Focus on nutrition, cardiovascular disease and cancer: ACNS National Meeting 1996

The ACNS Meeting was held in Sydney on the 30th September, 1996. It was, in part, a joint meeting with the Nutrition Society of Australia with common lecture sessions in the morning and an option of ACNS Symposia in the afternoon and an evening session primarily aimed at General Practitioners.

The Plenary Lecture in the morning was by Prof Alan Husband from the Dept of Veterinary Pathology, University of Sydney. His topic was 'Nutrition, stress and immune activation[1]. Professor Husband discussed how the response to stress (physical, social or microbial) provokes an integrated reaction involving the immune system (via cytokines), the cental nervous system (via nervous output) and the endocrine system (via hormones), each influencing and influenced by the other physiological responses to environmental change. He explained that in this context there was a close link between nutrition and immunity, in that nutritional deficiencies may cause stress or may alter CNS output and thereby impact on immune function. He also proposed that changes in immune status have a feedback effect on nutrient intake and utilisation partitioning such that inappropriate immune activation has deleterious effects on growth and development. His talk was complemented by examples from animal nutrition, but many of the issues may have common counterparts in human work.

In the subsequent symposium on Nutrition and Immunity, Dr Michael James from the Rheumatology Unit at the Royal Adelaide Hospital, spoke on 'Dietary polyunsaturated fats and inflammation'. Dr James discussed the influence of n-3 and n-6 fatty acids in our diet and the biochemical interactions that may effect the immune status. There is considerable evidence that increasing the amount of dietary n-3 fat can suppress inflammatory mediator production and thus inflammation. He suggested mechanisms for these effects and how high levels of n-6 fats in the diet may reduce the optimal anti-inflammatory effects of n-3 fats. On a practical level he was keen to explore the interaction of diets high in n-3 and lower in n-6, together with anti inflammatory drugs in inflammatory joint disease.

Dr Judy Carman from the Communicable Disease Control Branch, South Australian Health Commission then spoke on 'The relationship between anti oxidants and immune function in healthy men'. She described a study in which 64 healthy men were examined for the relationship between blood concentrations of the anti oxidants zinc, selenium, β-carotene and vitamins A, C and E with immune function (as measured by the CD4 number), whilst taking into account the potential confounders-- smoking, alcohol consumption, hepatitis and active exercise. They found that only β-carotene showed a relationship with CD4 number, a higher β-carotene being associated with a lower CD4 number.

Dr Ann Swain, from the Allergy Service, Dept of Immunology, Royal Prince Alfred Hospital in Sydney, spoke on 'Food allergy'. Anne discussed food allergy as a common cause of morbidity in children and the importance of recognition of the condition so that treatment can be effected. She acknowledged that this is sometimes difficult because the presence of hidden food allergens in commercially prepared and packaged foods may not be easy to identify. She also noted that distinguishing clinically between true allergic reactions and non-immunological intolerances is important, the latter being the more common cause of adverse reactions in the community.

The ACNS Plenary session consisted of four talks: 'Micronutrients and CVD' by Dr Samir Samman, 'Micronutrients and Cancer' by Dr Dorothy Mackerras, 'Macronutrients and Cancer' by Prof Stewart Truswell and 'Macronutrients and CVD' by Prof Madeleine Ball. These reviewed the areas of our knowledge and also some areas of uncertainty in our appreciation of the inter-relationship of diet and these two common diseases. The abstracts of these talks follow.

There were a small number of Free Communications and Posters, on food intake and cardiovascular risk factors in Beijing, presented by staff from the Monash Medical Centre. Abstracts of these papers and a couple of posters are included at the end of this report.

The ACNS evening program was entitled 'Dietary advice in the Nineties- Prudent, Practical, Preventative?' After a very pleasant dinner, the session opened with Dr Ross Walker giving a very amusing and practical description of sensible lifestyle advice for prevention of cardiovascular disease. His emphasis was very much on sensible eating and lifestyle, some red wine and taking time to relax and enjoy life! Associate Prof David Colquhoun then spoke on 'Dietary advice in the nineties' and promoted the use of the modified fat or Mediterranean diet as a palpable diet to reduce cardiovascular risk and to improve compliance to the dietary prescription. Dr David Sullivan discussed how the practical nutritional advice we are promoting was applicable to both cardiovascular disease and cancer, in that a reduction in saturated fat and an increase in fruit and vegetables had very likely benefits for both these disorders.

The ACNS committee was pleased with the results of the meeting, which allowed some exchange of ideas with members of the Nutrition Society and interaction with a number of local General Practitioners. The next meeting will be in conjunction with the Nutrition Society of Australia, in Brisbane in late November and again will provide an opportunity in the afternoon/evening for the involvement of local doctors. We thank the organisers, speakers and everyone who attended in 1996 for helping to make the meeting a success. Enquiries about the 1997 meeting should be addressed to David Colquhoun.

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Nutrition, stress and immune activation

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The response to stress (physical, social or microbial) provokes an integrated reaction involving the immune system (via cytokines), the central nervous system (via nervous output) and the endocrine system (via hormones) each influencing and influenced by the other physiological responses to environ-mental change. In this context, the concept of a link between nutrition and immunity is readily appreciated, in that nutritional deficiencies may cause stress or may alter CNS output and thereby impact on immune function. However, this paper addresses some facets of nutrition-immune interactions which are less obvious. While the selective effects on immunity of individual components of the diet, and the effect on selective components of the immune system of nutrient imbalance are addressed, the concept is proposed that changes in immune status have a feedback effect on nutrient intake and utilisation partitioning such that inappropriate immune activation has deleterious effects on growth and development. The potential mediators by which these effects occur are explored.

Dietary polyunsaturated fats and inflammation

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Dietary polyunsaturated fats are classified as n-3 or n-6 according to their double bond chemistry and these chemical differences confer differential biological effects on fatty acids from these two classes. In the modern Australian diet, the intake of n-6 fats exceeds that of n-3 fats by approximately 25-fold. This relative abundance of n-6 fat intake is reflected in the cell membranes where the ratio of n-6:n-3 PUFA is approximately 7:1. While this relative excess of n-6 to n-3 fat has been driven by agricultural and industrial changes as well as dietary changes aimed at lowering blood cholesterol levels, there is considerable evidence that increasing the amount of dietary n-3 fat can suppress inflammatory mediator production and can suppress inflammation. Animal studies using models of inflammatory disease have demonstrated that ingestion of fish oil, rich in n-3 fats, can suppress inflammation. In human studies, at least 11 double-blind, placebo-controlled clinical trials with rheumatoid arthritis patients have demonstrated that dietary supplements of fish oil can provide symptomatic benefits. The mechanisms for these clinical responses lie in the effects which n-3 fats have on the production of inflammatory mediators. Dietary fish oil which contains 20- and 22-carbon n-3 fatty acids and flaxseed oil which contains their 18-carbon n-3 progenitor fatty acid, can inhibit the production of the eicosanoid inflammatory mediators, prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) and the cytokine inflammatory mediators, interleukin-1β (IL-1β) and tumour necrosis factor-α (TNFα). Because n-6 fats can decrease the levels of n-3 fats in cell membranes, it is most likely that the optimum anti-inflammatory effects of n-3 fats will be within the context of diets also containing lower levels of n-6 fats than those in the current Australian diet.

The relationship between antioxidants and immune function in healthy men

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Antioxidant vitamins and minerals have been linked to immune function, with deficiencies linked to an impaired immune function and supplementation linked to an improvement in immune function. However, few studies have either considered this relationship in relatively healthy individuals in the community or accounted for potential confounders of the relationship, such as smoking, alcohol consumption, hepatitis and active exercise. This study explores, in 64 relatively healthy men, the relationship between the concentration of the antioxidants zinc, selenium, β-carotene and vitamins A, C and E with immune function (as measured by the CD4 number), whilst taking into account the potential confounders, smoking, alcohol consumption, hepatitis and active exercise. The results indicate that under these conditions, these antioxidants have no relationship with the CD4 number, except for β-carotene. β-carotene was found to have an inverse relationship with the CD4 number, so that a higher concentration of β-carotene was associated with a lower CD4 number.

Food allergy

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In young children food allergy is a common cause of morbidity due to its association with atopic eczema and gastrointestinal disorders. Although most children grow out of food allergies before puberty, highly sensitised individuals can have life-threatening reactions. Recognition is important since successful avoidance can prevent the occurrence of morbidity and mortality. However, the presence of hidden food allergens in commercially prepared and packaged foods can make this difficult, and mounting evidence of morbidity and mortality from accidental ingestion is a cause for concern. It is also important to distinguish clinically between true allergic reactions and

non-immunological intolerances, the latter being more common causes of adverse reactions in the community. Better understanding of this distinction by the public as well as health professionals should help to clear up much of the confusion surrounding food allergy, and should also help to eradicate many of the misguided testing and treatment methods which have plagued this area for over half a century.

Micronutrients and coronary heart disease

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A number of minor dietary constituents have been shown to affect coronary heart disease (CHD). These include some recognised nutrients as well as other substances, mostly derived from plants. A number of different mechanisms are involved including:

(i) antioxidant (or pro-oxidant) activity which impacts on the oxidisability of low density lipoprotein (LDL),
(ii) alteration in the lipoprotein profile or
(iii) effects on steroid hormone metabolism.

As an example, it has been reported widely that vitamin E is negatively associated with CHD. Vitamin E, or specifically α-tocopherol, confers its protective activity probably by reducing the oxidisability of LDL. Vitamin C acts synergistically with α-tocopherol by regenerating it from its major oxidised product, the tocopheryl quinone. In addition, vitamin C is associated (epidemiologically) with an elevation in the anti-atherogenic high density lipoprotein (HDL) which not only promotes reverse cholesterol transport but may also reduce the oxidisability of LDL. Flavonoids are a family of compounds found ubiquitously in plants and are considered non-nutritive. They are potent inhibitors of the oxidative modification of LDL and foods containing these compounds, such as tea and red wine, have been shown to be negatively associated with CHD. Flavonoids reduce the formation of free radicals, protect or regenerate α-tocopherol or alternatively, they chelate divalent metal ions and prevent them from participating in the Fenton reaction. Less is known about other minor dietary constituents, such as boron, which is found in fruits and vegetables.

Boron may have an impact on CHD by inducing small increases in the concentration of plasma oestrogen and testosterone but in animals it has been shown to reduce the concentration of HDL. The regular consumption of fruits and vegetables and moderate consumption of beverages such as red wine and tea may protect against atherosclerosis and thrombotic tendency. More research is required to elucidate the mechanisms by which minor dietary factors are absorbed, metabolised and interact with other nutrients *in vivo*.

The presence of a large number of minor dietary factors that protect against CHD reinforces the recommendation to eat a variety of foods and to increase the intake of fruits and vegetables rather than the consumption of high doses of any specific supplement.

Micronutrients and cancer

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The way diet-disease relationships are envisaged has changed over the last 20 years. Formerly, the focus was on diseases for which the lack of a nutrient was both the necessary and the sufficient cause of the disease. (Indeed, this is the basis of the definition of a nutrient.)

Nowadays, much attention is given to the possible role of a variety of food factors in preventing diseases such as cancer. These diseases are 'multi-factorial' in contrast to the 'uni-factorial' nature of the deficiency diseases. This means that lack or excess of the food factor is neither necessary for the disease to occur, nor, on its own, sufficient to make the disease occur. This makes relationships much harder to study - we have to talk about changes in the probability with which rare events are occurring. Studies have to be larger, because most study subjects, even those at high risk, will not get the disease. Studies have to last longer to deal with latent periods. Hence they are much more expensive and so there has to be reasonable evidence that a relationship might exist before it would be sensible to spend money on a trial to confirm or refute the hypothesis.

A series of case-control studies examining the effect of diet on lung cancer started with showing an inverse association for vitamin A. The hypothesis shifted to β-carotene and later to other nutrients in food. Other cancers were also studied, although the relationships found were not as consistent as those involving respiratory cancer. A number of different foods were examined, but the associations were not consistent for any one food type although there was a general pattern for people with high fruit and vegetable consumption having a lower incidence of cancer. Studies using biochemical data, rather than reported dietary intake, are not necessarily superior because the disease may alter the levels of the nutrient. At least one cohort study has shown that the association between lung cancer incidence and low serum β-carotene levels declines with time after measurement.

These data show that low dietary intake or serum levels 'predict' cancer incidence in a descriptive sense: they identify individuals at higher risk. They suggest that changing the level of dietary factors may lead to a change in the incidence of cancer, but they do not prove it. This hypothesis can only be proved by a study in which intakes are actually changed - a randomised controlled trial. Results from trials reported to date indicate that, with few exceptions, high doses of β-carotene, vitamin C and vitamin E do not alter the incidence of cancers or precancerous lesions. Indeed, two large studies have found that smokers or those with a history of asbestos exposure have a higher incidence of lung cancer if they took β-carotene. A third study, with only a small proportion of smokers, found no difference in lung cancer incidence in the active and placebo arms. Hence there is no evidence to date that taking high doses of these nutrients will reduce the incidence of cancer. Smokers should be dissuaded from taking β-carotene (although it should be remembered that the increase in risk is very small in relation to the risk due to smoking).

These trials do not answer all possible questions. For example, the effect of increasing intakes via the diet remains to be tested. However, the contradictory results from the lung cancer studies raise interesting questions for regulatory bodies concerning the type and level of proof that is needed for things such a health claims and functional foods.

Macronutrients and cancer (colo-rectal and breast)

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There is more information on colo-rectal cancer and breast cancer than other cancers. Doll and Peto (1981) considered that 90% of avoidable colo-rectal cancer deaths were related to diet; Willett (1995) revised the dietary contribution to 70% because of clear evidence that physical activity plays an important protective role. There are strong genetic contributions to colo-rectal cancer (CRca) with familial adenomatous polyposis and several other syndromes. CRca usually develops from adenomas. This precancerous condition makes research on the aetiology easier. The changes in DNA along the sequence from a tiny adenoma to metastasising carcinoma are better known than for other cancers. A characteristic change is hypomethylation of DNA. In half of over 20 case-control studies and in the US Nurses prospective study dietary fat was a risk factor. In the latter, this was animal, not vegetable, fat and the incidence of CRca is low in Greece despite considerable consumption of olive oil. The mechanisms for the effect of fat might be increased concentration of secondary bile acids, or free fatty acids in the large intestine or diacylglycerol. Red meat was a risk factor in 10/22 case-control studies and in the US Nurses prospective study. The mechanism here may be via heterocyclic amines (IQ, MeIQ, etc) on the surface of well-done meat, which are potent mutagens. Plant foods were protective in 15 of Trock's 23 case-control studies and in the US Nurses prospective. The latter was not statistically significant but fibre and folate were significantly protective against adenomas in the male Health Professionals prospective study. Resistant starch adds to the metabolic effects of fibre (or NSP) in the colon: dilution, lowered pH, production of butyrate, more rapid transit. Vegetable foods also may protect via anti-cancer compounds: glucosinolates in brassicas, sulphur-containing compounds in the onion family, carotenoids, etc. Alcohol appears to be a risk factor for rectal cancer, and calcium may protect against CRca by neutralising fatty acids. The Australian Polyp Prevention Trial (APPT) shows that low fat diet + wheat bran significantly reduce development of CR adenomas. At least 7 trials of similar design to the APPT are ongoing, with calcium or fibre or resistant starch or mixed vitamins or aspirin alone or in combinations being tested.

Breast cancer (BC) is estimated by Doll, Peto (1981) and by Willett recently to be 50% avoidable by dietary change– but which diet is so far elusive. In communities with high incidences of BC the majority of cases are post-menopausal. There is no easily sampled precancerous lesion and the genetic contribution only accounts for 2 to 5% of the aetiology. Early menarche and/or late menopause increase the risk, bilateral oophorectomy protects, and endogenous plasma oestrogens are higher in post-menopausal BC. Prolonged lactation gives some protection but only against the less common pre-menopausal BC. Between countries BC mortality correlates very well with national apparent fat consumption. But less than half of such consumption is by women (men eat more than half the food) and several other indices of affluence correlate nearly as well (eg. sugar consumption).

In rodents given chemical carcinogens (eg DMBA) fat acts as a promoter of mammary tumours. In humans however, plasma cholesterol does not predict BC in prospective studies and adipose fatty acid pattern is the same in BC cases as controls. Over 20 case-control studies have been reported. Overall, eg in a meta-analysis of 16 studies in 11 countries the summary relative risk (RR) was only 1.12 for fat and there was wide variability between studies. In the US Nurses study, however, and in another 6 prospective studies the summary RR for fat (by quintile) was a non-significant 1.05 and did not change down to fat intakes of 20% energy. It now seems therefore that fat is unlikely to be an important risk factor in adults. It may, however, have an influence in adolescent girls (height seems to be a risk factor for BC) or even in utero. Plant foods were reported protective in at least 10 case-control studies. A meta analysis of 12 such studies showed RR of 0.83 for fibre and 0.63 for vitamin C. Either of these may be surrogates for the active protective component(s). Promising possibilities include phytoestrogens (soya intake was protective in a Singapore study) or dietary fibre (interfering with reabsorption of oestrogens). Alcohol has emerged recently as a possible weak risk factor. The average RR is only around 1.25 and confounding cannot be excluded but alcohol showed up in case-control studies in different countries and in the US Nurses prospective study. Alcohol can raise plasma oestrogens. No intervention trials are under way with macronutrients except possible the NCI 20% fat trial, which has been on, off, on, off and maybe on again.

Macronutrients and cardiovascular disease risk

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Food consists of a mixture of macronutrients, micronutrients and non-nutrients. Differentiating the specific effects of individual nutrients can be quite difficult; for example, fat contains fat soluble vitamins and animal protein is rich in iron. There are also interactions between food intake and genetic predisposition and life style. Our evidence for the role of diet and its components on cardiovascular disease comes from a range of sources, including epidemiological and intervention studies. The end points have sometimes been cardiovascular disease (CVD) events, but more often have been changes in what we consider to be cardiovascular disease risk factors. These risk factors obviously include hyperlipidaemia, lipid

oxidation, raised blood pressure, central obesity, hyperglycaemia, insulin resistance, diabetes and thrombotic status. There is also now interest in the role of endothelial damage, endothelial local hormones, and vascular compliance with emerging evidence that diet may effect these parameters.

The association between saturated fat intake and CVD, and the different effects of saturated fats and unsaturated fats on lipid parameters have been known for many years. There is now further information that the various saturated fatty acids have very different effects on LDL, HDL cholesterol, and probably also on thrombosis risk, and that mono and polyunsaturated fatty acids alter the susceptibility of LDL to oxidation in opposite ways. Our detailed knowledge about the omega-6 and omega-3 fatty acids is also still evolving, although the beneficial effects of the omega-3 rich fish oils on triglyceride levels and haemostasis is well recognised. A few years ago concern was raised about trans fatty acids in the diet: the relevance for the overall Australian diet is unclear as intakes are estimated to be 2.7-4.8g per day, but moves to reduce the trans fatty acid content in some foods have been encouraged.

Concerning proteins, there have been a number of small studies published over the last 10 years looking at the possible benefits of soy protein. A recent meta analysis confirms the lipid lowering effects of soy compared to animal protein, particularly in hypercholesterolaemic individuals.

In the area of carbohydrate and carbohydrate rich foods, consumption of fruit, vegetables and whole grains is being promoted. The effects of various types of fibres, including soluble fibres in oat bran and beans, and of resistant starch on lipid and bile acid metabolism suggest these carbohydrates are beneficial. There is much interest in other forms of plant fibre, the lignans which can have phytoestrogen properties and also in other phytoestrogens present in foods such as soy products.

The relationship of alcohol to cardiovascular risk remains the topic of ongoing discussion in prime medical journals. There has been considerable debate about the role of alcohol per se and of the many other compounds in alcoholic beverages such as wine, where various flavonoids may contribute to the biochemical effects.

Our detailed knowledge of the influence of the various nutrients on metabolism and CVD risk factors, and the inter-actions related to the overall diet, lifestyle and the individual's genetic make-up is still increasing. The effect of the ApoE phenotype in determining fat clearance and LDL levels has been well reported but there is ongoing research on the role of genes for other apoproteins and lipoprotein lipase.

The Dietary Recommendations for Australians are a practical summary of our current knowledge on dietary measures likely to reduce the risk of CVD and a number of other chronic diseases.

Food intake patterns in Beijing Chinese

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Chinese food culture derives from one historical background. The eating patterns of Chinese people, however, is one that evolves and develops to interpret the very core food culture by adopting local and available resources into cooking, thus creating diversity of eating. Regional differences in cooking and eating have been documented in both ancient and modern Chinese. In this report, we study food intake patterns of 430 (men, 196; women, 234) Chinese adults living in Beijing, China. This study used a 156-item quantitative food frequency questionnaire. Last 12-month intakes were recalled. Subjects were randomly selected from the Chunwen district of Beijing.

Men and women differed in food consumed. Men had a significantly higher intake of wheat and wheat products and red meat, and drank more tea and beer than did women, while women consumed more leafy greens, fruits, nuts and seeds and eggs, and drank more milk than did men. Women also had a high food variety in the diet. Younger age groups and educated men and women incorporated a wider food variety in their diet. Household income was positively associated with red meat intake in men, and the consumption of non-staple wheat products and wine in women. The consumption of tea decreased with increasing education level, and increased with increasing age. The consumption of soft drinks was negatively associated with age. In women, the intake of legumes and products, leafy greens and cruciferous vegetables was negatively associated with education level and positively associated with age.

This cross-sectional study showed emerging evidence that soft drinks were favoured amongst the young and replace tea as a main beverage. In the past decades, China underwent major economic transformation. This begins to reflect in food intake patterns in sub-groups of the society (eg consumption of wine in women of higher income family). Education brought about changes in consumption of key culture marker foods (legumes, leafy greens and cruciferous vegetables) in women.

Cardiovascular risk factor prevalence in Beijing Chinese

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Degrees of economical development and environmental exposure have been associated with increased cardiovascular disease mortality in developed countries. Epidemiological evidence began to show increased cardiovascular disease risk factor prevalence in the fast growing Asia Pacific Region. 'People of Chinese ethnicity' is by far the largest population in the Region. While Chinese people share similar principles in food culture and perhaps genetic make-up, they differ in environmental exposure, such as local food supply. For these reasons, there is a case to study eating habits and cardio-vascular risk factor prevalence in Chinese people living in different parts of China and compare with those of overseas Chinese.

In this study, we report cardiovascular risk factor prevalence study of 433 (199 men and 234 women) Chinese adults living in Beijing, China. All subjects were randomly selected from Beijing Chongwen district according to the distribution of occupation reported in 1990 census.

The mean levels of age, systolic blood pressure (SBP), diastolic blood pressure (DBP), BMI, waist-to-hip ratio (WHR), CHOL, TRIG, HDL, LDL and LDL-to-HDL ratio, were 46 years, 121mmHg, 77mmHg, 23.4kg/m^2, 0.90, 4.59mmol/l, 1.29mmol/l, 2.77mmol/L, 1.22mmol/L and 2.44 in men, respectively; 45 years, 117mmHg, 73mmHg, 23.6kg/m^2, 0.82, 4.68mmol/l, 1.13mmol/l, 2.83mmol/l, 1.33mmol/l and 2.28 in women, respectively. The prevalence of overweight/obesity was 33.9% for men and 36.1% for women. 17% of men and 16% of women had defined hypertension. The prevalence of combined hyper-lipidaemia was less then 1% for men and women. 57% of men smoked regularly while 93% of women never smoked.

Compared to the collaborative study arms in Guangdong Province of China and Melbourne Australia, the Beijing Chinese had a moderate multiple cardiovascular risk factor (at least one of the three risk factors present) prevalence (men: Guangdong, 78%; Beijing, 62%; Melbourne, 40%; women: Guangdong, 35%; Beijing, 20%; Melbourne, 16%). Smoking accounted for differences in multiple risk factor prevalence in these Chinese populations; this is in spite of a high prevalence of hypertension and overweight in Beijing Chinese.

Dietary advice in the nineties - prudent? practical? preventative?

'Make food thy medicine' Hippocrates 400 BC

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An elevated plasma cholesterol and specifically low density lipoprotein cholesterol is the prime cause of coronary atherosclerosis. The plasma level is determined by genetic predisposition and environmental factors. The key environmental factor is the absolute amount of saturated fat (SFA). The most important cholesterol raising SFA are palmitic, myristic and lauric acids. The 25 year follow up of the Seven Countries Study has shown that risk factors are universal, but the force of a risk factor is determined by cultural factors. The 25 year follow up has shown that a cholesterol of 5.4mmol/l in the south of Europe is associated with a third of the risk as the same serum cholesterol in northern Europe.

Kritchevsky, in a number of experiments in the 1970's, demonstrated in the animal model that hypercholesterolemia does not always parallel atherogenicity. The recent Lyon Diet and Heart Study confirmed the cardioprotective effect of a Mediterranean diet. This post-infarction study, which compared a Prudent diet and a Mediterranean diet was to run for 5 years, but it was stopped prematurely due to the clear benefit of the Mediterranean type diet. There was a significant 70% reduction in total coronary events and a 70% reduction in total death rate. There is no difference in the serum cholesterol between the treatment groups.

The Seven Countries Study has also demonstrated that there is no relationship between percent of energy as fat in the diet and body weight. The NHANES I Study (7,000 subjects) compared body weight change over an 8-10 year period. Those with the lowest mean fat intake (27% E) gained weight the same as those with a high fat intake (47% E). The MONICA data on body weight across eighteen European countries also showed no evidence of an association between fat intake and median "BMI" among men and an inverse relationship among women. Over the last decade %E from fat in the USA has decreased from 36% to 34%. At the same time average adult weight has increased by 3kg. The key factors for obesity are genes and lack of physical exercise.

The dietary guidelines in the Western world almost universally suggest lowering fat as a percentage of calories to 30% or less. Recently the World Health Organisation (WHO) revised this to accept up to 35% of the calories as fat if the predominant fat is monounsaturated fat. Restriction of total fat is unnecessary on epidemiological, experimental and thera-peutic grounds. The emphasis on decreasing total fat was based on the misconception that this would have the benefit of lowering body weight and was the most effective for lowering cholesterol.

A low fat diet is associated with poor compliance and is unnecessarily restrictive. In the only direct comparative trial using clinical endpoints a high fat Mediterranean type diet was clearly superior in preventing CHD, and it of course tasted better.

Practical nutritional advice to avoid CVD and cancer

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The burden of disease has changed from communicable to noncommunicable diseases. Clinical nutrition has responded by looking beyond the problem of single diseases caused by deficiency of individual nutrients. Instead, it places greater emphasis on the relationship between the diet, as judged by the nutrient and energy balance of foods, and a broad group of degenerative disorders. Two of the most important of these are cancer and cardiovascular disease (CVD).

Diet may play an important role in the aetiology of a large proportion of cancers, although the proportion which are amenable to prevention by dietary intervention may not be quite as large. Unfortunately, others have made unjustified extrapolations concerning the possible treatment of established cancer resulting in unrealistic expectations amongst cancer sufferers. A summary of metanalyses of dietary risk factors for 6 of the most common forms of malignancy will be presented to demonstrate that the nutritional advice for prevention of CVD should also lessen the risk of cancer. Intake of plant food, avoidance of fat (especially of animal origin), and exercise reduce the risk of several types of cancer. The only inconsistency seems to be alcohol, which appears to reduce CVD risk, especially in men aged more than 40, whilst in women it mildly increases the risk of breast cancer.

There seems to be greater protection from foods rich in particular nutrients rather than from single nutrients used in isolation. Nevertheless, nutrients which may be important as far as cancer is concerned include antioxidants, phytochemicals, carcinogens and anticarcinogens. Antioxidants such as several vitamins and phytochemicals may reduce the oxidative damage to DNA, typified by the hydroxylation of guanidine to produce 8-OH guanidine.

Cancer appears to be less likely in individuals who maintain adequate plasma levels of Vitamin C (>50μmol/L) and alpha Tocopherol (> 30μmol/L). There is also evidence to suggest that smoking increases 8-OH-guanidine and that this can be rectified by consumption of large quantities of brussel-sprouts. Examples of food sources of several nutrients which reduce the risk of cancer will be discussed. Genistein will be discussed as an example of a nutrient which may prevent cancer by several mechanisms.

These concepts threaten to add to the complexity of nutritional counselling, so an alternative approach will be described. It is based on the fact that human genetic make-up has changed little since our ancestors relied on hunting and gathering for their food supply. In the meantime, there have been substantial changes in diet due to agriculture and modern food technology. The two most crucial elements associated with these changes are the decrease in dietary diversity and reduction in exercise. Lack of dietary diversity is associated with hypertension, obesity and diabetes. The simplified approach to nutritional advice relies on the concepts of the nutrient density and energy density of individual foods, and stresses the need for adequate dietary diversity and exercise. The outcome in individual patients depends on the interaction between genetic and environmental factors where the diet is one of the major environmental factors. We are in the early stages of identifying the interaction between genetic traits and nutrition, as illustrated by the increased risk of carcinoma of the colon in fast acetylators whose diet contains polycyclic hydrocarbons.

There is good evidence to assume that improved nutrition can reduce the risk of both cancer and CVD in individuals and the population at large.

Energy adjustment- the concepts underlying the debate

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Over the last decade, there has been quite a debate in the literature about how energy should be controlled in studies examining the relationship between diet and disease, especially when macronutrients are the focus of interest. The four methods commonly referred to-- the standard multi-variate, partition, density and residual methods-- are said to be mathematically interchangeable. Yet Kushi *et al*[1] have shown that different relative risks are obtained when each method is applied to the same data. How can this be?

The problem often relates more to the way study results are expressed than to mathematical minutiae. For example, we would expect the relative risk describing the association for an 80g difference in fat intake to be larger than the relative risk describing the association for a 20g difference in intake. However, most studies present the relative risk for the 'high' versus 'low' groups but do not always show what the difference in intake is between the groups. Once it is realised that the standard multivariate method calculates relative risk over a larger range than the residual method, the observation that the standard multivariate method generally gives larger relative risks than the residual method becomes self-explanatory. The difference in ranges between the two methods is much greater for the macronutrients than the micronutrients. Both results are correct and mutually consistent. However, if the results are to be used to estimate likely effects in the community, it is important to find the relative risk for the likely change and not to quote the relative risk for an unrealistic amount of difference.

There is a strong correlation- about 0.9- between fat expressed as a percentage of energy intake (density) and fat expressed as a residual (in grams). Hence both methods place individuals into the same groups and yield the same relative risks for 'high' versus 'low' intakes even though the units are different. However this may not apply to other nutrients.

This poster illustrates several other examples. In particular all methods except the partition method give a relative risk that summarises the effect of a change in all energy sources of the diet, whereas the partition method does not. Also, the fact that the micronutrients do not contribute to energy supply, which is the factor being adjusted for, alters some of the considerations related to this topic.

References:

1. Kushi *et al.* Dietary fat and postmenopausal breast cancer. J Natl Cancer Inst 1992;84: 1092-9
2. Mackerras D. Energy adjustment - the concepts underlying the debate. J Clin Epidemiol 1996 (in press)

Relationship of serum leptin to total and truncal body fat

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Leptin, the 16kDa protein product of the adipose specific obese (ob) gene, is postulated as being involved in the regulation of food intake and energy expenditure. Associations between serum leptin and BMI have been described. In this study the relationship between fasting serum leptin levels and measures of body fat distribution was determined in 183 women aged 20-80 years and BMI 17-43 kg/m^2. Body composition was assessed by both anthropometric measures and by Dual Energy X-ray Absorptiometry (DEXA) scans.

Serum leptin concentration was strongly positively correlated with most anthropometric and DEXA measures. The relationship between serum leptin (log transformed) and total grams of body fat r = 0.68 ($p< 0.0001$), and % body fat, r=0.76($p<0.0001$) were strongest. The relationship with most parameters, including that with BMI and waist circumference, ceased to be statistically significant when the effect of total body fat (measured by DEXA) or % body fat was adjusted for, using partial correlation analysis.

Truncal body fat, although a strong risk factor for cardiovascular disease and non insulin dependent diabetes, did not appear to have a strong relationship with serum leptin levels once total body fat was accounted for (r fell from 0.66 to 0.05).

The fate of postprandial TRL-apo(a) complexes

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The mechanism of clearance or catabolism of Lipoprotein(a) is not known. A proportion of apolipoprotein(a) appears to become associated with triglyceride-rich lipoproteins follow-ing fat intake. It is postulated that TRL-apo(a) may be cleared by receptor mechanisms, the more favourable being the LDL-receptor-related protein (LRP). The present study was undertaken to investigate the fate of TRL-apo(a) complexes following triglyceride (TG) hydrolysis by the enzyme lipoprotein lipase (LPL), to determine whether TRL-apo(a) remained intact or dissociated. This process may represent a pathway for Lipoprotein(a) catabolism.

Subjects consumed a fat-rich meal (1.5g fat/kg body weight) and venous blood was collected 3 h later. TRL of Sf > 400 was isolated by ultracentrifugation and incubated with d > 1.006 g/ml serum fraction (4 h, 37°C) to obtain TRL-apo(a). TRL-apo(a) was incubated with bovine LPL (67 U/ml mixture; LPL:TG ratio = 67) in the presence of fatty acid-free albumin (4% (w/v)) at 37°C for 5 h. The enzymatic reaction was terminated with diethyl p-nitrophenyl phosphate (2 mmol/l). In addition, negative controls containing heat-inactivated LPL, or no LPL were included. At each timepoint, aliquots were removed for measurement of triglycerides (without free glycerol), and quantitation of apo(a) by sandwich ELISA, and ultracentrifugation. The top and bottom fractions were recovered for apo(a) Western blot analysis and ELISA (Lp(a) measurement).

Qualitatively, Western blot analysis showed that TRL-apo(a) clearly dissociated between 50% to 75% hydrolysis of TRL-triglycerides. A range of 40% to 60% hydrolysis of TG caused an average of 50% dissociation of apo(a) from TRL as measured by ELISA. TRL-apo(a) did not dissociate with both negative controls. In conclusion, lipolysis leads to dissociation of TRL-apo(a).

Transport of lipoprotein(a) and low density lipoprotein across endothelial cells: effect of triglyceride-rich lipoproteins

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We assessed the transendothelial transport of lipoprotein(a) (Lp(a)) and low density lipoprotein (LDL), in the presence or absence of triglyceride-rich lipoproteins (TRL). Lp(a) differs from LDL in that Lp(a) comprises of LDL with the glycoprotein, apolipoprotein(a) (apo(a)), disulphide-linked to apoB-100. Differences in apo(a) size (400-900 kDa) result in many apo(a) isoforms and, in general, apo(a) size is inversely related to plasma Lp(a) concentration. Interestingly, larger molecular weight apo(a) isoforms preferentially bind TRL both *in vitro* and *in vivo* following a fat-rich meal. ^{125}I-labelled Lp(a) (affinity-purified by lysine-sepharose chromatography) and ^{125}I-labelled LDL were supplied to either the intimal or luminal surface (final ^{125}I-lipoprotein total cholesterol = 0.05 mmol/L) of a cell culture system containing human umbilical vein endothelial cell (HUVEC) monolayers grown on porous collagen-coated filters. When TRL were included, ^{125}I-labelled Lp(a) and ^{125}I-labelled LDL were incubated (4h 37°C) with TRL of Sf<400 (final triglyceride concentration = 1.5 mmol/L) before being applied to HUVECs. Intact Lp(a) and LDL were transported in both directions across the cell monolayers. The rate of luminal → intimal Lp(a) transport was similar to LDL transport (mean % [SD] at 24h; 12.9% [4.1] vs 10.7% for Lp(a) and LDL, respec-tively). Both lipoproteins were transported at similar rates from the intimal → luminal face of the monolayer. When ^{125}I-Lp(a) was pre-incubated with TRL, 20% of ^{125}I-label became associated with TRL. However, Lp(a) transport was similar (in both directions) with or without TRL (24h luminal →

intimal; 14.0% [4.0] vs 12.7% [1.9] for Lp(a) and TRL+Lp(a), respectively). LDL, in the presence of TRL, was transported at a similar rate to Lp(a), with or without TRL. When lipoproteins of d<1 .006 g/mL were isolated after incubation of ^{125}I-Lp(a) with TRL and applied to the luminal side of the HUVECs, there was a small (non-significant) reduction in transport (23.7% [4.6]) compared to Lp(a) transport in the absence of TRL (31.8% [4.0]). Although retention of ^{125}I-labelled material on the filters was similar for Lp(a) and LDL, it was significantly increased for Lp(a), but not LDL, in the presence of TRL ($P< 0.01$). We conclude that; i) Lp(a) and LDL are transported at similar rates across the endothelium, and ii) the rate which Lp(a) is transported across the endothelium does not appear to be affected by its association with TRL.

Effects of changes in plasma triglyceride and cholesterol ester transfer protein activity on cholesterol ester transfer and LDL particle size in hypercholesterolaemic patients

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We studied the effects of postprandial lipaemia and pravastatin therapy, which produce opposite effects on plasma triglyceride (TG) and cholesterol ester transfer protein (CETP) activity, on cholesterol ester transfer (CET) and low density lipoprotein (LDL) particle size in 19 patients (12 male, 7 female, ages 30 - 74 years) with primary hypercholesterolaemia (TC>6.5 mmol/L and LDL-C >4.5 mmol/L). After 6 weeks' therapy with placebo or pravastatin 40 mg nocte according to a double blind randomised cross-over design, samples were collected fasting and 6h after an oral fat load (0.88g/kg) comprising skim milk cream and peanut oil (90% fat, 5.2% protein, 4.8% carbohydrate). Lipids, lipoproteins, CET (modified from Mann' et al.), CETP activity (modified from Groener2 et al.) and LDL Stokes' diameter (gradient gel electrophoresis on 3-13% Gradipore gels) were assayed and statistical analysis was performed with BMDP software.

The oral fat load significantly increased TG but did not alter CETP activity during placebo and pravastatin phases. Pravastatin significantly reduced TG and CETP activity in both the fasting and postprandial state compared to the equivalent phases on placebo. CET increased significantly following the fat load but pravastatin attenuated both fasting and postprandial levels of CET. LDL Stokes' diameter did not change.

CET correlated with TG and high density lipoprotein-cholesterol (HDL-C), but the relationship with TG was curvilinear. LDL Stokes' diameter also correlated with TG and HDL-C. Relationships involving TG were enhanced during the postprandial phase, and LDL size was most strongly correlated with triglyceride rich lipoproteins (TRL) ($r = -0.54$ fasting, $r = -0.81$ postprandially). CET was only correlated with LDL Stokes' diameter during the postprandial phase ($r = -0.62$). Pravastatin weakened these correlations. Stepwise regression suggested that postprandial TRL accounted for 85% of the variance in CET, with a further 4% attributable to CETP activity. Postprandial TRL also accounted for 65% of the variability in LDL Stokes' diameter.

We sought to determine whether there was a threshold TRL level which would predict the transition between normal and small dense LDL particles. Even though LDL Stokes' diameter does not change acutely following an oral fat load, our results suggest that postprandial lipaemia exerts a strong cumulative effect on LDL size.

References:

1. Mann CJ, Yen FT, Grant AM, and Bihane BE. (1991) Mechanism of plasma cholesteryl ester transfer in hypertriglyceridemia. J Clin Invest 88:2059- 66.
2. Groener JEM, Pelton RW, Kostner GM. (1986) Improved estimation of cholesteryl ester transfer/exchange activity in serum or plasma. Clin Chem 32:283-6.