich lipoprotein triglycerides and ApoB48.

We also looked at a number of gene polymorphism's in the subject and we will show elsewhere some data.

I will show you the first set of data obtained after the 3 months dietary intervention in the two groups:

There was a small reduction in the BMI in the two groups, which was comparable to around a 4% or 5% reduction - a small change.

Looking separately at the two sexes, it appeared that the difference was found either in men or in women in the two groups.

Regarding fasting plasma cholesterol, in the two groups we have seen a significant reduction in this parameter after the Mediterranean diet or AHA-prudent diet, but the amplitude of change was noticeably higher after the Mediterranean diet than the AHAdiet.

Indeed, in both men and women in the Mediterranean diet, there was a significant reduction, but not in the women in the AHA group.

For fasting LDL cholesterol we also found a significant reduction in this parameter after 3 months diet in the two groups, but the amplitude of the reduction in the Mediterranean diet group was almost two fold as compared to the expected reduction after the AHAtype diet.
The men and women in the Mediterranean group showed a significant reduction but not the women in the AHAgroup.

For HDL cholesterol, there were basically no changes after the dietary intervention, and the amplitude of change was negligible.
For plasma fasting triglycerides, we have also seen upon the Mediterranean diet for 3 months a significant reduction of about 12% in fasting triglycerides. A somewhat lower change was observed after the AHA diet, with no significant difference.

The change was observed in both men and women, but especially in the women in the Mediterranean diet group.
For glycaemia, there was a very small reduction observed in this after the 2 diets, with no difference in the 2 groups, and the minor change was observed as well in men and women in the 2 groups.

For insulinaemia a significant reduction was observed in the 2 groups with more amplitude in the Mediterranean group as compared to the AHAgroup.
It is interesting to see that when separating the sexes, only the Mediterranean group men and the Mediterranean group women showed a significant reduction in insulinaemia, but not the men in the AHAgroup.

For postprandial parameters, we measured before and after the standardised test meal for 5 hours.

For plasma triglycerides, there was no reduction in the area-under-the curve after the AHAdiet but a reduction of about 15% after the Mediterranean diet was observed, with no specific difference in the two sexes.

For ApoB48, a marker of intestinally-derived triglyceride-rich lipoproteins, a standard marker relating diet and lipid metabolism, there was a significant reduction after both diets, but with a more marked amplitude after the Mediterranean diet and especially it is very interesting to note that both men and women on the Mediterranean diet showed this significant reduction, but not the men and women in the AHAgroup.

To draw some conclusions from this first set of data obtained after 3 months intervention, we can conclude that both diets improve metabolic status, especially for some risk factors such as BMI, fasting plasma cholesterol, LDL cholesterol, fasting triglycerides and insulinaemia, but also postprandial triglycerides and ApoB48.

It is interesting to observe that the Mediterranean diet clearly exacerbates some positive changes observed, especially for fasting plasma cholesterol and LDL cholesterol, but also postprandial triglycerides.
This data will be confirmed for a much longer and challenging period (12 months).

Our conclusion is that the data obtained from this study supports the concept that the Mediterranean diet, rich in MUFA, has a more beneficial effect than a low fat diet on metabolic parameters considered to be risk factors for cardiovascular disease.

Questions

Daan KROMHOUT
I would like to thank Dr Gerber and Dr Lairon for a very interesting presentation about the trial, I would also like to ask Dr Gerber to come back to the stage in order to answer some questions.
Are there any questions?

Member of the Audience
With the Mediterranean diet did you include wine?

Mariette GERBER
Yes, I did not mention this; for the drinkers, we recommended that they did not exceed two glasses of wine per day for the men and one glass of wine for the women.
In the AHA, the recommendation was only on the percentage of alcohol beverage by energy intake.
We did not counsel this, or recommend drinking wine, only for those who used to drink, which was probably the whole population, anyway.

Member of the Audience
With all the really fascinating metabolic data that you presented and that we have seen from other speakers, and the kinds of observational data that we have seen yourself or Dr Panico showing, has there been any consideration recently or in the past, of doing a primary prevention trial of cardiovascular disease end points with, say, the Mediterranean diet as the primary intervention?
Is that something that is at all feasible and should be considered?

Denis LAIRON
To my knowledge, there are no published studies like that and no trials done on this aspect. Of course, it would be extremely interesting and in fact we would like to carry out such a trial but the problem is one of money raising, so we started with this study, which is a short-term study on risk factors.
After the Lyon study, we would really like to have some data on primary prevention.
It would be very interesting, at a European level, to have a joint project in order to carry out such a primary prevention trial, in a cross-country situation.

Daan KROMHOUT
The problem of that type of trial is the financing. The numbers that you need in order to get any significant results are very large.
I am involved in different and –3 fatty acids and we have to randomise 4,000 MI patients in order to get significant results; we considered carrying out a primary prevention trial on Omega 3 fatty acids, but then we needed the sample size of about 40,000 and that was the reason that we went for secondary prevention, but we would have liked to go for a primary prevention trial.

Member of the Audience
I have a question on the results you presented comparing the effect of the Mediterranean diet versus the American AHA association. The conclusion was that both diets showed an effect on these markers, but I am still interested in the question as to whether the Mediterranean diet did better than the American Heart Association diet. This was not taken into account in the results you presented.
Have you done those analyses and, if so, what were the results of that?
Were they really comparable in their effects

Denis LAIRON
Our aim was really to compare two diets and not one single component of the diet.
Therefore, if we try to look more carefully at the data, we see that the two important changes in the two diets were MUFA content, with a high level in the Mediterranean diet as compared to the low fat diet and the dietary fibre content.
When looking at the data from the traditional Cretan diet, the two striking differences are MUFA content and dietary fibre due to legume consumption.
We are doing the statistical work now, because we have just got the data recently, so it was an opportunity to present the data, but we have a significant difference between the two groups with regards to the effect on cholesterol.
We need to check for some adjustments. In fact the reduction that we obtained on the cholesterol parameter with the AHA diet, was basically what we could expect to have with this kind of diet regarding previous publications.
Concerning cholesterol and triglycerides this is interesting, because it is generally thought that a low fat diet meaning that it is rich in carbohydrates, is expected to increase triglyceridemia – it is thought to be one of the major defects of this kind of diet. It is interesting with a Mediterranean type diet richer in MUFA and therefore less rich in carbohydrate, that there is no increase in triglycerides and there is even a reduction in fasting triglycerides, even moderate, but also postprandially indicating a better clearance of triglycerides from the circulation which is a very interesting point regarding cardiovascular risk.

Daan KROMHOUT
To add to the complexity of analysing this trial, if I recall correctly, is that you had a difference in both groups in Body Mass Index of about 1 unit so probably the weight loss was in the order of 3 to 4 kilos in both groups; to see what the effect of the diet is in a population that's losing weight is extremely complex, because there is also a strong effect of weight loss on different lipid and lipoprotein fractions so, I think, you have a hard task in figuring out all those things.
There was another question.

Member of the Audience
What was the amount of fat and the quality of the fatty acid in the test?

Denis LAIRON
It was about 30g

Member of the Audience
What kind of fatty acids?

Denis LAIRON
It was normal margarine.

Member of the Audience
Did you look and see if there was a correlation between insulinosensitivity and monounsaturated fatty acids or alphalinolenic acid or fibre, because it is quite important to know the main determinant of inter sensitivity?

Denis LAIRON
We are doing these kinds of analyses by now, in order to look at the individual component of the diet and to study the specific effect of some specific nutrients.

Member of the Audience
There may be an interaction between MUFA and fibre or …

Denis LAIRON
I cannot tell you that now, but we are expecting to have the data.
I would like to tell you that Stéphanie Vincent is presenting a poster n° 42, which contains some data of the study.

Member of the Audience (Speaker)
In relation to the question about the primary prevention trials, I would like to say that there is a study here in Crete, we studied with a cohort about 6,000 children all entering elementary school 10 years ago, and they had health and nutrition intervention for about 6 years during the primary school.
We have the follow up now and tomorrow I will present some preliminary results on the cardiovascular risk of infarctus in those children.

Daan KROMHOUT
Thank you – are there any other questions?
My final question is that you show weight loss in both groups. What is your explanation that on both diets people lost weight – did the amount of food that they consumed decrease during the intervention period or did they increase physical activity, what was the reason for the weight loss in both groups?
Do you have anything to say about that?

Mariette GERBER
Most of them were obese, so the dietician calculated the calorie restriction diets for both groups, so that was the reason or one reason for their loss of weight.

CONCLUSION

Serge RENAUD

The remarks I am going to make are based on what was written.
You reported before so, of course, we couldn't do a report on what has been shown this morning.
The Mediterranean diet and the coronary heart disease was the purpose of the presentation today.

One Mediterranean diet, per se, does not exist, since it is different and has different effects from country to country, even from region to region - this is the basis of what my colleague said and I think that it is quite important to understand. There was a discussion about that.

In a prospective study on 457,311 participants (men and women of the EPIC program) :
- a high intake of red meat, mainly processed red meat, was associated with an increased risk of fatal myocardial infarction,
- the protective effect of fish intake was not confirmed in this study,
- physical activity, alcohol intake and being a woman decreased the risk,
- and a decreased trend of fatal myocardial infarction was not observed with increased consumption of fruit and vegetables, except for leafy vegetables.

The concept of Mediterranean diet to prevent coronary heart disease mortality and morbidity, started in Italy (Napoli). This was after the Second World War when Ancel Keys and Paul Dudley White observed that the frequency of myocardial infarctions was much lower in the Napoli hospital than in the Boston hospitals, and that was really the starting point of all the studies.

A recent study which studied 32,000 women of the EPIC program in Italy, confirmed that the women with the greatest adherence to the Mediterranean diet had less than half of the risk of coronary heart disease than their controls.
In France Mediterranean region, many subjects depart from Mediterranean traditions. However, subjects at risk who changed to the Mediterranean diet improved their biological parameters and were maybe protected from coronary heart disease.

In the Lyon study with the Mediterranean diet, no sudden deaths were observed while there were 10 in the controls.
In the duplication in India of the French study, there were 16 sudden deaths in the controls and 6 in the experimental group attributed, as in Lyon, to alphalinolenic acid by inhibiting ventricular fibrillation.

Another type of fibrillation, much less severe, is atrial fibrillation, nevertheless a public health problem with the aging of the population. Trials have recently been initiated to evaluate whether this fibrillation will also be prevented by n-3 fatty acids.

The Lyon study, recently confirmed by an intervention trial and several prospective and case-control studies, has shown that dietary habits, easy to adopt, still compatible with a gourmet diet, cheap without side effects, protect from coronary heart disease clinical manifestations (non-fatal myocardial infarction and cardiac death) within days.

Conclusion of the conclusion :
Let us eat !
Mediterranean… or whatever,

but do not forget that it should include a higher intake of alphalinolenic acid, vegetables, fruit and cereals, even a moderate amount of wine.

Thank you very much for your attention.

Origin and interest of the alkalinizing effect of plant foods

Christian REMESY, Houdah SABBOH, Christian DEMIGNE
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Compared with the stone age diet, the modern human diet is excessive in NaCl and deficient in fruit and vegetables that are rich in K^+ and HCO_3, yielding organic anions like citrate. Consequently, a large part of the foods we daily consume has slightly acidic characteristics. The mismatch between the modern diet's makeup of these electrolytes and the still ancient renal capacity to process them gives rise to NaCl overload, low grade potassium deficiency and low grade metabolic acidosis. (Frassetto et al., 2001). The pH threshold under which acidity is perceived as aggressive is relatively low, in the range of 3-4. In fact, this acid perception is conditional because it may be modulated by other constituents of the food, such as cations or simple sugars. This acceptance of acidic foods is probably an inherited feature and might reflect the fact that acidic foods are generally better preserved, from a nutritional as well as from a safety point of view, compared to foods showing neutral or even slightly alkaline characteristics. In most cases, foods acidity reflects the presence of organic anions, mostly metabolisable (such as malate, citrate or lactate), together with some inorganic anions, chiefly phosphate.

The major part of organic anions is absorbed in the digestive tract and they are metabolised in various tissues, especially in the splanchnic area, and finally yields CO_2 and energy. Since they are present under partly neutralised forms, mainly as K salts, they may be considered as virtual precursors of $KHCO_3$, an important alkalinising chemical species especially for kidney function.

In the present review we will document the various sources of organic anions liable to be absorbed with staple foods (therefore available in the digestive tract lumen), as well as the different possibilities of in situ generation of some anions from other diet constituents (especially carbohydrates). The metabolic impact of these various anions will be examined, particularly as regards the control of the acid-base equilibrium in the body, and the various physiopathological consequences which might arise from disturbances of this equilibrium.

ORIGIN OFTHE ORGANIC ANIONS ABSORBED FROM THE DIGESTIVE TRACT

Anions :

Except ripped cereals, intrisically poor in organic anions and, to a certain extent legumes, most of the plant foods contain substantial quantities of organic anions, from 100 mg/100 g fresh weight (f.w.) up to 4000 mg/100 g f.w., for the richest sources such as citrus fruits. In terms of molar concentrations, these values represent 0.5 to 20 mmol citrate units/100 g f.w (Fig. 1). Malate and citrate are polycarboxylic anions (2 and 3 carboxylic groupings, respectively) but, at the acidic pH prevailing in plant products, they are partially neutralised by cations (for example, malic acid in apples is virtually under a [K-malate]1- form). These organic anions are frequently intermediate metabolites of the tricarboxylic cycle but, in some foods the prevalent anion may be different, for example oxalate (red beet, spinach, rhubarb) or tartrate (grapes). Besides these anions, relatively low concentrations of additional organic anions may be found, such as fumate or succinate, as well as various phenolic acids (quinate, ferrulate, caffeate, chlorogenate). Malate and citrate anions are frequently present simultaneously in a large variety of plant foods, but malate is the predominant anion in some fruits and vegetables (apples, cherries, plums, egg plants, cucumbers), whereas citrate is the major anion of citrus fruits, kiwi and solanaceae (potatoes, tomatoes).

Some anions, generally present in small amounts, are likely to exert pharmacological effects :

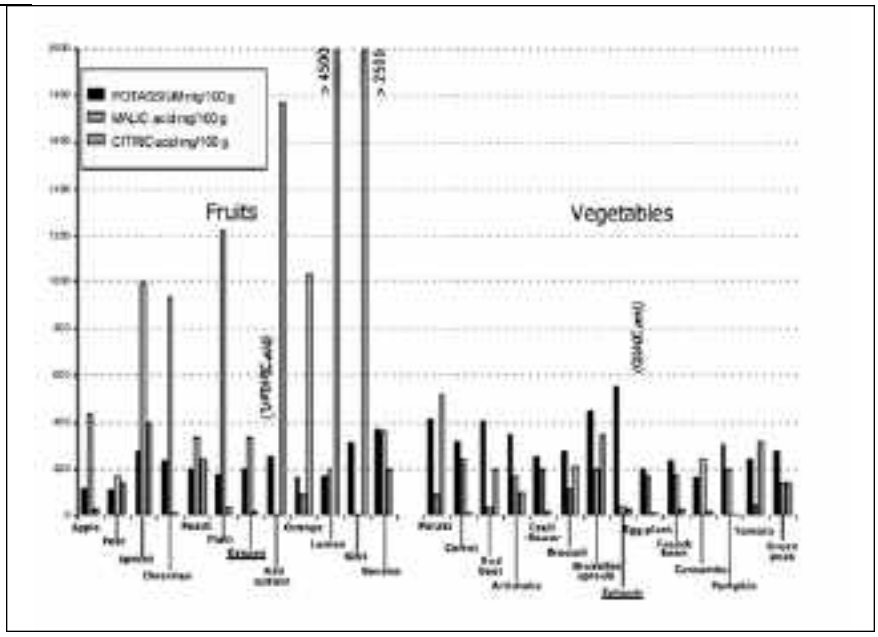

Figure 1 :
Composition in malic and citric acid, and in potassium, of some examples of usual plant foods (in mg/100 g fresh weight). The presence of particular organic acids (oxalic or tartaric acid) is mentioned for grapes and spinach.

Glucarate is found in plant foods such as cabbages, tomatoes, grapefruits or apples : this compound is a precursor of D-glucuro-1,4-lactone (1,4-GL), an inhibitor of b-glucuronidases which could promote the detoxification of carcinogens and other promoters of cancerisation through a decreased hydrolysis of their glucuronides (Walaszek et al., 1997). Cholesterol-lowering properties have also been ascribed to 1,4-GL (Yoshimi et al., 2000). Hydroxycitrate, present in some far eastern fruits such as Garcinia, is known as an inhibitor of lipogenesis and has also been examined as a possible anorexigen but the trials were not conclusive (Heymsfield et al., 1998).

Accompanying cations :

The K content of fruits is generally between 2.5 and 10 mEq/(100 g fw) and that of vegetables is usually greater, sometimes higher than 15 mEq/(100 g fw). In fact, the most salient feature which distinguishes fruits and vegetables is the [K]/[organic acids] ratio (in Meq) : this ratio is basically lower than 0.5 in most of fruits whilst it always exceeds 1 (up to 2.6 in pumpkins) in vegetables. In other terms, organic acidity of vegetables -even if somewhat lower than in fruits- is more completely neutralised by K ions and vegetables exhibit a greater alkalinising potency. Yet, it must be noted that, in each plant food group, some show an atypical composition, which has some nutritional and culinary background. A fruit such as banana exhibits a [K]/[organic acids] ratio > 1 (coherent with the possibility to use bananas as vegetable) and this ratio is much lower than that of most of the other vegetables for tomatoes, likely reflecting their possible utilisation as fruits (for juice confectionery, for, example).

The daily food supply of organic anions is obviously dependent on fruits and vegetable intake. It could be in the range of 1-2 g/d in low-plant food consumers, and easily reaches 3-4 g/d in subjects consuming a diversified omnivorous diet and more than 5 g/d in vegetarian people. These values of intake are close to those for K, which is consistent with the fact that organic anions are mainly K salts, but it must be kept in mind that K is also present in food products of animal origin.

Influence of cultivar, maturity or food processing :

The degree of maturity influences the concentrations of organic acids in plant foods, for example citric acid in Citrus fruits accumulates during the fruit development but tend to decline at the stage of maturity. Food processing such as boiling is probably a cause of organic salt losses, especially for vegetables since fruits are generally consumed uncooked. This point is still incompletely documented, but there is little doubt that steam cooking or brief frying are likely to maintain greater concentrations of K malate or citrate than cooking procedure leading to extensive leaching (Table 1).

Table 1 : Influence of the cooking process on the potassium level in potatoes.

Treatment	concentration (mg/100 g food)
Uncooked	530
Boiled	330
Oven cooked	540
French fries	700
Chips	1190

ABSORPTION AND METABOLISM

Most of the organic anions from foods are effectively absorbed in the small intestine, or even in the stomach by diffusion if the protonated form of these chemical species is lipophilic (for example acetate or butyrate). Citrate, as well as other intermediates of the tricarboyxlic cycle (succinate, a-ketoglutarate and probably malate) are transferred into the enterocytes through a secondary active transport requiring Na+ movements across the plasma membrane (Na-dependent dicarboxylate transporter). Various transporters of this type have been identified in the digestive tract, including the colon ; these carriers differ in their Km for substrates, from about 1 mmol/L down to a few ten of mmol/L(Pajor, 1999). The coupling of di- or tricarboxylate anions with that of Na^+ has been used in oral rehydrating formulations designed for treatment of diarrhoea. Other dietary anions such as lactate are also readily absorbed, through a relatively specific process since absorption of the L-isomer is markedly faster than that of its D-counterpart.

The other organic anions (oxalate, tartrate, phenolic acids) in the diet are generally less absorbable than malate, citrate or lactate, and the capacities of metabolisation in the body's tissues -hence of HCO_3^- generation- are very low and the absorbed part will be essentially channelled towards kidneys excretion. Oxalic acid is abundant in a limited group of plant foods (red beet, spinach and rhubarb) and its absorption exhibit some particularities (i) dependent on the dietary Ca availability, (ii) favoured in the colon by acidic fermentation's, together with possibilities of complete breakdown by the host microflora and (iii) possibly balanced by an opposite secretion of oxalate into the intestinal lumen (Hatch & Freel, 1995; Albihn & Savage, 2001). Hautmann (1993) has also reported that the stomach could be a critical site for intestinal oxalate absorption. Tartarate is essentially found, under its natural L(+) form, in grapes : this anion is partly absorbed from the digestive tract: it has been shown that bacterial colony metabolise the bulk of ingested tartarate in humans, and only 14% of ingested tartarate appears unchanged in urine (Chadwick et al., 1978). The other organic anions present in lesser amounts in plant foods (quinate, ferrulate, caffeate, chlorogenate) are part of the phenolic acids group, that may be absorbed at different levels of the digestive tract (small intestine, colon), and are then excreted by kidneys under various conjugated forms.

In fact, whatever the pathway for the metabolism of organic anions, their carbons are finally oxidised to CO_2 (in equilibrium with circulating HCO_3^- in extracellular fluids) at shorter or longer term. Insofar since these anions are present in foods as K^+ salts, which digestibility is generally high, it can be assumed that the end-product of their metabolism is HCO_3^-.

NUTRITION AND ACID BASE EQUILIBRIUM

Acidifying characteristics of the different types of foods

During their complete oxidation, carbohydrates or lipids do not generate unmetabolisable acidity, even if their partial oxidation (into lactic acid or ketone bodies respectively) may result in metabolic acidosis when it becomes excessive. In contrast, proteins contains various amino acids which catabolism is liable to affect the acid base equilibrium. The impact of the oxidation of proteins on this equilibrium is not unequivocal : basic amino acids ('cationic', such as lysine, histidine or arginine) yield various metabolites together with H^+ and sulfur amino acids (methionine, cysteine) are also acidogenic because they yield sulfate anions (unmetabolisable acidity), whereas the oxidation of the dicarboxylic amino acids ('anionic', such as glutamate or aspartate) 'consumes' acidity. Even if some proteins (for example rich in neutral and anionic amino acids) could theoretically be alkalinising, it remains that most of the dietary proteins (especially those well balanced for their amino acid composition) are acidifying (Remer & Manz, 1995).

Evaluation of the acidifying potential of the various foods

Basically, a food or a diet which provides of an excess of inorganic anions (or fixed anions) such as Cl^-, $HnPO_4^{(3-n)-}$ or SO_4^{2-}, compared to inorganic cations (Na^+, K^+, Ca^{2+}, Mg^{2+})

will be ascribed acidifying properties, in keeping with the classical 'dietary ash hypothesis'. The net excretion of acidity by kidneys is the referred to as :
(Cl + P+ SO_4 + organic anions)-(Na + K + Ca + Mg).
The urinary excretion of SO_4 and all the other ions may be estimated from data of food intake and food composition tables, using specific absorption coefficients, for example : proteins 75%, P 63%, Cl 95%, Na 95%, K 80%, Ca 25% and Mg 23%. In addition, the valences (2 for Ca and Mg) and the ionic charge of P at physiological pH (1.8) are also taken into account as well as, for proteins, an average percentage of methionine (2.4%) and cysteine (2.0%) (Remer, 2000). Practically, the above calculation may be simplified by considering that Na and Cl are essentially provided as NaCl in most foods.

According to these assumptions, it is possible to estimate a "Potential Renal Acid Load" (PRAL) for various foods, which ranges between 34 mEq/100 g (parmesan) and a -21 mEq/100 g (grapes). As a general rule, fruits and vegetable display negative PRAL values, milk and yoghurt are close to 0 and meat, fish, poultry and cheese (and some cereal products) exhibit positive PRAL values (Remer, 2000) (Fig. 2). At the scale of a complex diet fed during several days, renal acidity excretion may change from around 10 mEq/d up to 60-70 mEq/d during the transition from a lacto-ovo-vegetarian diet to an omnivorous diet moderately rich in proteins, with minute differences between the measured values and values obtained through the calculation of the PRALof consumed foods, whereas in parallel the urinary pH (6.7 in the initial conditions) may drop down to 5.9 (Remer & Manz 1995). These urinary balances also depend on the rate of organic anions excretion (especially citrate) which is relatively constant for each individual.

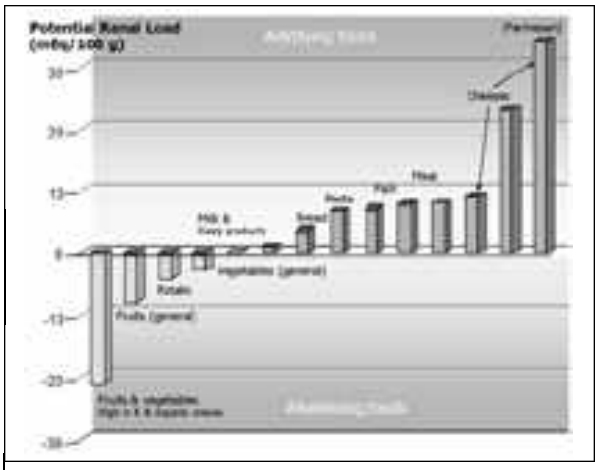

Figure 2 :
Potential Acid Renal Load values (in mEq/100 g food) for the major categories of foods (adapted from Remer & Manz, 1995 & Remer 2000).

Excessive dietary proteins from food with high potential renal acid load adversely affect bone (each additional gram of dietary protein results in an additional loss of >1.75 mg calcium per day), unless buffered by the consumption of alkali-rich foods or supplements (Barzel & Massey, 1998 ; Heaney, 2001). In 1992, Abelow et al. reported that the incidence of hip fractures in women aged 50 years and older correlates positively with a country's average annual per capita consumption of animal protein. In fact, the decisive risk factor for hip fracture would not be the rate of production of fixed acid from animal protein but the net rate of endogenous acid production, when all sources of dietary acid and base are considered. Thus, a vegan diet with protein derived equally from grains and legumes would deliver at least as many millimoles of sulfur per gram of protein as would a purely meat-based diet (Heaney, 2001), but it yields significantly lower rates of net endogenous acid production than do mixed animal and vegetable diets. Data from Frassetto et al. (2000) and Sellmeyer et al. (2001) strongly strengthen the generalisation of a worldwide association of hip fractures in women with animal protein consumption. Besides, although protein was associated with an increased risk of forearm fracture for women who consumed more than 95g per day, Feskanich et al. (1996) did not found any association between adult protein intake and the incidence of hip fracture and it has to be said that low protein intake may compromise bone quality, especially in the elderly, as well (Bonjour et al., 1997). In fact, the influence of dietary protein on calcium retention is complex, possibly transitory (Roughead et al., 2003), and liable to be modified by other nutrients in the food and total diet (Massey, 2003).

Role of potassium and sodium

The addition of exogenous buffers, as chemical salts (or as fruits and vegetables) to a high protein diet results in a reduction in net acid excretion and decreased calcinuria (Barzel & Massey, 1998). Metabolic balance studies have shown that restoring intakes of K^+ and HCO_3^- to levels approaching those in the diet of our prehistoric forebears with either supplemental $KHCO_3$ or with fruits and vegetables can alleviate hypertension, prevent kidney stones and protect against the occurrence of osteoporosis (Morris et al., 2000), whereas low K intakes increases daily and fasting urinary calcium excretion rates (Lemann, 1999). This calcium-sparing effect occurs through reduced bone resorption and increased formation (Sebastian et al., 1994), whether the potassium salt is citrate or bicarbonate. Indeed, commonly complex dietary proteins with a high phosphorus content (Spencer et al., 1988) or consumed together with $KHCO^3$ supplementation (Sebastian et al., 1994) do not cause calcium loss in adults. In the Lutz (1984)'s study, subjects had a similar calcium balance, when consuming either the higher protein diet (102g) plus bicarbonates or a moderate protein diet (44 g).
Humans have a very low need for salt but became addicted to the taste of salt as a consequence of its widespread and essential use as a food preservative. There is evidence that the range of usual dietary intakes is associated with urinary Ca loss, leading to adverse effects on bone metabolism (Shortt & Flynn, 1990), because renal clearance of both minerals is linked in the proximal renal tube. Even if calcium homeostasis is normally well regulated (such that increased calcium loss leads to a better calcium absorption from the gut), the duration of this adaptative process may not be sufficient. This is why only 3% of the variance in urinary calcium excretion could be explained by calcium intake (Matkovic et al., 1995). It has been estimated that a 100 mmol increment in daily Na intake is associated with an average loss of urinary Ca of approximately 1 mmol in free-living normocalciuric healthy populations (Nordin et al., 1993). Thus, if uncorrected, the extra sodium would lead to a skeletal loss of about 1% per year (Weaver et al., 1999). However, the limited studies which have investigated such an association with bone mineral density in humans have produced conflicting results. Actually, Ginty et al. (1998) have shown that adaptation of absorption may compensate for increased urinary Ca loss, at least in healthy young women. Results from Greendale et al. (1994) do not support either a detrimental effect (prospective study from the Rancho-Bernado cohort). Nevertheless, other studies in adults implicated Na as a risk factor for urinary calcium loss (Massey & Whiting, 1996), bone loss (Devine et al., 1995) and high bone resorption (Jones et al., 1997)

PHYSIOPATHOLOGICALASPECTS

Because urinary excretion of acid is insufficient, other

homeostatic systems, such as bone, are involved to buffer the excess dietary acid load (Barzel, 1995; Bushinsky, 2001; New, 2002). The skeleton serves as a substantial reservoir of labile base in the form of alkaline salts of calcium which can be mobilised to defend blood pH. Indeed, 80% of total body carbonate is in the hydratation shell, the water surrounding bone, as are 80% of citrate and 35% of sodium (Green & Kleeman, 1991). Osteoclasts and osteoblasts respond independently to small changes in pH, a light drop in pH causing a burst in bone resorption (Kriegger et al; 1992 ; Arnett & Sakhaee, 1996). Further metabolic acidosis even blunts the capacity of growth hormone (GH) to stimulate the systemic production of insulin-like growth factor I, which along with GH is a major determinant of bone mass (Green & Maor, 2000). Bone plays also a major role in the storage of phosphate, the phosphate buffer system being very important to relieve itself from fixed acid loads and to enable maintenance of pH within restricted limits (Pautard, 1961).

Thus, a negative calcium balance implies depletion of bone mineral. Actually, over 25 years ago Wachman & Bernstein, (1968) suggested that lifetime buffering of the low-level acid loading and metabolic acidosis that occur in humans eating ordinary diets was sufficient to impose a chronic demand for base of skeletal origin (Lennon et al., 1966). If bone is mobilised to buffer only 1 meq of acid each day, 15% of the total body calcium in an average person is lost in a decade (Barzel & Massey, 1998). This is why Nordin et al. (1987) suggested that age-related bone loss may be more attributable to excessive calcium loss than to inadequate calcium intake.

Chronic metabolic acidosis due to excessive intakes of sulfate and chloride anions increases with age at constant endogenous acid production, apparently due, in part, to the normal age-related decline of renal function (Frassetto et al., 1996). Thus, though mild, it leads to high losses of calcium in the urine and has been implicated in the pathogenesis of the physiological disturbances and degenerative diseases characteristic of ageing such as osteoporosis (through enhanced bone resorption) (Lemann et al. 1986 ; New 2002). Indeed, experimentally induced chronic metabolic acidosis by acid loading induced loss of bone mass (Barzel, 1969). In vitro, short term metabolic acidosis (3 h culture) elicits a Ca efflux, secondary to physiochemical bone mineral dissolution, while over 24h it results from cell-mediated bone resorption (Bushinsky, 1995) because, actually, acidosis causes a greater release of K than Ca and osteoclastic function is necessary to support this phenomenon (Bushinsky 2001).

Conclusions on the role of fruit and vegetables

Clearly, dietary factors affecting the amount of calcium lost in the urine have a major influence on calcium balance and may even be more important than those that modulate the intestinal availability of calcium. This is why the inevitable loss of calcium in urine is greater for Western-type diets that are high in unfavorable factors such as animal proteins, sulfates, sodium, coffee (Heaney & Recker, 1982; Guéguen & Pointillart, 2000). Critical determinant of hip fracture risk in relation to the acid-base effects of diet is the net load of acid in the diet. Thus, it is worthwhile to consider decreasing the rate of bone attrition by the use of a diet favouring alkaline ash. This type of diet would emphasize the ingestion of fruits and vegetables (Frassetto et al.,1996) because they are rich in bicarbonate and in organic anions that can be metabolised to bicarbonate (Remer & Manz, 1995). New et al. (1997, 2000) report that fruit consumption (which has a high potassium and magnesium content) predicted greater bone density at all 4 bone sites measured in postmenopausal women. And when potassium was considered, calcium intake was no longer significantly related to bone mass. Tucker et al. (1999) have shown, as well, in a cross-sectional and longitudinal study that alkaline-producing dietary components such as potassium contribute to the maintenance of bone density. An increase in fruit and vegetable intake from 3.6 to 9.5 daily servings decreased urinary calcium from 157 ± 7 to 110 ± 7 mg/d vs a drop of 14 ± 6 in controls (Appel et al., 1997). In conclusion, accordingly, greater attention needs to be given to eliminate the causes of calcium loss, which, in turn, should lower calcium requirement. Indeed, urinary losses remains an important and inadequately appreciated aspect of calcium nutrition. Adiet may be inadequate in calcium not simply because it is absolutely low but also because it is insufficient to offset prevailing excretory losses.

From a practical point of view, a supply of K in the range of 2-2.5 g/d as fruits and vegetables supposes a substantial intake of these plant foods, namely 0.6-0.8 kg/d (assuming a mean concentration of 3 g/kg fresh weigh). Such a supply would be possible in the framework of the ’10 per day’ recommendations for fruits and vegetable consumption, with servings of an average 80-100 g weight. These recommendations could seem high and not realistic, but it must be kept in mind that they arealready fulfilled by a noticeable percentage of the population, including not only vegetarians but also a fraction of omnivorous subjects.

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INTRODUCTION

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Understanding the contribution of the Mediterranean diet to health and longevity is complicated by the fact that we may not consider the Mediterranean diet as one single entity, but rather several dietary patterns with common features that are interweaved with, first, complex non-dietary Mediterranean life style patterns and, second, Mediterranean genetic profiles that differ from neighboring regions and other parts of the world.

Life style differences across Europe are illustrated with data from the EPIC study and translate into variation in, for example, smoking prevalence, physical activity, obesity, intake of folate, intake of alcohol and dairy products.

Genetic variation may modify the effect of life style and diet on health and longevity, and some of the variation in life style and diet may be due to genetic differences. We will briefly illustrate such phenomena with examples of variation in single nucleotide polymorphisms related to the metabolism of folate, alcoholic beverages and milk. The concept of 'Mendelian randomization' will be introduced and its usefulness in future work on diet, life style, genetic variation and health discussed.

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Diet and genes : interactions in the aetiology of chronic diseases

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Thank you very much, Mr. Chairman. I would also like to thank the organising committee, especially Saida Barnat, for inviting me to give this lecture on diet, lipid metabolism and genes, specifically regarding the interaction between diet and genes in the aetiology of cardiovascular disease.

As mentioned by the chairman, there has been a huge diversity in diet that can be traced back through human development from the ancient stage, the Palaeolithic stage, to the modern stage. There has been a trend in dietary pattern during the last century, especially in Western countries. The large diversity in dietary patterns is now well known.

When looking at the human genome there is an overall general stability. Our genome has not changed a lot over the past 7 million years. Some ethnic differences can be found for various traits – for example, it has been found that African-American children are more susceptible to cardiovascular disease than white American children.

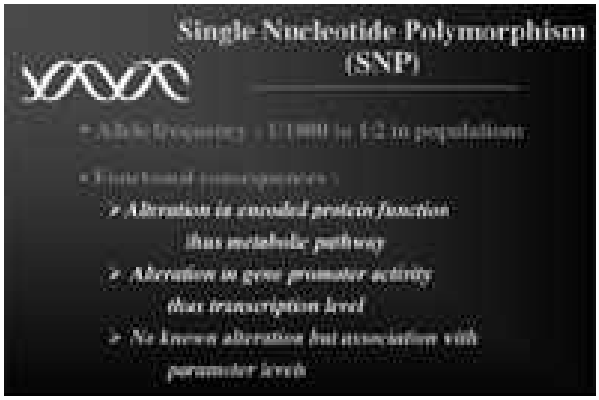
At the subject level, an important number of genetic polymorphisms can be found. For example, more than 200 SNPs (Single Nucleotide Polymorphism) have been found regarding about 15 key proteins involved in lipoprotein metabolism.

Some general knowledge has been obtained over the last decade regarding the metabolic response to diet. To summarise, we know the different effects of plant and animal proteins on lipid metabolism, the effect of carbohydrates with different glycaemic indexes on insulin responses or hypertriglyceridemia, the effect of soluble fibre on cholesterolemia and on glycaemic/insulinic indexes, the well-known hypercholesterolemic effect of saturated fats and LDLcholesterol. We also know for a fact that dietary cholesterol can increase cholesterolemia and LDL cholesterol in a part of subjects only.

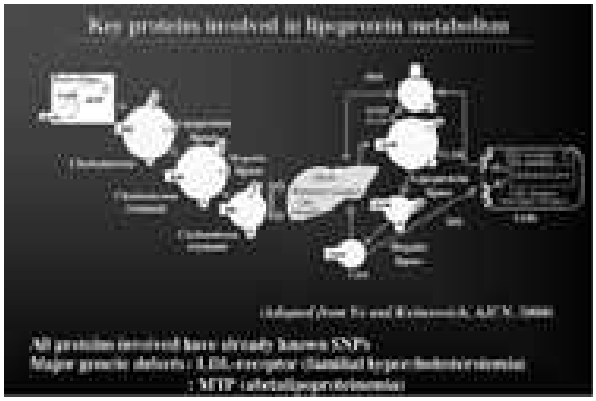
Nevertheless, people doing nutritional intervention in humans know very well that there is always a very large inter-individual variability. When doing nutritional intervention on humans, most subjects behave according to the main trait, but with more or less amplitude in their variation. A minority of subjects do not show any change on the same diet, and a few subjects can show an opposite trend. This leads to the general concept of the interaction between diet and genes.

I will focus on lipoprotein metabolism and cardiovascular risk. I will first show you a few examples of some known lipoprotein metabolism SNPs. Secondly, I will show you some preliminary data obtained from the Medi-RIVAGE study, which is presented elsewhere, where we studied the interaction between SNPs and responses to diet.

I will begin with some basic information about polymorphisms. In general, most of our interest in polymorphisms is directed towards single nucleotide polymorphisms (SNPs). This is, in fact, a very tiny change in the gene sequence. Only a single nucleotide in the gene sequence is changed and this, can or cannot, change the primary structure of the protein encoded by the gene Very importantly, the allele frequency of this change on one or two DNAstrands is very variable. In some cases, the SNP frequency is very low – 1/1000, or even less. In other cases, half of the population can have the so-called minor variant in their DNA.



The functional consequences of this SNP(the change in the gene sequence) can be very different. The most important change is due to the fact that a change in one nucleotide will change the primary structure of the protein. This can alter the function of the protein in some cases, which will then affect the metabolic pathway where the protein plays an important role. In other cases, this SNPis not in the encoding sequence of the gene , but in the promoter activity of the gene. This is modulated by different factors, thus stimulating or repressing the transcription of the gene and thus modulating the level of the protein in the tissue. This can alter the metabolic pathway concerned.
In other cases, there is no known direct relationship between a given SNP and a protein function. However, there can exist a known relationship between the SNPand some parameter levels, meaning that further knowledge is needed to identify the relationship.



This is a brief summary of lipoprotein metabolism in order to introduce some key proteins, which we will discuss a little later.

In the fasting state, i.e. before breakfast this morning, your liver was secreting very large-density lipoproteins, which are

triglyceride, cholesterol-rich lipoproteins (VLDL). These contain some apoproteins, especially ApoB100, ApoE and ApoC3.

Thanks to the action of 2 enzymes (lipoprotein lipase and hepatic lipase) present at the cardiovascular vessels, there is hydrolysis of the triglycerides leading to Intermediate-density lipoprotein (IDL) and then, the so-called low density lipoproteins (LDL), which are accumulating in the fasting plasma. Most circulating cholesterol is present in this type of particle.

These particles can then be taken up by the liver through a specific pathway, or by peripheral cells and tissues through different pathways. When LDL accumulate in the arterial wall, this is one of the early key step in atheroma deposition.

The liver is also able to secrete high-density lipoproteins (HDL). These are protective because they can take out cholesterol from the peripheral tissue and bring it back into the particle and then to the liver, which will be the main way of excreting this cholesterol from the body through the bile secretion.

Whilst having breakfast this morning, you ingested lipids, which have now been digested in your digestive tract. Many proteins are involved in the small intestine. These proteins manage the very large flux of lipids and cholesterol in your small intestine and drive the lipids into the circulation.

Some proteins are binding-proteins, like the intestinal fatty acid binding protein (I-FABP) which is involved in the trafficking of lipids, and the MTP, a very important protein for packaging of lipids in order to make particles that will be secreted into the circulation. These ones are very large triglyceride-rich particle containing cholesterol called chylomicrons.

These particles will transiently accumulate in the circulation, but hydrolysis of triglycerides present in the particles will lead to chylomicron remnants. These also contain some proteins on the surface, especially the ApoB48 protein that is a typical marker for the small intestine particles. These particles will finally be taken by receptor pathways in the liver and by some peripheral tissues, especially the artery wall.

For all these proteins I have mentioned, we know of some polymorphisms. In some cases, these gene changes are very dramatic. For instance, two very important diseases are directly linked to such changes. The well-known familial hypercholesterolemia, which is passed from parents to their children, is now known to be linked to a defect in the LDL receptor present in the liver; because the uptake of LDL is markedly decreased in the liver, the level in the circulation is higher and thus leading to you a higher risk for cardiovascular disease.
This is clearly a monogenic genetic disease. In some rare patients a mutation in MTP leads to inability of liver and small intestine to package lipoproteins and thus to secrete chylomicrons. This genetic disease is called abetalipoproteinemia.

In most people, genetic polymorphisms in various genes can only have discrete, but significant, metabolic effects. I will show you a few examples of the effects of such SNPs on some of the proteins.

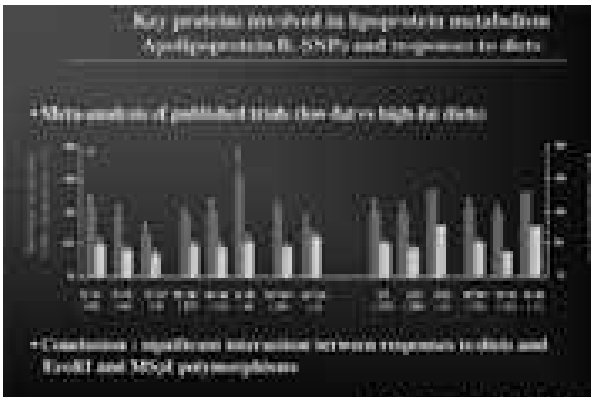
One of the most studied SNPs in lipoprotein is the case of apoliprotein E, which is on the surface of these particles. It is very important in the clearance of these particles from the circulation. The best-known polymorphisms are at residues 112 and 158 on the primary protein structure with three major alleles, E2, E3 and E4. As you can see, 80% of the population has an E3 form, while 10% has an E2 form and 14% has an E4 form.

When the so-called normal form – E3 – is present, the uptake of IDL in the liver is normal, the uptake of the LDL particle is normal and the LDL cholesterol level in the plasma is normal. When the E2 form is present, it stimulates the uptake of LDL particles by the liver due to a reduced affinity for the IDL liver receptor, thus decreasing the level in the plasma. The opposite is observed with the E4 form, thus leading to an accumulation of LDLin the plasma and therefore causing hypercholesterolemia.

An example in reference to diet is alcohol. Here, they compared healthy male and female drinkers and non-drinkers. The lowest LDLcholesterol level in men and women have been observed in E2 carriers. There is a dramatic difference in changes in plasma cholesterol upon alcohol drinking in men or women with the different alleles.

Another important protein is apolipoprotein B, which is the main protein on LDL particles with a number of best-known polymorphisms from this protein with variable frequencies. One can be found in almost half of the population while another is much less frequent.

In general, the polymorphism that occurs in the signal peptide region of the gene alters the ApoB level, while the other polymorphisms are generally associated with LDLcholesterol in the circulation.



A recently published meta-analysis has reviewed the effects of apoB polymorphism on the change in LDL cholesterol in subjects changing from a low-fat to a high-fat diet.

There are changes in LDL cholesterol levels depending on the type of diet, which is well known. However, the amplitude of change is different depending on the type of polymorphism present in the subject. The conclusion of the meta-analysis is that the significant interaction between LDL response to a high fat diet and two polymorphisms should be considered.

Another key protein is apolipoprotein ApoA4, which is specific to the small intestine and associated with chylomicrons entering the circulation after fat digestion. Two common SNPs have been identified on the ApoA4 gene. The result in the case of the 1 2 SNPis an increased affinity of the A4 for lipids, thus leading to increased competition for the binding of all other apoprotein and lipoprotein particles. This was associated in women with higher fasting triglycerides and lower LDLcholesterol in homozygotes. These polymorphisms are also related to a higher LDLincrease on a high-fat diet.

Another interesting example is the intestinal fatty acid binding protein (I-FABP) that plays a key role in the lipid moving from the small intestine to the circulation. An important SNPhas been found in this protein. It was first discovered in the Pima Indians with a frequency of about 30%. The same frequency has been

found later in European students. It is therefore not specific to Pima Indians. This SNPleads to three genotypes.

For instance, in vitro, the minor variant of the I-FABP has a higher binding affinity for the fatty acids. Using an intestinal cultural model, the minor variant induces an increased triglyceride ApoB synthesis and secretion from intestinal cells.

In humans, this polymorphism has been associated with higher fasting insulinaemia, insulin resistance and fat oxidation. In other studies, it was found to be associated with higher LDL cholesterol in men and women. It is also associated with higher BMI and triglyceride.

Here is an example of the response to diet. In a study, an increased postprandial insulin response was found in the homozygote carrier of the minor variant. Also there was a striking postprandial accumulation of triglyceride in the circulation after a test meal in the carrier of the minor variant. This is also very detrimental in terms of being a risk factor for cardiovascular disease.

Another example can be taken from a key enzyme involved in lipoprotein metabolism i.e. lipoprotein lipase. This enzyme ensures the clearance of lipids from the circulation. Some SNPs have already been identified in the gene sequence of the protein with different allele frequencies in the population.

In general, it has been shown that these polymorphisms are associated with reduced LPL enzymatic activity and increased plasma triglycerides, which results in a reduction in HDL cholesterol level.

Finally, it has also been shown in other studies that another LPL polymorphism is associated within the total cholesterol response to some diets. In the postprandial state, carriers of this minor variant show higher triglyceride-rich lipoproteins accumulation in the circulation.

I would now like to show you some preliminary data we have collected in the Medi-RIVAGE study. The literature review made supports the concept that SNPs can influence metabolic response to diet. The protocol design of the Medi-RIVAGE intervention study is presented elsewhere. A certain number of polymorphisms have been determined in all subjects of the cohort such as: ApoE, ApoB, ApoCIII, ApoAIV, intestinal fatty acid binding protein, MTP, CETPand HL. Other polymorphisms are under investigation.

In general, the subjects in the two arms (Mediterranean diet or prudent-type diet). have been pooled. In some other cases the two groups are shown separately.

We observed a greater reduction in cholesterolemia in the carriers of the two variants ApoE3/3, which is the normal variant and ApoE 2/2 allele. Additionally, the reduction in triglyceridemia in the subjects is more marked if the subject is a carrier of the two e2 alleles. We did not find any influence of the SNPs on the postprandial triglyceride response.

Thus, in subjects with an e2 and e3 allele, i.e. about 18% of the subject population, a better lowering of triglyceride and cholesterol is observed.

Another example is the ApoA4 polymorphism regarding LDL. We found a significantly more important LDL cholesterol reduction in the T carriers. When looking at the sub-cohorts separately, the T carriers show a much greater reduction on the Mediterranean diet compared to the low-fat diet. This indicates that in this case, the carrier of the minor variant (15% of the cohort subjects) is associated with better lowering of LDL cholesterol with some specific interaction with the Mediterranean diet.

Fortunately, as mentioned also by our chairman, we did not find any differences in the allele frequency of polymorphisms in this study. The difference is not due to a different number of subjects.

Another example is MTP polymorphism. We observed that the T/Tcarriers show a more significant decrease in cholesterolemia than the rest of the subjects. The same has been observed for triglyceridemia, with a threefold lower reduction in T/T homozygotes.

When looking at the two sub-cohorts separately, it was striking to observe that the T/T carriers showed an impressive reduction under the Mediterranean diet which was greater than the reduction observed after the prudent-type diet.

Thus subjects with the MTPT/T(10% of the cohort) show better lowering of plasma cholesterol and triglycerides. There is some possible specific effect of the Mediterranean diet.

Finally, the example of CETP polymorphism's shows that the subjects homozygote for the B2 variant have a significantly greater reduction in LDLcholesterol on both diets. This was also the case for the reduction in triglyceridemia on both diets. These subjects represent 23% of the cohort.

In other cases we have not found a relationship between polymorphisms and some metabolic parameters (ApoC3, hepatic lipase).

In conclusion, in the Medi-RIVAGE study, we found that in this rather small cohort some gene polymorphism's are associated with different metabolic responses to dietary intervention in the fasting or postprandial state. We are searching for the interaction with other SNPs.

A very important future goal would be to use more complex statistical analysis to look at the interaction between different SNPs. A given subject can have different types of SNPs and not only one SNP.

Gene-interaction is a key emerging field. Given the huge number of polymorphisms potentially available, we need to identify the key relevant SNPs implicated in lipid metabolism and cardiovascular risk.

Finally, as mentioned by our chairman, we can expect that better knowledge in this field in the near future could help to identify specific groups in the population who could benefit from specific recommendations, given their specific susceptibility to dietary compounds. Maybe this could also be envisaged for individual subject advising, given we can expect to have a genetic information in the close future.

Questions

Elio RIBOLI
Thank you, Doctor Lairon, for an interesting presentation.
We can now take questions for both Dr. Vollset and Dr. Lairon: one covered a more theoretical area and the other a more practical area concerning gene nutrient interaction.
With the next presentation we will move to a different field.

Member of the Audience
You talk about individual lifestyles on the one hand, and on the other hand you mention that this could have public health implications. I can agree with the latter in a clinical context, but I think that the major implication of this work on a public health level is that we need to consume a varied diet in order to make up for all the possible deficiencies we may have in some of our metabolic roots. This may be a way to substantiate a varied diet and not picking out one nutrient as the major factor in a specific population.

Denis LAIRON
This is why we compare diets and not single nutrients in the Medi-RIVAGE study. An interesting issue in terms of public health is to be able to identify different propensities for SNPs in the population.
For example, as the chairman mentioned, we already know that some populations have a very high or a very low level of SNPs or polymorphisms for a given pathway. This is very important in terms of public health because we can then draw general recommendations given this knowledge. In other cases, the relevance in terms of public health is much weaker. However, this knowledge can be very important for certain sub-populations, such as those at risk of cancer or cardiovascular disease. For instance, there is a clear public health implication regarding ApoE for which we have a great deal of data. It is clear that people with ApoE2 and ApoE4 polymorphisms, who make up about a quarter of the population, benefit more from a Mediterranean or low-fat diet. These polymorphisms are associated with abnormalities in lipid metabolism. Our colleagues in hospitals and clinics now systematically screen for ApoE genotypes. As soon as they have a patient with a lipid abnormality they do a polymorphism check. As hospital genetic unit, they check for about 5,000 ApoE polymorphisms per year.
I think that this is a very important step in dietary recommendation.

Member of the Audience
I just want to comment on what Steinman said about Mendelian randomisation. If you think about the different SNPsearch strategies, the Mendelian perspective is a rather environmentalist approach – you select the dose because you know something about the metabolism in a given case.
This has been used in a study on the circulation of hybrinogin, although I do not know if it was called Mendelian randomisation in this case.
It is important to emphasise that Mendelian randomisation uses a small sample size, which can be very critical. Many of these studies are really, in fact, of small sample size. This elegant way of conducting studies offered by nature will only be viable in very large studies like EPIC, for instance.
This field of methodology will most likely evolve. Up until now it has not been used extensively.

Denis LAIRON
You have made a very good comment - methodology is indeed evolving. Over the last year, we were able to do about 50 polymorphisms using the traditional RFLP method.
With the new methodologies available, we will be able to do thousands of polymorphisms daily. This is already the case with the best automats. We will be able to check very huge cohorts. For instance, we can check 500 people for one polymorphism in a day and possibly many more in the future. This will open up the possibility for study on very large cohorts.

Childhood, obesity and physical activity

Anthony KAFATOS
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Thank you, chairman. Good morning Ladies and Gentlemen.

Today, the topic of my talk will be the relationship of physical activity and obesity in childhood.

The ancient people on this island during the Minoan era realised how important physical activity was in maintaining good body size and body composition.

In the Minoan frescoes, bull playing (not killing) was very popular with both men and women also indicating how very lean and athletic these men and women were.

Dancing was a very intensive physical activity. If you have the chance to observe some of these dances today, you can see that a lot of energy is expended.

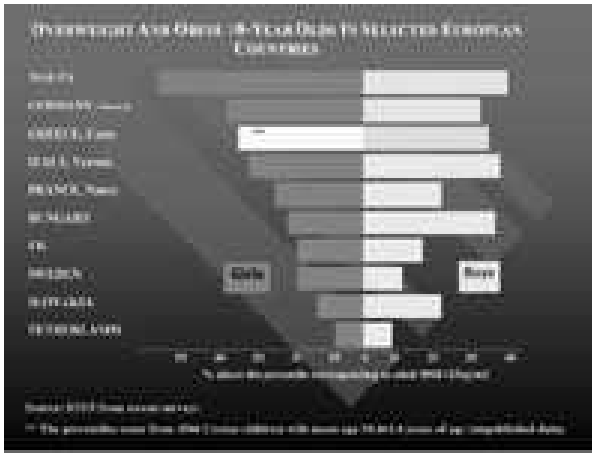
You can see that this Minoan palace Prince has a perfect body composition. The ancient painter seems to have exaggerated the waist circumference, which is very small. I asked my colleagues in the department of mathematics if they could measure the waist circumference. They explained that it could not be done without making numerous assumptions. Some parts of the painting were added by archaeologists over time.

The women were also very nice looking. They were thin and also had small waists. The breasts were a sign of breast feeding and mothering in contrast to the more anorexic models we see on television today.

Physical activity is a very important topic for young children. Physical activity leads to weight control and improved body composition by reducing blood pressure, raising HDL cholesterol, reducing the risk of diabetes and preventing some kinds of cancer and osteoporosis. It is during adolescence that we acquire our bone mass.

Physical activity is also important for psychological reasons. It leads to more self-confidence and higher self-esteem. There are also social benefits.

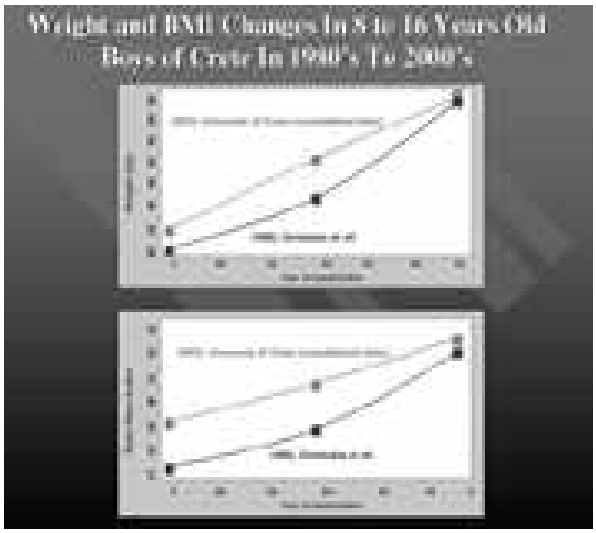
Unfortunately, there is a pandemic of obesity in adults and children. Greece has one of the highest percentages of obese children – almost 30% to 40% of them are overweight and obese. There are differences among countries.



Since the early 1970s to 1993, the percentage of obese and very obese children has risen in Japan.

If we look at a cohort of children in the early 80s from urban and rural areas in Crete and compare it with a cohort of children examined in Crete last year, you can see the difference in weight – weight is higher in recent years. The body mass index is also much higher in recent years.

The opposite was the case in the early 80s, with a much higher HDL cholesterol level compared to the children's HDL levels today, whereas LDLcholesterol was much lower.



If you look at the triglyceride level in children, it is much higher today in comparison to 23 years ago.

Overweight and obesity in children is related to a lack of physical activity, unhealthy eating habits, consumption of a great deal of high energy density foods (McDonalds and other similar fast-food chains), genetics and lifestyle.

A very sedentary lifestyle contributes to this problem. Children spend a lot of time in front of the television – 43% of adolescents spent more than 2 hours a day watching television and they spend much more at weekends.

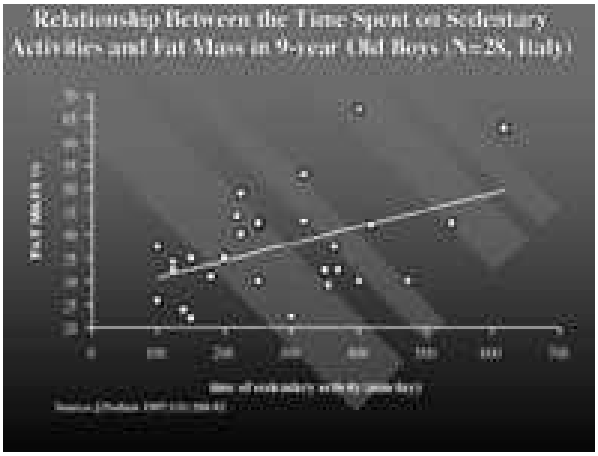
Children become less active as they reach adolescence. About 1 in 5 children in the United States, and 1 in 4 in Greece, are overweight or obese. The number of obese children between the ages of 6 and 17 has risen within the last three decades. Up to 50% of obese adolescents remain obese as adults. Parental obesity more than doubles the risk of adult obesity among 10-year-old children, whether they are obese or not.

We are presently investigating the role of genes in obesity, but there are over 250 genes related to obesity. The main problem is how to relate the genotype to phenotype. We have to do a long-term follow-up on children with certain phenotypes and genotypes to see how genes are related to this problem. There is certainly a lot of work ahead of us.

Children with overweight parents have lower levels of physical activity and their diets are higher in fat and lower in carbohydrates. Low levels of physical activity have been associated with cigarette smoking, marijuana use, lower fruit and vegetable consumption, longer hours TVwatching, and a poorer academic performance.

There are very few long-term studies on how obesity and physical activity are related to mental health. The Harvard adolescent study is one. It began in 1922 and examined mortality records in 1988. It was found that the levels of physical activity as adolescents related to an increased risk of all causes of death in men, but not in women – 1.8. The risk of coronary heart disease is 2.3 compared to 0.8 in women. The risks for cerebrovascular disease and colorectal cancer are also higher.

There was a small study in Italy which compared body fat mass to the rate of activity or inactivity. There is a clear relationship between the two.



There are only two or three primary prevention studies internationally concerning children. One of them is the CATCH study in the United States. They observed that the children in the intervention group have significant more moderate to vigorous physical activity in comparison to the control group. There was no correlation with the serum lipoprotein or obesity in this instance.

The Heart Smart study is another study on children. It concerned a small number of children over a short period of time. There is a correlation with the resting pulse rate in girls and in subcapular skin fold and right arm circumference. There is no actual correlation between HDLand other lipoproteins. The outcome is generally poor.

We looked at Cretan children and thought that if we went to an isolated village in the mountains to the South of the island, the lifestyle might be more traditional and the Mediterranean diet might be predominant. Unfortunately, the results show the opposite.

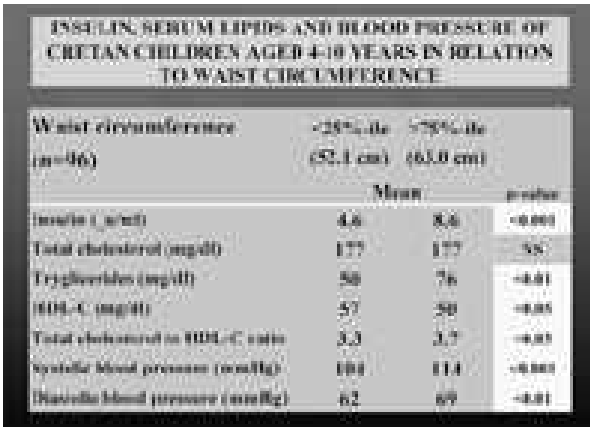
The children in this village have the same lifestyle as those in Athens or Heraklion. We looked at all 200 children aged 4 to 10 with various waist circumferences – from small to large. We observed that the insulin level in those with smaller waist circumferences (below the 25th percentile) is almost half of the levels found in those with larger waist circumferences (above the 75th percentile). The lipoprotein levels were significantly lower except for HDL, which was significantly higher in the low waist circumference group. In these small villages meat, hamburgers and slouvaki is eaten almost daily.

We decided to start a primary prevention trial with a cohort of 6,000 children entering elementary schools of Crete in 1992. They were all the same age – 6 to 7 years old. The control group was composed of 1,500 children in the Western part of the island near Chania. The areas of Heraklion and Rethimnon was the intervention group. This intervention package was teacher delivered and included teacher training on nutrition and health basics such as the importance of vegetables and fruits and physical activity. We also placed emphasis on traditional dietary patterns including the fasting rituals of the Orthodox Church. The package included a physical activity component and information about tobacco smoking and alcohol consumption. The study lasted ten years.

We compared the children's weight at base line (1992), the first follow up (1995), the second follow up when the children graduated from elementary school (1998), and four years after graduation (2002). The mean weight in the intervention group was four kilograms less than the control group. As a result the body mass index was significantly lower in the intervention group.

These results are very encouraging. This is the only international study which shows educational intervention as having an effect on the body mass index and also a discernible effect on lipoproteins.

The waist circumference to height ratio is significantly different in the two groups. Serum cholesterol is much lower in the intervention group. There is a general reduction of cholesterol at mid puberty in both groups because testosterone and oestrogen levels are increasing, but the reduction was much greater in the intervention group.


INSULIN, SERUM LIPIDS AND BLOOD PRESSURE OF CRETAN CHILDREN AGED 4–10 YEARS IN RELATION TO WAIST CIRCUMFERENCE

Waist circumference (n=96)	<25%-ile (52.1 cm)	>75%-ile (63.0 cm)	
	Mean		p-value
Insulin (μU/ml)	4.6	8.6	<0.001
Total cholesterol (mg/dl)	177	177	NS
Triglycerides (mg/dl)	56	76	<0.01
HDL-C (mg/dl)	57	50	<0.05
Total cholesterol to HDL-C ratio	3.3	3.7	<0.05
Systolic blood pressure (mmHg)	101	114	<0.001
Diastolic blood pressure (mmHg)	62	69	<0.01

LDLcholesterol was much lower in the intervention group. The total cholesterol to HDL ratio is lower in the intervention group compared to the controls. These significant effects continue 4 years after the end of the intervention.

We also looked at the endurance run test, which is a standardised test to check cardio-respiratory fitness. Once again, the intervention group had significantly better performance as compared to the control group. Here we only used children that had participated in all the follow-up examinations, from the baseline to the final check in 2002.

In the endurance run test, those children with a BMI over 25 performed much worse in comparison with those whose BMI is less than 25.

Another important finding in this study is that the number of children who smoked in the intervention group was half that of the control group. This is a very encouraging result.

Of course, there are weaknesses in this study: we used the principle of black box epidemiology; we used some variables at the beginning for the baseline data and looked at the outcome. Although we organised seminars aiming to introduce interactive courses on health promotion to teachers at the beginning of each school year we did not monitor teacher delivery and therefore do not have much information about the kind of intervention the children actually received given that some teachers were more motivated than others. We are now looking at the teachers to see what their health beliefs and attitudes are. This is a very large cohort (300 teachers) and it is difficult to get qualitative epidemiological data.

We recommend a minimum of 60 minutes of physical activity a day for children. Motivation at school is very positive and tends to motivate children to continue being physically active later in life.

Even greater levels of physical activity may be necessary for the prevention of weight gain and a hypocaloric diet is not recommended for weight loss because growth will be discontinued. It is important to decrease time spent on sedentary activities, like watching TV. Children who participate in organised sports are often persistent exercisers.

Thank you for your attention.

Questions

Stein Emil VOLLSET
Thank you for your presentation Mr. Kafatos. It is good to see that intervention studies work sometimes.

Member of the Audience
I have a small technical question about your graphs. You show three points over time. I suppose that there was one point at the beginning when the two groups were equal.

Anthony KAFATOS
The graphs were showing the difference from the baseline data, which is why we did not show the original 1992 measurements.

Member of the Audience
I see you define moderate intensity activity. How do you define that in practical terms?

Anthony KAFATOS
We have a list of activities with a standart score for each category - very light, moderate and vigorous. We used the list of activities with the moderate activity score and we then multiplied it by the amout of time spent in physical activity in order to get the energy expenditure for a particular activity.

Cultural and social analysis of the mediterranean diet among the Mediterranean muslims

Françoise AUBAILE-SALLENAVE
CNRS - Museum National d'Histoire Naturelle , France

I would first like to warmly thank the organisation for its kindness and the organisers, especially Dr Barnat, for having invited me.

The Mediterranean diet

In this paper, I will first try to show different features of the Mediterranean diet, and then how social and cultural representation can explain the nutritional disorders and their consequences, which are observed today in some Mediterranean populations.

The Mediterranean diet is both unique and very diverse. Though the cooking are many, depending on the country, city, or even the family, we can nevertheless note that there are a certain number of prevalent features, which enables us to speak of one Mediterranean diet. These features are the consumption of large amounts of cereal products such as bread (leavened and unleavened), pasta and couscous, vegetables, legumes, fruits (both fresh and dried), water and fruit juice. The consumption of these products varies according to the season. Protein comes from dairy products, cheese and, along the coast, from fish. Meat is rarely eaten fresh and reserved to feast occasions ; it is often traditionally preserved in mutton or pork fat, depending on religion. In Christian countries, great quantities of dried fish are imported from the Northern seas for the many fasts.

Those kinds of diets which could have been considered as model diets were often either outside the limits of frugality or striving for a certain nutritive imbalance : cereal fields are not large, gardens, providing vegetables and fruits are small, gathering wild herbs is still vivid and cattle are used for ploughing and carrying rather than as a source of meat.

Another false idea concerns olive oil. Olive oil is not universal in the Mediterranean diet : it is predominant in Greece, hazelnut oil is widespread in Turkey and now sunflower oil is predominant in all countries. It is mutton fat among the Muslim countries and Orient Christians, and pork fat among the many other Christians.

The traditional preserves, in particular those made from meat, are preserved in mutton fat which is melted and salted butter. Butter is an important fat among the Maghrebians.

A sweet culture

This Mediterranean diet can be qualified as being hyperglycaemic. It has acquired such an importance amongst the Muslims that we can speak of a 'sweet culture'.

Effectively, it was the Arabs who, at the beginning of the 8th century, introduced sugar cane from South Iraq and acclimated it to the hottest Mediterranean countries – the near-East, Egypt, Sardinia and Andalusia – after their victory over the Sassanides, the last Persian dynasty.

With the conquest of that kingdom, they gained access to a most refined culture in which, among many refinements, the cuisine and sweet confectionery were very highly developed. Since then, the technique of sweet confectionery, which is mostly associated with honey, fat and melted butter, has been one of the cultural bases of the Mediterranean diet, whose many practices and knowledge are common between the near East and Maghreb societies.

The great development of sweet confectioneries amongst the Muslims goes hand in hand with the great social and cultural valorisation of sweetness involved in rich symbolism.

On many occasions, honey or dates are the traditional offerings of welcome because of their pleasant taste and for what they represent. They are highly generative, they push away bad influences and they are the best sign of welcome.

Rituals of welcome the newborn baby

This welcome ritual involves dates and milk in the Bedouin society of the Middle East and the Sahara, as well as in rural and urban societies of the Mahgreb.

In Palestine, they give honey to a newborn baby because he is considered a host of the family. The newborn baby, begins his life with sweetness. Often, a few hours or somæetimes a few moments after his birth, he is given his first food, which is often something sweet, so this, then, is his first taste. This ritual is called '*tahnik*', meaning the rubbing of the palate with a sweet product. This Islamic practice is realized throughout the Muslim world. At the same time, it has a transmission value and is a propitiatory act.

This ritual has a transmission value because people think that a person who is holy and full of baraka can pass his virtue on by putting his saliva in someone else's mouth. This is never done by the mother but by the midwife, another family member or someone else who is known for his moral qualities, happy life or good looks.

The *tahnik* also has a propitiatory value. It is done with the aim of making the baby's mouth sweet. This means that he will speak fine words to his parents or that his life will be as sweet as the sweet he is given.

In Tunisia, this ritual is carried out in hope that the baby will have a nice life and that he will speak well to his parents. In certain households of Morocco, the midwife dips her finger in sugar oil or oil in which dates have been soaked and then puts it in the baby's mouth.

Dates and oil are also highly beneficial and good omens. The baby often has many other experiences with sweetness, such as during the feast celebrating his birth, the first seven days. On the 40 th day of his life, a little bit of the ritual confectionery is put on his lips.

Social and cultural importance of the sweet

The many recipes of these confectioneries are another proof of their social importance. The occasions when the sweet confectioneries are made are moments with social applications : marriage, birth, circumcision (which often lasts a week), religious feasts, the fasts of Ramadan (which lasts 28 days), - the feast celebrating the birth of the prophet, Ashûra (the first day of the year) and the Ayd el-kebîr (when sacrifices take place and which can least 2 or 3 days).

I can quote the Arabic names of more than 80 kinds of sweet confectioneries found in the Maghreb and I can do the same for Syria, Lebanon, Turkey and Andalusia, and I know of 77 in Sardinia.

Most of these confectioneries are generally made from almonds, chickpeas, wheat flour and semolina, with white cheese (like ricotta), eggs and flavourings. Aniseed is the most popular flavour, but orange-flower, lemon and cinnamon are also

popular. Honey is the traditional sweetener, but in the last century sugar has begun to be used, too.
Besides the social and religious implications, the feast confectioneries are a way to forge identity. This is evident in immigration, but also in the home country. Each city has its speciality and each family also has its speciality passed on from mother to daughter. Sweet confectioneries are invested with the feeling of prestige, since the preparations are time consuming and involve all the women of the house.
Young immigrated Maghrebian women who do not know the preparations of the sweets used for the great marriage or birth feasts will ask their mother, or an aunt to come from the home country to teach them all the rituals, including how to prepare the prestigious sweet confectioneries.
A literary example showing the importance of sweetness is an Egyptian allegorical tale from the beginning of the 16th century. In the Egyptian story the battle takes place between King Mutton, who reigns over meat, and King Honey who reigns over sweets, vegetables and cereals.

Tea and sugar

Though knowledge of sweetness is old, we can say that the large use of sugar from sugar cane is fairly recent. In my opinion, it is strongly linked to the Muslim society's recent adoption of tea.
Apart from water, fruit juice and, over the last 50 years, sweetened industrial drinks, two drinks dominate Mediterranean countries – coffee and tea. Coffee is an old drink adopted by the Turks in the 16th century who introduced it in their colonies – the Middle East, Greece, Arabia, Egypt, Tunisia and Algeria. Traditionally, it was, and still is, often drunk without sugar. The same is true for tea in the areas of ancient use, such as Central Asia, where it is even frequently salted.
Tea appeared in the Mediterranean area at the end of the 19 th century and was strongly sweetened with sugar. This specific drink had an immediate success in Muslim society, which had already developed a taste for sweets. The English first introduced tea and sugar into Morocco in the middle of the 18 th century, and they then introduced into the other Maghrebian countries. In the 19 th and 20 th centuries, tea and sugar were introduced into the Gulf Emirate and Arabia. Everywhere, they brought tea jointly with sugar.
We can now note the different ways of drinking tea and coffee in the countries where coffee is of ancient use and tea a new adoption. For example, in the Emirates, they always drink a very sweet black tea with or without milk. The sweet tea is in opposition to the bitterness of the nearly unsweetened coffee.
In Qatar, coffee and tea are offered to guests. After fruit and biscuits they offer three cups of hardly sweetened coffee and then three cups of very sweet black Indian Lipton. Here in Greece, there are four ways of drinking coffee : *sketos* is coffee without sugar (it is the equivalent of the egyptian saada), *me oligi* is slightly sweetened, *metrio* is sweetened and *glikes vrastos* is very sweetened.
Tea now represents welcome and hospitality among societies with no coffee, such as the Saharan population and many Maghrebian groups. Remarkably, in all societies that have recently adopted tea, the drink has brought great changes in diet, mainly concerning the quantity of sugar ingested. Consequently, it has also created economic changes where the purchase of tea and sugar is a burden on the household budget, which till then were in an autarcic economy such as were the Touaregs.
The testimonies are disparate, but nevertheless very significant. In the Sahara in the 1950s, sugar and tea were the two main trade articles (the third being cotton fabric). Among the Bedouin around 1960, the consumption of tea and sugar might have taken up a third of the household budget. In Tamanrasset, strongly sweetened green tea has become a drug – on average, a couple consumes 3 kilograms of green tea and 20 kilograms of sugar a month .
In about 1960 in Tabalbala, another oasis, the annual consumption of green tea for a very poor family of 6 was 5 kilograms of tea and 40 kilograms of sugar. A wealthy family consumed 19 kilograms of tea and 300 kilograms of sugar. A rich family (although the children do not drink much tea) consumed 30 kilograms of tea and 540 kilograms of sugar . Evidently, we have to take into account that these people have an intense welcoming tradition where tea is extremely important and its consumption ruled by strict rituals dictating the number of cups offered, 3, 4, 5 or more.
We sometimes know the quantity of tea and sugar for each cup. Among the Hamid of Southern Egypt in 1988, an adult drank at least five cups of tea a day, which is about 800 millilitres of liquid (135 grams of sugar and 8 grams of tea). When they use a glass they put more than 30 grams of sugar in it. In Tunisia, 7 grams of tea and 60 grams of sugar are necessary for 4 cups of tea a day. To this must be added the honey and the lipids used in the sweet confectioneries eaten with the tea.
What I saw in Morocco and Egypt is also very significant. In Egypt, they often pour more than 100 grams of sugar in the teapot for 5 or 6 glasses of tea. Each person drinks 4 or 5 glasses of tea a day.
In the beginning, the consumption of sweet tea had a positive effect – it provided calories often missing from the diet. However, the evolution and easier access to other kinds of sweetness, especially sweet confectioneries that combine carbohydrates and lipids, have physical effects. The first is weight gain, and sometimes becoming overweight. This is perfectly in tune with the female standard of beauty.

The fat body image as a sign of beauty, wealth and prestige

Effectively, in these societies that were frequently confronted with an irregular diet or under nourishment, the fattened body has a strong symbolic value – it is a sign of strength, wealth and beauty. It also gives prestige. These representations are still valid in many Mediterranean societies where women and mothers are in charge of the family diet and the family image. Let us remember the fat body image, which is present in many of the 1001 Nights tales. These are mainly Egyptian from the 17 th and 18 th Century. The standard of beauty is to have wide hips and a thin waist. For a long time, women looked for all possible ways to become fatter and therefore more attractive to their husbands.
Arabic scholarly literature about fattening nutrients is very rich. The suggested plants are numerous : fenugreek (arab *holba*, also used everywhere by the pregnant women), sesame, black cumin, gladioli roots, iris root, wild artichoke, salep (a starch from orchid's bulbs providing a very popular drink in Turkey and Egypt), nut grass and English galingale.

The literature also suggests using dangerous plants, such as henbane, mandragora, sarghina and hemp. Minerals, like arsenic, were used to gain weight, as were animals, such as lizard fat. *Ma'jûn* and *abbûd*, which lead to stoutness and favour pregnancy, were used as confectioneries.
In some Saharan populations, becoming fat is a practice that has social implications and is realized according to strict ritual. In some Moorish families of Touareg, before marriage, young girls are subjected to a special diet based on the ingestion of enormous quantities of milk reaching 12 to 15 litres a day. This is done to gain weight quickly – in three or four months, a little ten-year-old girl can weigh 80 kilograms. This represents perfect beauty and the family's wealth at the same time. Consequently, her dowry will be higher. Similar practices happen to-day among whealthy families of Arabia.
In *Djerba* the practice was described in the 1980s. It is called hajba, which means confinement. The mother makes her daughter attractive for marriage by fattening her and confining her inside the house so she has a perfect white complexion. The practice actually means much more than this. It is the link between beauty and wealth, since fatness is the sign of an

honourable family, and that the girl will give birth to nice children and feed them well. This could explain the observation by nutritionists, who say that schoolgirls are significantly more overweight than boys in the same regions.
Correlatively and traditionally, in all classes of society except for the lowest, women do not go out. In the most traditional societies the man does the shopping for food and all other household needs. The only occasions for women to go out were, and still are, the weekly bath (where women will spend a whole day), to the cemetery during certain feasts, to visit friends during births, marriages or circumcisions, or deaths.
Even now in towns, women from the middle and even upper classes do not go out, nor do they partake in physical exercise. Excepting those who have received schooling, they still equate beauty and health with a fat body. But this is changing with the young generation who go to school.
People who value sweet confectionery and sweet beverages in general might do so to compensate for the religious prohibition of alcohol. The main recommendation would be the promotion of education for all, especially for girls.
Dietary suggestions, physical exercise and sports should be included within the education system to promote a more balanced lifestyle.

Thank you.

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Questions

Stein Emil VOLLSET
Thank you, Dr. Aubaile, for a very interesting introduction to a Mediterranean culture and diet that has not received attention from Western nutritionists.
Are there any questions, or comments?

Member of the Audience
Thank you very much, Dr. Aubaile for an excellent presentation. I think it is good to get away from purely scientific presentations.
I would just like to make a comment regarding the consumption of sweets in most Mediterranean countries. We should understand that all these sweet confectioneries are part of ceremonies. These preparations were traditionally eaten only during ceremonies, which were rare. Where we should make the link with the nutrition transition happening in many of the countries at the moment is with the rise in economic status of some families and increased urbanisation. This has led to some people having ceremonies everyday. Therefore, they have more occasions to eat these sweets.
I would like to make another comment concerning tea. In Morocco, I know that refined sugar makes up 40% of the daily calorie intake. In many rural areas this is the main source of energy. These people never reach 2000 calories a day. It is an accompaniment to bread. It is true that the English introduced it, but it is part of their energy. The tea is less sweetened in urban areas where other things are eaten.

Françoise AUBAILE-SALLENAVE
Thank you for those useful comments. The lifestyle in this area is completely changing and excess consumption of sweet confectioneries is much more frequent.
Before, these sweets were eaten only during feasts, especially during Ramadan : now, more sweets are consumed than before, even during Ramadan.
We can see the same situation here – when we had dinner yesterday it was practically a feast. This type of meal is eaten much more frequently.

Member of the Audience
I have a question for the speaker, but also for the audience. Does anybody know of the health consequences of this extremely high sugar intake? The World Health Organisation has labelled soft drinks an energy dense food, which should be avoided. This came out a few months ago and caused a very strong reaction from a number of companies. Coca cola contains about 140 kilocalories. An Orangina contains about the same number of kilocalories. This is much less than the tea you have described. Should we envision any health education for the people who drink this type of tea?

Member of the Audience
There have been a limited number of small-scale clinical trials on the Arab diet, particularly related to nut consumption. Except for the period of Ramadan, nuts have historically been consumed as parts of dessert. Historically, a lot of Arab desserts (and desserts in

Greece as well) will have a high ratio of nuts to sugar and other ingredients. This has lead to a lower glycaemic response, but that is very much in the process of changing.

Member of the Audience
I think that in the Muslim countries and Greece, sage was drunk a great deal. This was a very good habit, because sage has high flavanoid content.

Françoise AUBAILE-SALLENAVE
They drink a lot of tea or infusions made from different kinds of aromatic herbs. The tea is frequently only mint. These infusions are very good only if they are not over-sweetened.

Member of the Audience
Good morning Professor Aubaile. Thank you for your presentation, it was very enlightening and interesting.
One of the things we were discussing yesterday evening was that we know what is happening, but how do we give good nutritional advice to this population?
The cultural and familial factors will have a huge impact on the changes, whether or not people are sick. We know that the recommendation will probably be to lower sugar consumption to prevent health problems in the future.
How do you think the message should be delivered? What are the best channels to use to bring the message to the people?

Françoise AUBAILE-SALLENAVE
I did not understand all the things you mentioned. It is a difficult question, because the cultural thinking is very difficult to change.

Member of the Audience (as above)
The question was : what communication channels must be found or improved? It has been seen that there is more sugar consumption, that there is obesity and other risk factors. Therefore, how can we bring the message to the families?

Françoise AUBAILE-SALLENAVE
Everyone now has a television and they see a different standard of beauty. I think that education is the first step, but I do not know if it is a real possibility. I do not have many answers to your question.

Member of the Audience
There is an Institute of Nutrition in Tunisia. During Ramadan, they run messages on television three times a day and about ten or twelve times a day on radio to make people aware of their diet. The accent is placed on sugar, fat and a healthy diet. Whether or not it is followed is another problem.

Member of the Audience
I would like to come back to the issue raised by Elio Riboli about the health consequences of sugar. Apart from the relationship between sugar and calories, when you have a high sugar intake your HDLcholesterol will decrease. However, I have not seen many papers where sugar was related to coronary heart disease or cardiovascular disease in general. The HDL lowering effect has been shown in metabolic studies and is quite well documented.
One of the reasons the WHO document pinpointed soft drinks was because of their relationship with obesity. In my opinion, the direct relation between sugar and obesity is not very strong. One of the big problems behind obesity, as you see in the United States, is the lack of dietary fibres. If people go on a diet and consume high numbers of soft drinks, they generally eat very little dietary fibre. This promotes the occurrence of obesity.

Member of the Audience (Elio RIBOLI)
I agree 100% with what was just said. The main worry regarding sugar is that it adds calories without you realizing that you are eating. When you drink a soft drink, you do not think you are eating, yet with four drinks a day, you are getting more than with a large portion of meat or pasta.
About 10 or 12 years ago, some colleagues from North Africa came to see us in Lyon. They told us they had the impression that breast cancer was very frequent in the Maghreb, but there was no data supporting this. What I am showing you now is the most recent data based on a quite good cancer registration system that has been set up over the last decade. It shows that the Maghreb areas have an incidence of breast cancer which is double that of the dark green area. It shows that the Maghreb has the same incidence of breast cancer as the most affluent parts of South Africa, where there is a very high incidence of breast cancer among the white population. As of today, probably one third of the breast cancer incidences can be attributed to obesity, particularly after menopause.
For the time being, we only have this observation that confirms that breast cancer is very high in the Maghreb region compared to the rest of the continent. The main characteristic, which stimulated this observation, was the high prevalence of obesity among women in that region. Sugar might be one important contribution to obesity and therefore, here it is not healthy sugar. It would be interesting to investigate this more.

Stein Emil VOLLSET
I think we have to move towards closing this session. We will take one final comment.

Member of the Audience
I teach a seminar on food and culture at the University of North Carolina. I teach only honours students, so we get the best students in the United States. I had a paper done just this past semester suggesting the addiction to sugar as a possibility in the United States. What interests me is to see these students who are incredibly bright walking around with a Coke in one hand and an energy bar in the other saying they do not have time for a meal. Underlying a great deal of what we have talked about these last three days is the issue of how to sustain a healthy diet, as there is an erosion of those rituals which transmit the practices for eating. As one looks at a diet, it cannot be looked at without looking at the social structures that maintain it.

Conference ending lecture

Elio Riboli

We have had the liveliest discussion of the conference in the last hour. This shows how difficult it may be to agree on the scientific evidence supporting a dietary recommendation, not because scientists are difficult, but rather because the evidence is often not as clear-cut as the evidence we have for tobacco, for example.

I am not going to summarise what has been done here. This conference has been a very interesting experience on how you can logically proceed through a series of questions, find some answers, and possibly end up with the conclusion that you do not have answers to all your questions.

The first question was : is there a benefit to the Mediterranean diet? There were a lot of presentations showing that both epidemiologically and experimentally there are elements of the Mediterranean diet that are associated with decreased risk of chronic diseases. The first question can therefore be answered and the answer is yes, there is a benefit.

The second question is what explains the benefit? Here we enter a much more delicate type of research.
It is quite difficult for observational epidemiological studies to clearly separate the benefit of different foods, all belonging to the Mediterranean diet. Recent epidemiology has however been based on much larger number of study subjects and different populations and has helped in obtaining more specific data on the benefits which can be expected from fish, vegetables, olive oil and so on. We know of all the difficulties in this case. Still, the data we have and which has been presented here clearly indicated that there is at least a general agreement that fruit and vegetables, whole grain cereals and fish are beneficial since they are associated with the reduced risk of disease. Other foods may be associated with increased risk of disease.

The problem is whether we know enough to make quantitative recommendations. We have seen the difficulties of going from a qualitative statement that something is good and something else is less good to a quantitative recommendation of the relative proportion. I am going to have to use a very common statement to further make this point: more research is needed. So far, research has focused on single components and not on relative proportions of nutrients and foods. This is a scientific issue and cannot be deduced just by looking at what people do.

There is a dimension upon which we have not touched on at all. We cannot, ethically speaking, propose that people change their diet unless we have compelling evidence to do so. This may seem obvious, but there are other areas of our life where we make a big fuss when someone proposes that we make a change. We should be very careful in having the pretension to tell people that they have been eating a certain way for the last 30 centuries because they live in Scotland, but they really should eat like a Cretan. People in a certain region are used to eating what they find and produce in the area: people in Scotland do not use olive oil, because it was very hard to find until recently. We have to be extremely prudent in interfering in tradition. Southern-Mediterranean are proud of their Mediterranean tradition, but there are people who are proud of their Nordic tradition. We should not propose that one tradition is better than another. Progress is based on the construction of strong traditions; otherwise we would still be living in caves. There is a balance between what tradition is and what is right. Not all tradition is good.

When we offer something to eat, we can do so because it looks nice, it has a nice smell, nice colours, because it can be eaten quickly. If we propose it because we think it is good for our health we enter into a completely different dimension: commercial advertisement. To what extent are we authorised to attach a health claim to a food? This is an issue which is very much open for debate.

There we come back to square one, because if you want to advertise a food because we attach a health claim to that food, then we need scientific evidence. Otherwise, the health claim will eventually be shown to be bogus and damage the food itself and those selling the product.

Not everything that is proven scientifically is ethically acceptable. We know for sure that the best-known risk factors for breast cancer are age at menarche, age at first child, number of children and lactation. There is not a single country in the world recommending that women have their first child before age 19, have at least 5 children before age 32 and lactate all of them, even though there is strong evidence that this reproductive behaviour would reduce breast cancer risk.

This means that we should be prudent with dietary recommendations and this is why I am in favour of giving broad, general recommendations based on the currently available evidence. If these recommendations were implemented, there would be a huge benefit in terms of health.

There is another question we have not addressed but which may be the object of a future discussion: Is the cost of promoting a new diet socially acceptable? Everything has a cost. If cheese makers advertise on television, the cost of the advertisement is reflected in the cost of cheese or in the lower price paid to the dairy farmer – the producer or the buyer has to pay.

The final question is whether anybody is going to be damaged by the changes. If people buy more of one type of item, they will buy less of another type? We are not the government; we do not make political or economic decisions. At the European Commission level this is an issue.

I have enjoyed this conference very much. We have had very good sessions of high scientific quality. We have had this unusual experience of having scientists, public health experts, health promotion experts, fruit and vegetable producers in the same room.

I would like to thank the organisers. This is a courageous initiative that we have all enjoyed very much.

These two best posters have been selected by the Scientific Committee EGEA for an oral presentation on Saturday, June 8th ,2003.

The impact of the traditional Cretan Mediterranean diet on the Management of Type 2 Diabetes Mellitus

C. Itsiopoulos[1], **M. Cameron**[2], **M. Kaimakamis**[3], **K. Rowley**[4], **JD. Best**[4] & **K. O'Dea**[5]

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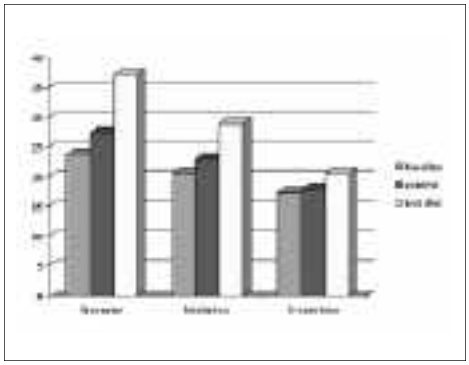
5. Menzies School of Health Research, Darwin NT, AUSTRALIA.

Objective : To investigate the impact of the traditional Cretan Mediterranean diet on metabolic control and weight management in Australian-born people with type 2 diabetes.

Study Design : Randomised cross-over study with 3 months on control usual diet or a reconstructed Cretan Mediterranean diet followed by 3 months of the other diet. The subjects were 27 Australian-born people, aged 47-77 years, with type 2 diabetes. The traditional Cretan Mediterranean diet was provided ad libitum in a wide variety of prepared modules.

Methods : Markers of glycaemic control, lipid profile, blood pressure, biochemical markers of dietary quality (plasma carotenoids, plasma fatty acids, red cell folate), homocysteine, body weights and body composition (by DEXA) were monitored.



Results : Following intervention on the test diet subjects experienced improvements in glycaemic control and insulin resistance (HOMA method). Significant increases in serum carotenoid levels (lycopene, lutein/zeaxanthin and b-carotene) were observed as shown in Figure 1. No significant changes in body composition were observed.

Conclusions : The traditional Cretan Mediterranean diet can be successfully implemented in the treatment of diabetes with modest benefits in metabolic control. Furthermore, we have shown that this relatively high fat diet provided ad libitum did not lead to weight gain.

Indicators of oxidative stress in elderly men aged 80 and over : differences between Crete and Zutphen (NL)

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Introduction : Levels of circulating hydroperoxides and indicators of iron status are commonly used as markers for oxidative stress and high levels may increase coronary heart disease risk and decrease survival.

Objective : To evaluate potential differences in hydroperoxides, total serum iron, ferritin and non-transferrin-bound iron (NTBI) in sera of survivors of two cohorts of the Seven Countries Study, Crete and Zutphen (NL).

Materials and methods : For this cross-sectional analysis blood samples were collected between March and August 2000 from 147 men living in or near Zutphen and 128 men living on Crete, all aged 80 years or over. Sera were assayed for hydroperoxides, total serum iron, ferritin and NTBI in one laboratory. Analyses of covariance was used to calculate age-adjusted means and to test for significant differences between the cohorts and between smoking status within cohorts.

Results : The geometric mean level of serum hydroperoxides was significantly lower in men from Crete (34.2 mmol/L) compared to men from Zutphen (55.4 mmol/L) ($p<0.01$). Likewise, men from Crete had lower serum ferritin (65 mmol/L and 137 mmol/L, respectively) and iron levels (15.0 mmol/L and 17.7 mmol/L, respectively) (both $p<0.001$), whereas NTBI levels differed not significantly (0.63 mmol/Land 0.72 mmol/L, respectively) ($p>0.10$). In Zutphen, current smokers had significantly higher levels of serum iron compared to non-smokers ($p<0.05$). In Crete, no statistically significant differences in indicators of oxidative stress between smokers and non-smokers were observed.

Conclusions : Elderly men from Crete are exposed to less oxidative stress than elderly men from Zutphen as measured by serum hydroperoxides and serum iron and ferritin.

Fish protein improves total antioxidant statusof streptozotocin-induced diabetes in spontaneously hypertensive rat

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Hypertension is the major risk factor in the developement of cardiovascular heart diseases. This risk is enhanced when this pathology is associated with diabetes.

The aim of the study was to determine the effect of fish protein on blood pressure, glycemia and antioxidant status in streptozotocin-induced diabetes in spontaneously hypertensive rats. 20 SHR were fed, for 2 months, diets containing 20% casein (C) or fish protein (FP). After 1 month of experiment, 10 rats were intraperitonealy injected with streptozotocin (65mg/Kg body weight).Total antioxidant status of blood, antioxidant enzyme activities [glutathione peroxidase (GPx), glutathione reductase (GSSH-Red) and superoxide dismutase (SOD)] and antioxidant substances (glutathione (GSH), nitric oxide (NO)) were determined on organs. Plasma vitamin C was analyzed.

The results showed a decrease (-14%) of blood pressure in SHR rats fed fish protein as compared to those fed casein but an increase (+13,9%) of the pressure values in STZ-SHR compared to SHR-FP. Total antioxidant status was increased (+5,5%) in plasma STZ-SHR as compared to SHR-C and in STZ-SHR versus SHR-FP. Plasma antioxidant status was significantly higher (+8%) in SHR-FP as compared to casein and in STZ-SHR-FP versus casein (+10,5%). The resistance of blood red cells (RBC) to hemolysis was threefold lower in STZ-SHR compared to SHR-FP and in STZ-SHR-FP as compared to those fed casein. The activities of GSH-Px and GSSG-Red in liver and kidney were significantly elevated in STZ-SHR as compared to SHR-C or FP. SOD activity in liver and heart was increased in STZ-SHR-FP than in STZ-SHR-C and in STZ-SHR-FPas compared to SHR-FP. In kidney SOD activity was enhanced (+31%) in STZ-SHR-FPthan in STZ-SHR-C. Total GSH concentration was 1,79-fold higher in STZ-SHR-FPthan in STZ-SHR-C and 1.51-fold higher in STZ-SHR-FPas compared to SHR-FP, in heart. In liver, SHR-FPpresented higher values of total GSH than SHR-C. Nitric oxide concentration was significantly increased (+46%) in STZ-SHR-FPversus C in kidney and in STZ-SHR-FP compared to SHR-FP (+59%). In heart, NO level was significantly higher in FP compared to C groups. FP diet involved a higher plasma C vitamin concentration as compared to C diet.

In conclusion, fish protein diet has a benefit effect on blood pressure only in SHR group and on the antioxidant status in particular in STZ-SHR and we must give a great interest to this protein as antioxidant supplementation in future treatment of degenerative diseases.

Moroccan diet : is it Mediterranean ?

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Diets around the Mediterranean Sea have long been praised for their health merits. However, these diets are not practiced uniformly across the Mediterranean countries (north/south, west/east) nor within countries (urban/rural, regional, poor/rich).
In Morocco, while most Mediterranean foods are produced and consumed, their intake level remains low and largely disparate among population groups. This paper will examine available data from national surveys to assess the practice of the Mediterranean diet by the different population groups.
The 1984/85 food consumption survey, obviously out-dated but the only available, showed that total intake of fish was 17g/p/day (27g urban and 9.5g). That of vegetables was 289g/p/day (356g urban and 238g rural). That of fruits was 87g/p/day (113g urban and 68g rural). Total fat intake was 67 g/p/day, two-thirds of which were provided by oils and only 12% by olive oil. The latter's intake was below 8g/p/day (5g urban and 10g rural). All these figures were naturally lower for low-income classes.
The 1998/99 expenditures survey indicates that fish expenditure reaches barely 3.3% of total food expenditures in urban areas and only 1.7% in rural areas. Those of Fruits and vegetables are less than 10% in urban areas and less than 15% in rural areas.
Morocco is going through a nutrition transition, occurring simultaneously with demographic and epidemiological transitions. Indeed, the 1998/99 national survey showed that overweight affects 36% of the adult population and 50% of urban women. The rate of hypertension in 2000 was 33% and that of diabetes is more than 10%. This situation calls for an examination of the risk factors, among which, diet and physical activity. Therefore, the evaluation of the Moroccan diet will help determine how it compares with the appreciated healthy Mediterranean diet. The results could be used as a base for nutrition education among the population.

Fruits and vegetables consumption in relation to cardiovascular disease profile of Greek medical students

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Introduction : Diet is related to the onset and progression of many chronic diseases, including cardiovascular diseases (CVD). Fruits and vegetables are considered important food groups in terms of chronic disease risk reduction. To date, however, their association with the CVD profile of young Greek adults has not been examined.

Objective : To investigate the fruits and vegetables intake of medical students in Greece and its relationship to major CVD risk factors.

Materials and Methods : A total of 523 third-year medical students (299 men, 224 women) aged 22 ± 2 years were examined in the context of the Clinical Nutrition class of the University of Crete School of Medicine. Trained nutritionists conducted a 24-h dietary recall to each participant, and anthropometric measurements and blood chemistries were performed. Purpose designed lifestyle questionnaires were used to assess the use of tobacco.

Results : Ninety percent of the male and 94% of the female students reported consuming fruits and/or vegetables, 327 ± 273 g/day and 358 ± 285g/day respectively. Only 31% of those who smoked reached the population goal of 400g/day consumption, in contrast to 41% of the non/ex-smokers ($p<0.05$). Fruits and vegetables consumption was inversely related to the obesity status of the medical students. After controlling for age, sex, tobacco use, and body mass index, fruits and vegetables consumers had lower total cholesterol (175.9 ± 1.5 vs. 192.1 ± 5.2 mg/dl, p=0.003) LDL-cholesterol (110.8 ± 1.4 vs. 126.9 ± 4.5 mg/dl, p=0.001), and TC:HDL-C ratio (3.68 ± 0.05 vs. 4.26 ± 0.17, p=0.001) than non-consumers. No differences were observed in blood pressure values.

Conclusions : Fruits and vegetables intake was associated with a better CVD profile in Greek medical students. Encouraging the consumption of these food groups may be an important step in reducing the prevalence of CVD risk factors in early adulthood.

Lutein, lycopene and beta-carotene reduce the oval cell reaction in the resistant hepatocyte model of hepatocarcinogenesis

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Introduction : The interest in carotenoids as potentially active substances for the cancer prevention is increasing. In the resistant hepatocyte model of carcinogenesis, when liver damage is so severe that hepatocytes are extensively killed or that their proliferation is prevented by exposure to hepatotoxins/carcinogens, oval cells appear in the periportal areas of liver lobules.

Objective : The effect of beta-carotene (BC), lutein (LU) and lycopene (LY) over oval cell proliferation was investigated.

Materials and methods : Wistar rats were separated in four groups (n=10). The groups treated with LY or BC or LU received the carotenoids (70mg/kg BW) and the control group (CO) received only corn oil. At the end of 8 weeks, livers were processed for light microscopy. Ductular cells could be distinguished on H&E and were GSTP positive. Images of 15 periportal zones per slide were considered and a reticule of 500 points was superposed to the image on the monitor. The number of intersections coincident with oval cells was counted. The volume fraction occupied by oval cells was obtained by the percentage of points counted in relation to the total intersections.

Results : A statistical difference was observed between the volume fraction in periportal zones of rats treated with corn oil and the groups treated with carotenoids. The values were: 17.1±13.6a (CO); 10.4±7.0b (BC); 12.4±7.5c (LY); and 9.8±6.8b (LU).
Conclusion: These results are in agreement with the quantification of GSTP-positive lesions in these livers, comet assay data, and reinforce the chemopreventive activity displayed by these carotenoids.

Evaluation of the nutritional situation determinants in Tannant (Morocco) using Positive Deviance approach

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In each community, there are individuals whose not very common practices and behaviors allow them either, to avoid a problem or, to find better solutions with the problems than their neighbors, who have access to the same resources. The survey on these positive deviants makes it possible to know what they do moreover than the other members of the community: unusual strategies and behaviors to deal with the threat of malnutrition.

To identify the causes of malnutrition in Tannant population, we first investigate the food habits and practices, care and health concerning the young children and their parents.
The nutritional status is measured using anthropometry. The food consumption is evaluated using 3 twenty-four hour recalls. Behaviors and practices are followed using ethnography.

The findings of studying the positive deviants will contribute to develop an education package aimed at alleviating nutritional stress in the studied community.

The advantage of the Positive Deviance approach is that is based on the local resources, it respects the cultures and knowledge of the populations and it allows a better use of the local resources to alleviate stresses.

Coronary risk factors and their relationship with saturated fat consumption in young adults born in Warsaw (preliminary data)

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Objective : The main aim of the study is to investigate the relationship between birth weight and coronary risk factors in young adults aged 24-28 years. In the current preliminary presentation we show data concerning the incidence of coronary risk factors and their relationship with saturated fat consumption and BMI.

Materials and methods : 1900 young adults born in one district of Warsaw in 1974-77, whose mothers participated in the prospective follow-up from the first visit during pregnancy till delivery, take part in this study. 1600 were invited until now. 327 (17,3%) underwent the examination.

Results : In the whole group of 327 people the most common were such risk factors as high (>10% en) consumption of saturated fatty acids (66,6%) and sedentary life-style (60,6%). Overweight (BMI 25-29,9) occurred in 22% and obesity (BMI≥30) in 9,5%. Hyperlipidemia occurred in 21,4%. Almost all risk factors were correlated with BMI. Serum LDL cholesterol, glucose, HbA1c and WHR correlated positively with saturated fatty acids consumption.

Conclusions : 1. Frequency of coronary risk factors in young adults is significant. 2. Serum insulin, glucose, TG, LDL, fibrinogen, HBA1c and blood pressure were positively correlated with BMI; HDL-chol was negatively correlated. 3. Serum LDL cholesterol, glucose, HbA1c and WHR correlated positively with saturated fatty acids consumption 4. It is important to implement prevention and treatment of coronary risk factors in young adults. It concerns mainly life-style measures. 5. The relationship of coronary risk factors with birth weight will be the subject of separate report.

Intake of linoleic and alpha-linolenic acids, and dietary origins in a French population. Relations with plasma and adipose contents

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Introduction : In France, the optimal levels of linoleic acid (LA) and alpha-linolenic acid (ALA) in the diet for optimal growth and health have been revised in 2001 and set at 4% of LA and 0.8% of ALA(% of total energy : en%). However, scarce data of actual dietary intake are available, especially regarding ALA.

Objectives : The objectives of this study were to assess the levels of LAand ALAsupplies of a French population and to investigate relations between intake and occurrence of LAand ALAin plasma and adipose tissue.
Materials and Methods: One hundred forty women (79 non-pregnant women and 61 pregnant women) recorded the foods they consumed for 7 days.

Results : The average values were found to be 8.9 g/d (i.e. 4.4 en%) for LAintake and 0.7 g/d (i.e. 0.34 en% for ALAintake. The main dietary source of ALAwas animal fats (75% of total ALA) among which dairy produce were the most prevalent (46%), with 13.2% represented by cheeses, 11.5% by pastries, 9% by yoghourts, 8.3% by butter and 4% by milk. 8% of ALAwere provided by fruit and vegetables, 9% by vegetable oils. LAand ALAlevels were respectively 14% and 0.5% of total fatty acids in adipose tissue ; they were respectively 27% and 0.5% in plasma total lipids. In plasma, these fatty acids were mainly incorporated into cholesterol esters (CE) (53% LAand 0.42% ALA). Moreover, LApercentage value in CE was positively correlated with that found in adipose tissue ($r = 0.606$; $p<0.001$).

Conclusion : average LAsupply (4.4 en%) is actually in accordance with the recommended value, whereas ALAsupply is too low. Strategies, such as food enrichment, must be found to increase the ALAintake.

The nutritional value of sprouted seeds in the diet

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Introduction : We present a review of studies on the nutritional value of sprouts grown from various seeds including legumes, broccoli and sunflower seeds. There is evidence of the content of vitamins A, B, and C as well as minerals including calcium, iodine, and zinc in the sprouts of mung beans, sunflower seeds and other beans and seeds. Broccoli sprouts are a rich source of phytochemicals such as sulforaphane, making them many times more potent than broccoli itself.

Objectives : Our objectives in this survey of the literature were to compile published information and to validate the nutritional worth of various sprouts as assessed in these studies.

Materials and Methods : This research started with our thesis "Viable Responses to World Food Concerns of Production and Distribution: Aquaculture and Hydroponics" (Master's Degree 1989) Since then, we have followed the field and added information regularly. The later studies are on work done with broccoli sprouts isolating sulforaphone because of its ability to protect cells against cancer. Continuing investigations involve helicobacter pylori. The preliminary results offer an exciting possibility of an effective treatment of ulcers with broccoli sprouts.

Results : In addition to their nutritional value, sprouting seeds in households provides fresh, organic produce in 3 - 5 days, a big step toward food security. For bioavailability, sprouts rank very high. Because of possible contamination with E.coli, some experts now advise cooking them rather than eating them raw. In climate extremes; in conditions of drought and flooding when fields are not available for cultivation; in times of war; in situations where transportation is a problem due to availability or cost, sprouting seeds locally can provide fresh food on a sustainable basis.

Conclusions : This technique of producing food is a positive contribution to health, as well as to social and environmental concerns.

Folic acid, vitamin B12 and homocysteine status in elderly men aged 80 and over : differences between Crete and Zutphen (NL)

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Introduction : Low levels of folate, vitamin B12 (cobalamin) and corresponding high levels of circulating homocysteine have been associated with an increased risk of coronary heart disease, colon cancer and cognitive disorders in elderly people.

Objective : A comparison is made of the levels of folate, vitamin B12 and homocysteine in sera of elderly men in two cohorts of the Seven Countries Study, the Cretan (GR) and the Zutphen (NL) cohort.

Materials and methods : For this cross-sectional analysis blood samples were collected between March and August 2000 from 147 men living in or near Zutphen and 128 men living on Crete, all aged 80 years or over. The sera were assayed for folic acid, vitamin B12 (clinical autoanalyzer) and homocysteine (HPLC) in one laboratory. Analyses of covariance were used to calculate age-adjusted means and to test for significant differences between the cohorts.

Results : The geometric mean level of serum folate was significantly higher in men from Crete (6.42 ng/ml) compared to men from Zutphen (4.80 ng/ml) ($p<0.001$), whereas vitamin B12 levels were lower (355 pg/ml and 432 pg/ml, respectively, $p<0.001$). These differences can probably be explained by the higher vegetable and fruit consumption in Crete and the higher consumption of meat and dairy products in Zutphen. Analyses of consumption data have not been performed yet. Surprisingly, serum homocysteine levels were higher in men from Crete (23.7 mmol/l and 18.4 mmol/l, respectively; $p< 0.001$). Within the Zutphen and Cretan cohorts, however, inverse correlations were found between serum folate and homocysteine as expected (Spearman correlation coefficients were –0.37 and –0.34, respectively, both $p<0.001$).

Conclusions : Elderly men from Crete have significantly higher levels of serum folate and lower levels of serum vitamin B12 probably due to dietary differences.

Nut intake in the EPIC study and colorectal cancer risk : gender and site specific protective effects

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Introduction : Nuts are an important part of a Mediterranean Diet and have been shown to be protective of heart disease and prostate cancer, probably due to their unsaturated fatty acid or phyto-nutrient/estrogen content. However, the effects of nuts on colorectal cancer (CRC) are unknown.

Objective : To determine the effects of nut intake on CRC risk within the EPIC study.

Methods : Total nut intake was determined from country-specific dietary questionnaires for 855 (Male(M)=327; Female(F)=528) colon cancer cases, 474 (M=215; F=259) rectal cancer cases and 476,711 controls (M=141,446; F=335,265). A multivariate Cox proportional hazard model, stratified by center, controlling for fruit and vegetable intake, energy from alcohol, energy from lipids, energy from other sources, height, weight, sex and age, was used. Relative risk estimates were obtained from nut intake (i) categorized in non-sex-specific, cohort wide quintiles and (ii) in a linear log transformed model.

Results : No significant protective effects of nut intake on CRC risk were observed in either males or females. However, division of the data into colon and rectal cancers showed a significant protective effect of nut intake on colon cancer in females at the highest quintile of intake compared to the lowest in the categorical model (OR=0.687; 95% CI=0.499-0.945), and with increasing intake of nuts in the continuous model (OR=0.878; 95% CI=0.789-0.977). No significant effects were observed for colon cancer in males or for rectal cancer in either gender. From this data, it is not evident why a sex difference exists or why it may be limited to the colon and not the rectum.

Conclusions : The EPIC study has shown a significant protective effect of increased nut intake on colon cancer in women, with no effects on rectal cancer for either gender.

Ready-to-eat cereals consumption in relation to anthropometric indices, physical fitness, and dietary intakes among Cretan high school pupils, Greece

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Introduction : Diet in childhood is considered important for the development of chronic disease in later life. Little is known, however, with regard to the association between consumption of ready-to-eat cereals (RTEC) and diet and health indicators in children and adolescents.

Objective : To investigate the consumption of RTEC among adolescents in Crete, Greece, and examine associations with anthropometric indices, physical fitness level, and nutrient intakes.

Materials and Methods : A representative sample of 392 pupils (183 boys, 209 girls) aged 15 ± 0.4 years, attending high schools in Crete, was selected for the purposes of the study. Antropometric measurements were performed, and the physical fitness of the pupils was evaluated with the 20m-endurance run test. Trained nutritionists conducted 24-h dietary recalls interviews and addressed a set of questions to determine the frequency, patterns, and type of RTEC consumed by the pupils.

Results : Regular consumption of RTEC (at least once/week) was reported by 42.1% of boys and 43.1 of girls. 43.7% of the pupils ate RTEC at meals other than breakfast. Compared to non-consumers, daily RTEC consumers (≥ 5 times/week) had lower values of the body mass index (mean ± SD; 21.9 ± 0.6 vs. 23.4 ± 0.3 kg/m2), waist circumference (73.2 ± 1.5 vs. 76.6 ± 0.7 cm), waist-to-height ratio (0.43 ± 0.01 vs. 0.46 ± 0.01) ($p<0.05$ in all analyses), and fasting plasma glucose (70.1 ± 2.2 vs. 77.9 ± 1.1, $p<0.01$). Subjects who ate RTEC had also better performance on the endurance test. Cereals-consumers had significantly higher intakes of calcium, iron, fibre, folate, and vitamin B2 than non-consumers.

Conclusions : Regular consumption of RTEC was favourably associated with many health and diet indicators in Cretan high school pupils. RTEC consumption may be a beneficial factor in the diets of young children and adolescents, with potential implications for improved health status and reduced risk of disease in adult life.

Vegetables and fruits consumption in young people in North of France

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Introduction : It is usually said that school boys and girls do not eat vegetables and fruits because they don't like it. But few data are available on that topic, especially on sociological factors which could explain that situation. Since dietary habits during youth may be important for forthcoming health of children it is worth to know that food intake.

Objective : The purpose of that study was to know the vegetables and fruits consumption in young people, the nutritional consequences and the factors explaining that consumption.
Material and methods : We have investigated 201 schoolchildren from 10 to 20 years with a four days dietary record in some schools in North of France with the SUVIMAX photo-booklet.

Results : The mean consumption of fruits was 67,9 ± 79,9g/d and the mean consumption of vegetables was 178,3 g/d including 103,2 g/d of potatoes (which are vegetables but often excluded from that group by some public health and nutrition committee in France). 28,8 % of the subjects do not consume fruits. Girls consume more fruits than boys (78,2 g/d vs 53,9 g/d). Children whose mother is born in France eat less fruits than children whose mother is born in another country (65,3 g/d vs 94,2 g/d). Children whose father is born in the North of France eat less fruits than children whose father is born in another region of France (59,1 g/d vs 118,4 g/d). Children whose parent's job is socio economically higher eat more fruits than children whose parent's job is socio economically lower. Children who eat more than 117 g/d fruits have higher intake in fibers, vitamins A, D, C, B, B5, B6, B9, magnesium and potassium than those who eat less than 25 g/d of fruits. Children who eat more than 248 g/d vegetables have higher intakes of energy, carbohydrates, proteins, fats, fibers, vitamins A, E, C, B1, B3, B5, B6, B9, magnesium, phosphorus, potassium, iron, than those who eat less than 98 g/d vegetables. There is no relation between fruits or vegetables consumption and height, weight and body mass index of the subjects (9,5 % of school children are obese and 12,4 % are overweight)

Conclusion : Among school children from 10 to 20 years in North of France, fruits and vegetables is low and particularly fruits consumption is linked to many sociological and cultural parameters. Higher consumption of fruits and vegetables is associated with higher micronutriments and energy intakes but there is no relation with body mass index. That could reflect also some differences into lifestyle. These data will be discussed

Health and dietary self-assessment of Greek medical students as an educational tool on nutrition teaching

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Introduction : The search for more effective modes of teaching nutrition appears to be receiving increasing attention. The Clinical Nutrition course at the University of Crete emphasizes the role of health and dietary self-assessment in teaching nutrition to medical students

Objective : To report our twelve year experience in using health and dietary self-assessment as an educational tool.
Materials and Methods: During the course, a 24-hour dietary recall questionnaire is administered by dieticians to all third-year medical students and anthropometric measurements and blood analyses are performed. Individual nutrition analyses and health reports are discussed by the students and the staff in small-group interactive sessions. At the end of the course students are asked to evaluate the course.

Results : From 1989 to 2001, 989 third – year medical students attended the Clinical Nutrition course. The response to the nutrition assessment was overwhelming positive and it was gratifying to receive a high participation (98%) and cooperation from the students. In the course evaluation 91% of the medical students enjoyed dietary assessment exercise and 90% benefited from it, while the percentages of negative responses were only 2% and 4% respectively. The discussion following health and dietary assessment was considered useful in terms of acquiring knowledge and improving students'health habits.

Conclusions : Dietary assessment can play a significant role in teaching nutrition to future medical practitioners. The use of personalized data provided by self-assessment can give more relevance to the content of clinical nutrition and enhance the learning process.

Dietary fibre intake in relation to diet and health status of Greek medical students at the University of Crete School of Medicine

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I**ntroduction :** Dietary fibre, an essential component of the Mediterranean diet, is associated with important health benefits in adulthood, especially in terms of reducing the risk of cancer and cardiovascular disease (CVD). There is no published data on fibre intake in young adult populations in Greece and relationships with diet and other health indicators.

Objective: To assess dietary fibre intake by Greek medical students and examine associations with daily nutrient intake and CVD risk factors.

Materials and Methods : A total of 951 third-year medical students (500 male, 451 female) from various regions in Greece, aged 22 ± 2 years, were examined in the context of the Clinical Nutrition class of the University of Crete School of Medicine. Trained nutritionists conducted a 24-h dietary recall to each participant and anthropometric measurements, blood pressure recordings, blood analyses and smoking assessment were performed. "High" and "low" fibre intake was defined using upper and lower energy-adjusted fibre intake quartiles for each sex.

Results : The mean fibre intake was 16.9 ± 10.7g for males and 13.7 ± 10.1g for females ($p<0.001$). The lower quartile cut-off for energy-adjusted fibre intake was 4.3g/1000kcal for men and 4.5g/1000kcal for women and the corresponding upper quartile cut-off was 9.2g/1000kcal for men and 11.1g/kcal for women. "Low" fibre eaters had higher intakes of energy, total fat, saturated fatty acids, cholesterol, protein and sodium ($p<0.001$ for all nutrients) than those with "high" fibre intake. Energy-adjusted fibre intake was positively associated with intakes of iron ($p<0.001$), folate ($p<0.001$), magnesium ($p<0.001$), potassium ($p<0.001$), vitamin C ($p<0.001$), vitamin A ($p<0.001$), vitamin E ($p<0.05$), and vitamin B6 ($p<0.001$). No statistical association was observed between fibre intake and anthropometric measurements, systolic blood pressure, serum lipoproteins or tobacco use.

Conclusions : Our data suggest that fibre intake among medical students was strongly related with a healthier dietary pattern. Medical students should be encouraged to consume fruits, vegetables, legumes, cereals and other food groups with high content of fibre.

Monitoring food habits in the Mediterranean region through the DAFNE databank

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Introduction : The term “Mediterranean diet” is used to denote dietary practices in the olive-growing areas of the Mediterranean basin, as these were described in the 1960s.

Objective : To monitor dietary practices in four Mediterranean countries (Greece, Italy, Spain and Portugal) and to further compare them with the characteristics of the 'Mediterranean diet', as originally described.

Materials and methods: The DAFNE (Data Food Networking) databank, which includes information collected in the household budget surveys, was used to retrieve nationally representative dietary data for the four countries. To allow comparisons over time both within and between countries, data were harmonized according to the DAFNE methodology.

The mean availability (g/person/day) of eight components of the Mediterranean diet (olive oil/cereals/vegetables/fruits/legumes/dairy products/meat/fish) were estimated, based on data covering the period from 1980 to 1999. Food availability was studied to describe dietary practices in the region, to assess overtime changes and to identify deviations from the traditional diet.

Results : Data confirm the diverse expressions of Mediterranean diet. When compared to Greece Portugal and Spain, Italy is the highest consumer of cereals, mostly attributed to the high pasta consumption. Vegetables are preferably consumed in Greece; Spain and Portugal favor the consumption of fish and seafood. Olive oil is the prevailing added lipid in Greece, Italy and Spain, while in Portugal seed oils are commonly consumed. In contrast to the past, meat availability is high in all countries and exceeds 130g/person/day.

In recent years, a general decrease is noted in the availability of olive oil and plant foods. The bigger deviation from the traditional diet concern the increase in meat and cheese and the decrease in fruit availability.

Conclusion : Despite the worldwide acceptance of the beneficial effects of the Mediterranean diet, people in the region have progressively departed from their long-standing tradition. The need for a nutritional policy that would address this issue has become imperative.

Factors influencing changes in dietary habits at family level in a middle-income neighbourhood of Beirut, Lebanon

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Introduction : In a middle-income neighbourhood of Beirut, Lebanon, with high levels of obesity (38%) a community health promotion project was initiated. The nutrition education activities consisted of classes and cooking sessions on preparing a healthy diet for groups of women.

Objective : The aim of the study was to understand the conditional factors for changing the family diet in a group of women that participated in the nutrition activities.

Materials and methods: In a qualitative study 28 participants of the nutrition activities were interviewed (semi-structured). Coding and analysis of the transcripts was done in two rounds and was assisted by QSR-Nudist5 software package.

Results : Major reported dietary changes were reduction of fat intake and increase in vegetable consumption. Two major determinants of change in family diet were identified: the initiating cause for change and the family dynamics.

Most families where one of its members had a cardio-vascular disease had already experimented with dietary changes. When the incident befell the husband, changes were more often extended to all family members. The educational sessions were beneficial for reinforcing or incrementing the already introduced changes.

Women in families that were incident-free participated in the educational sessions for reducing future risks or for increasing their "healthy feeling". Most of these families experimented with changes during the course of the project. Children had a powerful voice in maintaining the changes.

Conclusions : Acceptance and maintenance of dietary changes introduced via the mother depend on her negotiating power. Depending on the initiating cause she will get support from different family members. Effectiveness of interventions could be enhanced by learning women to better manage family dynamics. Specific interventions should also be designed for each causal condition.

Association between cardiovascular diseases and additional eating during the night

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Introduction : Additional eating after evening meal is widely known in the Saharan woman in south of Morocco and consists in a traditional overeating related to the perception culturally valorising obesity. Does this subculture can lead to cardiovascular diseases? Objective : The aim was to examine the association between obesity, hypertension, diabetes and additional eating during the night in a urban women of south Morocco.

Materials and Methods : 180 urban women aged 15-70y, of mostly Saharan origin, not pregnant. Body weight, height, plasma total cholesterol, triacylglycerols, glyceamia and blood pressure are measured. All subjects completed a diet and lifestyle questionnaire at recruitment, giving details of their usual diet. The sample was divided in two groups according to their answers to the food habit questionnaire as either consuming additional eating after evening meal (group1) or not (group2). Obesity was defined by body mass index BMI>or=30.

Results : The results showed that the prevalence of obesity, hyperglycaemia, hypercholesterolemia and hypertension was higher in group 1 than group 2. Energy intake after evening meal was significantly correlated with BMI.

Conclusion : The results demonstrated that over-consumption with additional energy intake after
evening meal may be one explanation of the apparent higher prevalence of cardiovascular diseases risk in Saharan women in south Morocco.

Cor relation of physical activity with diet and obesity in children

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Introduction : Childhood obesity is reaching epidemic proportions in Greece. Increased physical activity and improved dietary habits are the main strategies for primary prevention and treatment of obesity.
Objective: The aim of the present study was to examine the relationships of children's physical activity with obesity indices and dietary data.

Materials & methods : A total of 634 children (14-16 years) were examined in 2001-2. A 24h-physical activity questionnaire was obtained by interview and the shuttle run test (SRT) was performed. The dietary analysis was based on the 24h-dietary recall, while obesity was defined by BMI. The 24h-Physical activity was divided in 3 major categories: A:Reclining/Sleep/Watching TV, B:Very Light/Light activities, C:Moderate/Vigorous activities.

Results : Triglycerides and LDL-C were inversely related to C and B activities respectively, while the ratio TC/HDL-C was positively related to A activities ($p<0.05$). Although BMI was inversely related to A activities, our findings showed that obese children had a significantly lower score in SRT. SRT was negatively associated with A activities and positively with C activities ($p<0.05$). Boys, as compared to girls, spent fewer hours on A and B activities and more hours on C activities both in weekdays and weekends.
However, when the food consumed was categorized into food groups, it was found that a) large consumption of dairy products was related with significantly less hours spent on A activities and more on B activities, b) consumption of cereals and meat was positively associated with A activities while the latter was also negatively associated with B activities. No correlations were found for other food groups like fruit and vegetables, snacks, sugar, soft drinks, fats and oils, fish and seafood, and eggs, or nutrient intake.

Conclusions : The above findings indicate that high physical activity reduces the levels of triglycerides and LDL-C while endurance capacity is inversely related to obesity.

The Mediterranean diet score: a useful means of evaluating dietary change ?

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Introduction : The traditional Mediterranean dietary pattern, recognised for both its palatability and health benefits, has been quantified as a simple to use diet score calculated from 8 dietary components [1]. A high score is defined as ≥ 4.

Objective : The aim of the present study was to evaluate the usefulness of the Mediterranean diet score (MDS) in detecting dietary change.

Methods: Subjects were Scottish patients who had survived an acute myocardial infarction, were attending cardiac rehabilitation and participating in a randomised-controlled study to examine the effectiveness of intensive dietary counselling. Seventy-five post-myocardial infarction patients were randomised to receive either usual care or intensive dietary counselling, of 4 hours duration, encouraging the consumption of a diet in accordance with the principles of the Mediterranean diet.

Results : At baseline and 12-weeks after the completion of cardiac rehabilitation all subjects completed 7-day weighed intake food diaries which were used to calculate the MDS at these time points. At baseline there was no difference between the groups either in the proportion of subjects achieving a high MDS (≥4) or in the mean MDS. However, at 12 weeks a greater number of subjects in the intervention group achieved a high MDS compared with those in the control group (38% vs 16%, p=0.039) and the mean MDS was significantly higher for the intervention group (3.24 vs 2.31, p< 0.000).

Conclusions : The MDS has been used to compare the diets of groups but this study was the first to examine the value of the MDS to evaluate changes in eating habits following a dietary intervention. While the results of this study are promising, further research using the MDS as an evaluation measure is warranted before its widespread use for this purpose is recommended.

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The influence of a mediterranean-like diet with and without red wine on the criteria related to the metabolic syndrome

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Introduction : The metabolic syndrome is a cluster of cardiovascular risk factors linked to insulin resistance. There is evidence that life-style changes may prevent the expression of the insulin resistance phenotype.

Objective : This study examined whether a Mediterranean-like diet supplemented with red wine had an impact on the criteria related to the metabolic syndrome.

Materials and methods : In a cross-over study, 10 healthy men and 10 women between the ages 25 to 59 years, without severe dyslipidaemia and on no medication, consumed a Mediterranean-like diet for 6 weeks respectively with and without red wine. During the experimental periods the subjects increased their intake of vegetables, cereals, fruit, mono-unsaturated fatty acids and fish at the expense of red meats and dairy products. Dietary control was through 4 times 3-day dietary record during the study period. Fasting blood samples were taken at base line, after the diet and after the diet plus wine periods. Total cholesterol (TC), triglycerides (TG), HDLC, LDLC, and LDL particle size, insulin, glucose, uric acid, as well as the BMI and blood pressure were measured.
Results: The serum lipids, uric acid and insulin did not change significantly during the study, nor did the LDLparticle size compared to baseline values. The blood pressure recordings also remained unchanged. The changes in the BMI (p= 0.0023) and fasting glucose (p= 0.0435) were significant as compared to the base line values. The addition of red wine to the Mediterranean-type diet did not significantly add to these beneficial changes.

Conclusions : The Mediterranean-like diet had a protective effect against the metabolic syndrome, and more specifically on the impaired glucose regulation by significantly lowering the fasting glucose level and BMI after the diet plus wine period. This study indicates that minor lifestyle changes can markedly influence the impaired response to the physiological effects of insulin including those on glucose and lipid metabolism.

Traditional foods : health, cultural and standardization aspects

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Introduction : Nutritional investigations have provided strong support that a diet that adheres to the principles of the traditional Mediterranean one is associated with longer survival. This could be partly attributed to Mediterranean traditional foods, which this diet implies.

Objectives : There is a need to study traditional foods in order to enrich and improve our diet and at the same time preserve important elements of our cultural inheritance. Our research team has implemented several research projects, with the objective to formulate a framework for the systematic investigation of traditional foods and recipes, aiming primarily at the elucidation of the role of traditional Greek diet on health.

Methodology : The study of traditional foods is multifaceted and includes :

- Determination of the nutrient and non-nutrient value of primary and composite traditional foods.
- Recording of the traditional production methods with audio-visual means.
- Technological study on their potential industrial or semi-industrial production.
- Formation of integrated records related to their traditional character, which may serve as identification files for potential use in proprietary claims.
- Historical and folkloric review, which documents their traditional identity.

Results : We present some highlights of traditional foods studied :
Pasteli (sesame bar): Compared to snacks with similar energy value, pasteli has a higher protein and dietary fiber content and in spite of its high proportion of lipids, cholesterol is absent and saturated lipids are low.
Green pies : In recipes, where the only added lipid was extra virgin olive oil, 55% of the food's energy value derived solely from olive oil. Moreover, edible greens provide significant amounts of antioxidants.

Conclusion : The study of traditional Greek foods provided evidence of their beneficial effects on health, indicating that they meet current criteria for a prudent diet. The registration and standardization of traditional foods could provide incentive for their reinstatement into the daily diet.

Influence of fish protein on blood pressure, plasma, lipoprotein metabolism and on hepatic enzyme activities in spontaneously hypertensive rats

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Hypertension is a major risk factor in the development of coronary heart disease, along with other factors as dyslipidemia, obesity and diabetes. It has been reconized that the incidence of cerebral stroke lesions was lowered, and lifespans were elongated by improving the protein source, even if this has no effect on blood pressure (Murakami et al., 1994). The aim of the study was designed to determine if dietary protein sources (casein and fish protein) are related to changes in blood pressure, lipoprotein metabolism and liver enzyme activities in spontaneously hypertensive rats at 5 wk of age.

Hydroxy-methyl-glutaryl Coenzyme A (HMG CoA)-reducatse, cholesterol 7a-hydroxylase, acyl-CoA:cholesterol O-acyltransferase (ACAT) activities were measured in liver.
After 2 mo of experiment, fish protein diet lowered blood pressure (-14%) and plasma angiotensin II concentration (-65%) compared to casein diet. Fish protein diet decreased the concentrations of triacylglycerol (TG), phospholipid (PL) and cholesteryl esters (CE) in liver. Plasma, liver and HDL2-total cholesterol contents were respectively, 1.25-, 1.71- and 1.79-fold lower in fish protein group than in casein group. Consumption of fish protein decreased HDL3 amounts and apolipoproteins, HDL2-PL, -unesterified cholesterol (UC) and -CE and VLDL-TG. Furthermore, fish protein reduced VLDL-apo B48 (-44%) and increased -apo C (+49%). HDL3-apo A-I, A-II and A-IV were respectively, 1.62-, 2.20- and 1.70-fold lower, whereas, apo A-II and A-IV of HDL2 were 3.44- and 1.71-fold higher in SHR fed fish protein than in those fed casein. As compared to casein, fish protein enhanced cholesterol 7a-hydroxylase (+79%) and HMG CoA-reductase (+33%) activities but decreased (-39%) ACAT activity in liver. The 16 :0 was lower in VLDL-TG but higher in LDL-HDL1-CE in fish protein group. Total monounsaturated fatty acids, especially, 16 :1(n-7) and 18 :1(n-9) in VLDL-TG were lower in fish protein than in casein groups. The proportion of 18 :2(n-6) was 3.21-fold lower in HDL3-PL and 2.54-fold lower in LDL-HDL1-CE in fish protein fed rats. The proportion of 20 :4(n-6) in VLDL-TG, LDL-HDL1-CE and HDL3-PL was significantly lower in fish protein than in casein fed groups.

The results show that administration of fish protein to SHR has a favorable influence on blood pressure, plasma angiotensin II and cholesterol concentration as compared to casein. It stimulates HMG-CoA reductase and 7a-hydroxylase activities but has different effects on HDL2 and HDL3 lipid metabolism. This investigation reported a novel argument on the importance of fish protein in the prevention and the development of hypertension and its complications.

The blood ethanol curve after drinking red or white wine

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Introduction : Gustafson and Källmen (1988) were the first who demonstrated that the general form of the blood ethanol curves are dependent on the consumed beverage. Comparing the curves of the same individuals drinking either beer or white wine (La Garonne) they found, that after beer the time to reach the maximal blood concentration was longer than after white wine and they postulated that this may result in different physiological effects.

Objective : In our study we compared the concentration-time curves of ethanol in plasma of volunteers after drinking red or white wine.

Material and Methods : 6 apparently healthy volunteers ingested after an overnight fasting either 400 ml red wine (Spätburgunder or Lemberger) or 400 ml white wine (Weissburgunder). The experimentals were carried out at a 4 weeks distance with the same subjects in a randomised cross-over design. Plasma was collected before and 15, 30, 60, 90, 120 and 180 min. after drinking. Ethanol concentration in plasma was estimated with clinical methods and a noncompartmental pharmacokinetic evaluation was performed according to standard methods using the WinNonlin® Professional Software. The following parameters were evaluated: tmax, cmax, AUC (0-3).

Results : The results of this pilot study are pointing to the fact that there are differences in the pharmacokinetics of ethanol depending on the ingested beverage. In our study there was a significant ($p < 0.05$) difference in the time to reach maximal blood ethanol concentration (tmax) between the red wine Lemberger (0.792 ± 0.332 hr) and the white wine Weissburgunder (1.50 ± 0.00 hr). The total elimination time of ethanol after Lemberger was 6.8 ± 1.3 hr and after Weissburgunder 7.5 ± 1.7 hr.

Conclusion : Ethanol and alcohol beverages are known to affect upper gastrointestinal motility in humans. White wine has been reported to diminish gastric emptying compared to beer and there seems to be the same effect comparing the absorption rate of white wine with that of red wine.

Effects of german red or white wine drinking on the antioxidative capacity of human plasma

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Introduction : The cardioprotective effects of red wine consumption have been attributed to its high content of polyphenolic compounds. These substances are potent antioxidants capable of scavenging free radicals and inhibiting lipid peroxidation in vivo. In Germany, people mostly prefer white wines instead of read ones but white wines contain only small amounts of polyphenolic compounds. Therefore it is astonishing that recently several authors described cardioprotective effects resulting from white wine drinking in humans and animals.

Objectives : In the present study, the effect of an ingestion of red or white wine from German production on the total plasma antioxidant status and the profile and content of some selected polyphenols was determined in plasma of human volunteers.

Material and Methods : 6 apparently healthy volunteers ingested after an overnight fasting either 400 ml Spätburgunder or Lemberger or Weißburgunder or tap water (control) together with a standard meal. The experiments were carried over a 4 weeks period in a randomized cross-over design. Plasma was collected before and 15, 30, 60, 90, 120 and 180 min. after drinking. The total plasma antioxidant capacity was measured with 3 different methods (TEAC-test, TRAP-test, PCL-test).

Results : Both red wine and white wine drinking resulted in significant ($p < 0.05$) increases of the initial antioxidative capacity of plasma. This plasmatic increase did not correspond neither to the antioxidant capacity of the wines nor to the total phenolic content nor to the concentration of selected polyphenols in plasma. White wine ingestion resulted in a greater rise of the antioxidant capacity when measured with the PCL- test and a lower rise when using the TRAP-test than the red wines.

Conclusion : The results document an enhancement of the antioxidative potential in human blood after drinking white wine which is not only induced by polyphenols.

Dietary alpha-linolenic acid intake, heart disease and prostate cancer

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Background and aim: The protective effect of very-long chain n-3 fatty acids as present in fish on heart disease is well-documented. However, much less is known about the effect of alpha-linolenic acid (C18:3n-3), the n-3 fatty acid present in vegetable oil. We reviewed results of human studies on ALAintake and heart disease and addressed possible adverse effects on prostate cancer.

Subjects and Methods : We identified 5 prospective cohort studies that reported on the intake of ALA and endpoints of cardiovascular disease. We further reviewed data from clinical trials on ALA intake and cardiovascular disease. In addition, we identified 10 prospective cohort and case-control studies that reported ALA intake or levels and prostate cancer. We calculated combined risk estimates of disease by using a random-effects model.

Results : The combined relative risk estimate for fatal coronary heart disease, adjusted for other risk factors, is 0.79 (95% CI: 0.60-1.04) for a high versus a low intake. The average difference of ALA intake was 1.2 g/d. Results from three secondary trials also suggested that a high intake of ALAlowers the risk of coronary heart disease.
Combined data from 10 observational studies show a relative risk of prostate cancer of 1.50 (0.99-2.28) for men with either a high intake or high blood levels of ALA. Results from different studies were heterogenous, without apparent reason.

Conclusion : Evidence from prospective cohort and clinical trials together suggests that ALA protects against fatal coronary heart disease. Observational studies suggest that ALAintake is associated with increased prostate cancer risk. This association may not be causal, and prostate cancer is 5-10 times less frequent than heart disease and occurs at an older age. Nevertheless, the association is too large to ignore, and the role of ALAin prostate cancer deserves further study.

Mediterranean diet as a model diet in prevention and treatment of CHD. Graphic method for doctors to explain this diet to patients with CHD

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Introduction : Eurodiet Report (2000) recommends creating national Food-based Dietary Guidelines.

Objective : 1. To design a graphic method for doctors to explain the principles of Mediterranean diet in CHD prevention and treatment to the patient. 2. Evaluation of consistency of nutrition value of designed model diets with Mediterranean diet and comparision of their vitamin and mineral content with RDI for females and males at various ages.

Materials and methods : Polish Food Guide Pyramid and 10 Dietary Guidelines were used for graphic demonstration of the diet's principles. In the Pyramid food products were divided into 5 groups: cereals, vegetables, fruit, milk products, meat and its substitutes. An average measure of 1 serving was calculated. Additionally, vegetable fats and sweets were placed outside the Pyramid.

Results : 1. A table, graphically associated with the Pyramid, was designed. It shows the number of product servings from respective food groups for healthy nutrition of females and males at various ages. 2. An average nutrition value of model diets: protein ~16% E, fat <30% E, MUFA ~10% E, SFA ~7% E, cholesterol <200 mg, linolenic acid 2.2-2.6 g, EPA 150 mg, DHA 150 mg, n-6/n-3 < 3, fibre 25-40 g. Diets >1800 kcal supply vitamins and minerals on RDI level, diets of lower energy value may need supplementation.

Conclusion : This graphic method of explanation the principles of Mediterranean diet is well understandable by patients and helps them to follow diet prescribed by their doctor. Nutritional values of proposed diets are consistent with principles of the Mediterranean diet.

N-3 fatty acids and markers of cardiac ar rhythmia in healthy middle-aged subjects

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Introduction : N-3 fatty acids may reduce the risk of sudden death by preventing life-threatening cardiac arrhythmias. Two possible mechanisms are through an effect on cardiac autonomic regulation and through electrophysiologic effects on heart cell membranes. Heart rate variability (HRV) and baroreflex sensitivity (BRS) reflect cardiac autonomic regulation; reduced values predict arrhythmic events and mortality. Effects of n-3 fatty acids on these risk indicators of arrhythmia have not widely been studied. A standard electrocardiogram (ECG) may be used to detect clues as to the mechanism by which n-3 fatty acids affect the electrophysiology of the heart.

Objective : To investigate the effect of supplemental intake of n-3 fatty acids on autonomic regulation and electrophysiology of the heart in apparently healthy subjects aged 50 to 70.

Materials and methods: After a run-in period of 4 weeks, 84 subjects were randomized to receive capsules with either 3.5 g fish oil or placebo oil daily for 12 weeks. Before and after intervention, ECGs and blood pressure were recorded for 10 minutes with standardized respiration of 15 breaths per minute.

Results : HRV and BRS did not significantly improve by the intake of n-3 fatty acids. HRV decreased by 3.05 ms or 7.7% (95% confidence interval, -8.91 to 2.82 ms) and BRS decreased by 0.92 ms/mmHg or 0.1% (95% confidence interval, -2.66 to 0.81 ms/mmHg) in the fish oil group compared to the placebo group. ECG characteristics were hardly affected by n-3 fatty acids.

Conclusions : N-3 fatty acids have no substantial effect on indicators of autonomic regulation in healthy subjects. Also, these results can not support the hypothesis that (n-3) fatty acids prevent arrhythmia through electrophysiologic effects on heart cell membranes. Potential effects of n-3 fatty acids on autonomic regulation and electrophysiology of the heart should be studied in more susceptible populations.

Stability and radical scavenging capacity of heated olive oil

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Introduction : Virgin olive oil has a remarkable radical scavenging capacity. However, very little is known about the loss of this capacity, when the oil is used in domestic frying.

Objective : The objective of this study was to evaluate the stability of heated olive oil in relation to its radical scavenging capacity (RSC) and to compare these properties with those of other plant oils used in frying.

Materials and methods : Oils (virgin and refined olive oil, refined sunflower, cottonseed and soybean oil and a commercial mixture special for frying) were purchased from the retail market or donated by a plant located in the area of Athens. The oils were heated at 180∞C for 10 hours. RSC was measured spectrophotometrically using the 2,2-diphenyl-1-picrylhydrazyl radical. Results were expressed as Trolox equivalents. Polar compounds (PC) content was measured using the standard IUPAC method.

Results : Initial RSC values were practically similar for all the oils tested. Virgin and refined olive oil reached rejection point (27% PC) after prolonged heating (more than 10 hours), but they lost their RSC in 5 hours. Sunflower, cottonseed, soybean oils and the commercial frying mixture reached rejection point in a shorter period of heating in relation to virgin olive oil, but they retained longer their RSC.

Conclusions : It is clear from the results that a different evaluation is needed if the oil is seen only as a frying medium and a different one if the frying oil has to be also a source of natural antioxidants. Virgin olive oil, which is widely used as a salad oil, seems to retain its nutritional value if used in frying for a limited number of frying operations.

Bioactive constituents from *Cichorium pumilum*

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Introduction : Leaves and roots of *Cichorium species (C. intybus L., C. endivia L., C. pumilum* Jacq., Asteraceae) have been not only used as a food supplement but also for medicinal purposes to promote appetite and digestion. The plants elaborate sesquiterpene lactones, including bitter lactucin-like guaianolides, as their characteristic secondary metabolites, some of which possess interesting biological activities. For example, the eudesmanolide magnolialide present in roots of the three above mentioned species and in leaves of some *C. intybus* cultivars appeared to induce differentiation of human leukemia cells to monocyte/macrophage cells [1].

Objective : Phytochemically, roots of *C. pumilum* have not been analysed in detail. To date only magnolialide and its derivative artesin have been reported. Therefore, we decided to search for further secondary metabolites that might be present in the plant material.

Materials and Methods : The compounds were extracted with ethanol and separated from the extract using column and thin layer chromatographies on silica gel followed by semiprep. RP HPLC. They were identified by spectral comparison with reference compounds from our collection or with reported values.

Results : As well as re-isolating the two previously known root constituents, we were able to obtain two further closely related eudesmanolides along with eight lactucin-like guaianolides and five phenolics.

Conclusion : Roots of *C.pumilum*, *C. intybus* [2] and *C. endivia* [3] appear to be comparatively uniform in their sesquiterpene lactone compositions; six of the isolated compounds, i.e. magnolialide and the bitter lactucin-like guaianolides 8-deoxylactucin, lactucin, 11b,13-dihydrolactucin, lactucopicrin and picriside B, are held in common.

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Lipoprotein profile and prevalence of cardiovascular risk factors in an urban women population of El jadida in Morocco

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Aim : In Morocco, dietary habits are exclusively managed by the women, that is why we have to implicate them to touch all the population. The objective of the study was to determine the lipid and apolipoprotein profile, evaluate the prevalence of cardiovascular risk factors and their relationship to age in an urban adult women population of El jadida in Morocco.

Design : A total of 213 women, aged 25-55 were included in this study. All the women included were teachers or manual workers, which is representing the most important part of urban women in El jadida. Lipid and apolipoprotein profiles were studied including measurement of plasma triglycerides (TG), plasma cholesterol (TC), triglyceride-rich lipoprotein triglycerides (TRL-TG), TRL-cholesterol (TRL-C), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), apolipoproteins A1, B, B48, CIII and E. Body mass index (BMI) and blood pressure (BP) were assessed.

Results : The women studied showed the following pattern: elevated (over normal levels) TC and LDL-C levels in 10% and 19.4%, respectively; low HDL-C levels in 45.3%, elevated TG levels in 11.8%. Obesity (BMI > 30) and hypertension were highly prevalent ie in 23.9% and 16.5%, respectively. Elevated TRL-C level (> 0.6 mmol/l) was present in 13.4% and elevated TRL-TG level (> 0.8 mmol/l) was present in 13.4%. Plasma triglyceride concentrations were closely correlated with plasma TRL-TG (R=0.86, P=0.0001), apoB (R=0.50, P=0.0001) and apoCIII (R=0.52, P=0.0001) concentrations and moderately correlated with HDL-C levels (R=-0.3, P=0.0001) and BMI (R=0.4, P=0.0001). The association between BMI and systolic blood pressure was statistically significant (R=0.3, P=0.0001). Obesity, BP, TRL-C, TRL-TG, TG, apoB and apoCIII significantly increased with age.

Conclusion : High prevalence of some risk factors for cardiovascular disease including altered lipid and lipoprotein profiles was found in the Morocco urban women population studied. In addition, we found important associations between age, especially above 35 y, and some risk factors. This suggests that such Mediterranean populations are at high risk for cardiovascular disease and that dietary interventions should take an important place in prevention strategy.

Is Omega–3 PUFA of benefit for Mediterranean patients with coronar y artery disease ?

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Introduction : A low frequency of ischaemic heart diseases in Eskimos has been related to polyunsaturated fatty acids.

Objective : To examine fatty acid patterns associated with coronary artery disease (CAD) for a possible relationship between fatty acid profile and CAD diagnosis in Mediterranean patients

Methods : A total of 50 consecutive patients were included. The gas chromatography method was used to analyze the membranes of erythrocytes from patients. The patients without coronary stenosis were used as controls (n=24).

Results : Patients with CAD (n =26) showed increased percentages of saturated fatty acids (35.8 vs. 34.2%, $p<0.001$) and monounsaturated fatty acids (14.6 vs. 13.6 %, $p<0.01$), as well as reduced percentages of polyunsaturated fatty acids (38.5 vs. 41.3% $P<0.001$). The decrease in polyunsaturated fatty acids percentages was due to the series of n-3 fatty acids (9.2 vs. 11.4 %, $p<0.001$) and docosapentaenoic acid (C22 :5(n-3)) (2.99 vs. 3.94%, $p<0.001$), mainly at the expense of docosahexaenoic acid (C22:6 (n-3)) (4.92 % vs. 6.41 %, $p<0.001$). The study shows altered n-3 fatty acids in Mediterranean patients with CAD. Our data suggest that the percentage of docohexaenoic and docosapentaenoic acids in erythrocytes could be used as indicators of an independent risk factor for coronary artery disease.

Conclusion : Fatty acid dietary interventions could be of benefit for mediterranean patients with CAD.

OMEGACOEUR ® a mediterranean nutritional complement, inhibits monocyte adhesion to human endothelial cells

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Introduction : Mediterranean diet is known to protect from cardiovascular diseases. The Mediterranean nutritional complement Omegacoeur® (Laboratoire Holistica International, France) consists of a mixture of different natural oils enriched in w3, w6 and w9 fatty acids. Objective :As w3 polyunsaturated fatty acids exhibit anti-inflammatory properties, we evaluated the effect of Omegacoeur® on monocyte adhesion to endothelial cells.

Materials and Methods : Monocyte adhesion was induced by exposing human endothelial cells to TNF (tumor necrosis factor, 50 ng/ml). Endothelial cells were incubated with either standard culture medium, TNF alone or TNF + Omegacoeur® for 24h. For the adhesion assay, the amount of fluorescent monocytes attached to the endothelium was measured using a fluorimeter.

Results : Exposure of human endothelial cells to TNF increased monocyte adhesion by 37%. The use of Omegacoeur® decreased the TNF-induced monocyte adhesion (-13%).

Conclusion : Omegacoeur is able to antagonize monocyte adhesion to TNF-stimulated endothelial cells and may be effective in preventing or treating cardiovascular diseases.

Vegetable consumption (*Daucus carotta* and *Lactuca sativa*) improves cholesterol metabolism and antioxidant status in animals fed cholesterol enriched diet

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Introduction : Epidemiological studies have shown that a high consumption of fruits and vegetables is associated with a decreased risk of cardiovascular diseases, hypertension and cancers. Vegetables are a rich source of fiber, which can modulate cholesterol metabolism, other antioxidant compounds (vitamins, carotenoids and polyphenols) and minerals.

Objective : We investigated the effect of vegetable, focusing on lettuce and carrot consumption, on biomarkers of risk for cardiovascular diseases. Vegetables are a rich source of dietary antioxidants which may be implicated in the prevention of risk factors development. Risk factors include high blood pressure, high serum cholesterol or LDL-cholesterol.

Materials and methods : Different animal models (Wistar, C57BL/6J mice or SHR) were fed either a control diet or a vegetable enriched diet (20% of dry weight) for 4 weeks (8 weeks for SHR). Besides lipemia and cholesterol metabolism parameters, we evaluated the plasma and urine levels of malondialdehydes and isoprostanes as markers of oxidative stress.

Results : In all models, apparent absorption of cholesterol decreased leading to lower total plasma cholesterol and triglyceride concentrations in animals fed vegetable diet, especially when dietary cholesterol was added to the diet. Lettuce also induced a decrease of cholesterol content in low density lipoproteins.
Antioxidant status was improved in mice fed carrot diet as shown by FRAP, vitamin E, carotenoids, vitamin E/TG ratio measurements. In SHR rat, we observed a reduction of lipid peroxidation in lipoproteins (LDL) when fed carrot diet. Vegetable consumption influences other biomarkers of oxidative stress: we observed a decrease of TBARS and isoprostane urinary excretion or a decrease of peroxidability of heart tissue in rats fed lettuce diet.

Conclusions : The daily consumption of vegetable has hypolipemic effects and improves antioxidant status. The complex and multifunctional effects of vegetables can contribute to several benefits impacts on human health, not only on cardiovascular diseases but also on oxidative pathologies including some cancer and osteoporosis.

Blood pressure and hypertension in the greek population in relation to diet : a study of 28000 adults across the countr y

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Introduction : Hypertension is responsible for a substantial fraction of cases of cardiovascular diseases, particularly stroke. From a public health perspective, hypertension ranks together with smoking, obesity, physical inactivity and inappropriate diet as principal targets. Hypertension may be particular important for Greece since mortality from stroke in this country far exceeds the European average.

Objective : To identify predictors of systolic and diastolic blood pressure, as well as hypertension.
Subjects & Methods: Data at enrolment concerning blood pressure and possible sociodemographic and dietary determinants were retrieved for over 28000 Greek adults who participate in the Greek component of the European prospective investigation EPIC. Two approaches were used in the analysis: multiple regression of blood pressure (alternatively systolic and diastolic) among individuals not on anti-hypertensive treatment on a series of possible predictors; and multiple logistic regression contrasting hypertensives to non-hypertensives on the basis on the indicated possible predictors.

Results : The prevalence of hypertension is high in Greece and increases with age. The findings from the alternative analysis converge in indicating that predictors of high blood pressure or hypertension are: older age, low socio-economic status, rural residence, high body mass index, high waist to hip ratio, and low consumption of plant foods.

Conclusions : From a public health perspective, control of hypertension is as important for the Greek population as control of tobacco smoking and obesity. The beneficial role of high intake of plant foods underscores the importance of the traditional Meditteranean diet.

Flavonols and flavones in Bulgarian vegetables and fruits

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Introduction : Multiple scientific surveys prove the preventive role of Mediterranean diet against the development of modern diseases. This fact could easily be explained with the abundance of bioactive components contained in it, represented mainly by flavonoids. They appeared on the scientific nutrition scene after the French Paradox was unveiled. It turned out that the foods typical for Mediterranean and Balkan diets are a rich source of flavonoids. The knowledge on their diverse range is of great importance for the assessment of foods and nutrition.

The aim of the study is to determine flavonols and flavones in vegetables and fruits, typical for Balkan Diet, and to assess their intake in Bulgarian nutritional habits.

Materials and methods : Flavonols myricetin, quercetin, kaemperol, and flavones luteolin and apigenin were determined in 25 vegetables belonging to 7 different plant families and in 17 fruits belonging to 3 families. The food samples were collected within the period 2001-2002, according the requirements for a representative sample (origin, variety, season, and quantity).
Flavones and flavonols were determined as aglycones after acid hydrolysis of freeze-dried food material, using an RP-HPLC method with UV detection.
The dietary intake of analyzed flavonoids was determined on the basis on Household Budget Surveys food data.

Results : In vegetables the highest flavonols contents were determined in red onion (538.0 mg/kg), followed by lettuce (169.7 mg/kg) and green pepper "Kapia" (117.8 mg/kg). Among the fruits the flavonols reach their maximum value in blueberry (144.1 mg/kg), share out for myricetin (43.4 mg/kg) and quercetin (100.9 mg/kg). Relatively high is their level in apricot (30.4 mg/kg), blackberry (30.5 mg/kg), and strawberry (25.1 mg/kg).
Flavones show high values in parsley (708.0 mg/kg) and in celery leaves (325.4 mg/kg) and celery roots (48.0 mg/kg).
The results for dietary intake of flavons and flavonols in Bulgaria show that the highest intake is from onion, followed by pepper, apples, tomatoes, grape, and pears – 5.5, 1.9, 1.5, 0.7, 0.2, 0.1 mg/day/person, respectively.

Conclusion : The presented results will enrich the database with values for flavonols and flavones content in foods typical for Mediterranean and Balkan diet. The possibilities for assessment of the food intake and for exchange of near information related to nutritive as well as biologycally active role of the food will be enhanced.

Quantitation of olive oil bioative components in Greek cultivars; Method devolepement for their determination in bilogical fluid

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Introduction
The olive oil, principal ingredient of the Mediterranean diet, contains a number of highly active metabolites such as oleuropein (OE), hydroxytyrosol (HT), tyrosol (T), elenolic acid (EA). These substances have been shown to possess antioxidant, anticancer, antibacterial and antiatherogenic properties. Therefore, the determination, as well as the bioavailability of the aforementioned substances is of vital importance.

Experimental
Quantitatitation of HT, T, OE and EAin various Greek cultivars : Weighted dried leaves from various Greek cultivars of Olea europea were extracted using various solvents. The extracts, after appropriate dilutions, were analyzed by a validated HPLC-DAD methodology for the quantitative determination of OE, HT, T and EA.

Biovailability studies of OE, T, HT and EA : OE and olive oil has been administered to rats for a six-month period and the total amount present in their biological fluids was assessed. The sample pretreatment was based on an SPE method using OASIS HLB cartridges. The identification and quantitation of the analytes (HT,T and EAfrom rat urine and plasma) was carried out by a validated HPLC-DAD methodology on a C8 column using gradient elution. An increased sensitivity GC-MS/MS MRM quantitative method was also developed, where the analytes were converted to their corresponding trimethylsilysl ethers prior to the analysis.

Results and discussion
– There is a significant variation of the bioactive substances content in the various Greek Olea europea cultivars and therefore careful selection must be done in order to achieve the highest human health benefits.
– The methodologies developed for the bioanalytical studies have been shown to be accurate, precise and sensitive enough for the detemination of the aforementioned analytes in biological fluids. Moreover initial results obtained show that HT and T are excreted in rat urine. Further LC-ESI-MS/MS research is underway to improve the sensitivity and specificity of the analysis.

Apple polyphenols inhibit secretion of intestinal triglyceride-rich lipoprotein

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Introduction : Several epidemiological studies have suggested an association between a diet rich in fruits and vegetables and a lowered risk of cardiovascular diseases (CVD). Polyphenols contained in fruit and vegetables are reported to be protective against CVD, possibly through the modulation of the lipid metabolism, e.g. inhibition of lipoproteins oxidation or hepatic cholesterol synthesis. Furthermore, consumption of three apples per day has shown to decrease hyperlipemia. More recently, post-prandial hypertriglyceridemia, which depends partly on intestinal lipoprotein secretion, has been proposed as a major risk factor of CVD.

Objective : We therefore hypothesised that apple polyphenols could control intestinal triglyceride-rich lipoprotein (TRLP) synthesis and secretion.

Materials and methods : Using human intestinal Caco-2/TC7 cells cultured on a microporous filter, we analysed the effect of apple-polyphenol addition on lipid absorption and TRLP secretion under an apical supply of complex lipid micelles, the composition of which mimicked that of post-prandial duodenal micelles.

Results : We showed that polyphenols inhibit the secretion of TRLPin a dose-dependent manner without any modification of fatty acid and cholesterol absorption and of triglyceride and phospholipid intracellular levels. In contrast, intracellular and secreted cholesteryl-esters were decreased by 32% and 58% respectively in the presence of 200 µg/ml of total apple-polyphenols extract, suggesting that polyphenols inhibit acyl-CoAcholesterol acyltransferase (ACAT) activity. In order to characterise which components could mediate the blockade of secretion, total apple polyphenols extract were fractionated by HPLC. Only the procyanidin-enriched fraction was able to inhibit TRLP secretion. Moreover, using CP-113818, an ACAT inhibitor, we were able to mimic the effect of apple polyphenols.

Conclusion : Together, these results suggest that apple procyanidins are responsible for the observed inhibition of TRLP by a mechanism which could implicate ACAT activity.

Beneficial changes in CHD risk factors in subjects on a Mediterranean diet for 3 months : The Medi-RIVAGE study

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Aim : The Medi-RIVAGE study is a dietary intervention where we randomly study a Mediterranean-type diet (MED : plenty of vegetable food and fish; 35% fat with half as monounsaturated) and the usual low fat prescribed diet (AHA-type : 30% fat, one third as monounsaturated), derived from the American Heart Association recommendations, in 212 volunteers who already have one or more cardiovascular risk factors for coronary heart disease (CHD). From the Seven Countries Study, the Framingham study and others numerous ones, risk factors for CHD have been identified. In this poster, we investigate the change in main CHD risk factors in the 88 subjects of the Medi-RIVAGE study which chronically ingested the Mediterranean diet for 3 months.

Design : The subjects, men or women, are included with at least one cardiovascular risk such as high plasma cholesterol or triglycerides level, BMI>27 kg/m2, cigarette smoking, etc… 212 subjects have been enrolled, and their new dietary habits are maintained for 3, then 12 months. The benefits of the diet are estimated by clinical evaluation, fasting biochemical parameters quantification and investigation of postprandial lipid metabolism.

Results : Comparison of subjects with defined risk factors at inclusion and after intervention with the Mediterranean diet for 3 months shows the following significant changes ($p<0.05$ t-test for paired samples): Body Mass Index (BMI) 28.7 kg/m2 at enrolment vs 27.7 kg/m2 after 3 months (55.7% above 27 kg/m2 at enrolment vs 46% after 3 months); fasting total blood cholesterol 6.6 mmol/l vs 6.1 mmol/l (53.4% above 6.6 mmol/l vs 33.3%); fasting LDLblood cholesterol 4.4 mmol/l vs 3.9 mmol/l (58.9% above 4.1 mmol/l vs 32.2%); fasting blood triglycerides 1.6 mmol/l vs 1.5 mmol/l (20.5% above 2.0 mmol/l vs 13.6%); fasting blood insulin 10.5 µU/ml vs 8.3 µU/ml (31.8% above 12 µU/ml vs 12.0%); fasting blood glycemia 5.3 mmol/l vs 5.1 mmol/l (14.8% above 6.1 mmol/l vs 6.9%). No significant change in fasting HDLcholesterol was observed 1.5 mmol/l vs 1.5 mmol/l (36.4% below 1.3 mmol/l vs 38.6% after 3 months).

Conclusion : After 3 months diet, the Mediterranean diet significantly reduces the risk to develop CHD by decreasing numerous risk factors for the disease.

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