Linking the field to the laboratory in nutrition research

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The aim of this presentation is to see how the interests of those in public health nutrition (nutritional epidemiology or the 'field') come together with those working in metabolic and clinical nutrition (laboratory) to address important public health issues in the best possible way. What I mean by the field is population-based research on groups of humans. I will use the terms nutritional epidemiology and 'the field' interchangeably.

GENERAL PRINCIPLES

Research, as usually presented to the Nutrition Society, seeks to answer questions aimed at understanding what causes a particular phenomenon, to explore cause–effect relationships.

In a laboratory setting this would usually be undertaken by use of an animal model or on a small group of individuals using an experimental approach where a specific dietary component was changed, under controlled conditions, and the effect on a specific outcome assessed. In a nutritional epidemiological setting this would usually involve recruiting larger numbers of individuals who are selected so as to be a representative sample of a wider population and who are subsequently observed to explore the nature of the relationship between the cause (exposure) and the effect (outcome). The conduct of the research is organized by a protocol which is set out beforehand and standardizes the way in which subjects or animals in the study are recruited and treated or observed during the study (Margetts, 1991).

In any study the aim is to measure the relevant exposure with sufficient accuracy to be able to determine whether the original question can be answered without being influenced by bias. Before commencing the study it is necessary to determine how the recorded exposure relates to the relevant exposure; if there is no relationship then it is likely that the wrong estimate of the effect of the exposure on the outcome will be assessed. Therefore, to define the relevant exposure requires an understanding of the causal pathway and the mechanisms by which the exposure may influence the outcome. It also requires an understanding of the effect that potentially confounding variables may have on the relationship.

The interface between the field and the laboratory is at this level: laboratory research identifies potential mechanisms and clarifies relevant exposures which field research applies to populations to test whether there is any association between the measured exposure and the outcome. These results can then feed back to the laboratory to refine understanding about the mechanisms and clarify further relevant exposure measures which can be applied in new studies in the field, and so the cycle continues.

Both field and laboratory studies require appropriate study design and measures of exposure.

Relevant measure

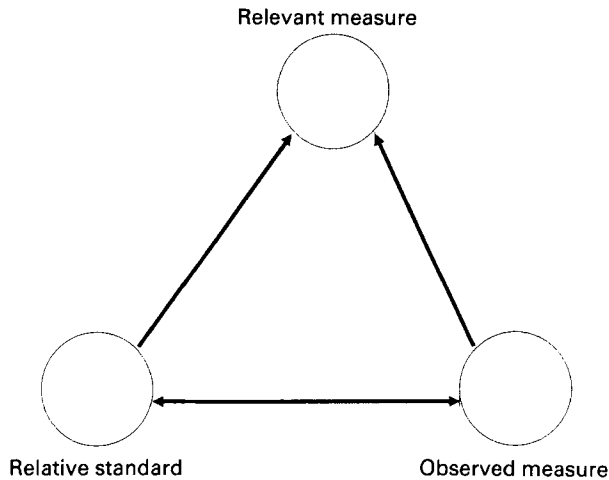Relative standard   Observed measure

Fig. 1. Validation studies compare the observed measure with a relative standard and assume both relate to the relevant measure of exposure. If this does not apply the validation is inappropriate.

Study design

Studies should be designed with due consideration of the effects that chance, bias and confounding may have on the results. The number of subjects required to achieve a given statistical power should be determined first; standard formulas are now available to calculate appropriate sample sizes (Cole, 1991). These formulas usually require three pieces of information: the required power, the expected difference between groups, and a measure of the variability of the estimate of exposure (or the proportion exposed).

While animal experimental studies can explore mechanisms in great detail, the animal must be an appropriate model if the results are to be useful in furthering our understanding of the human situation. There must also be some consideration of the doses given, to determine whether these are likely to be applicable to humans.

Measures of exposure

Measuring the wrong exposure or measuring the exposure with errors will distort the estimate of effect. Clayton (1994) and other workers have considered the effects of measurement error on accuracy and have suggested some ways to remedy the effects of measurement error (Clayton & Gill, 1991). There will always be measurement error but it is possible to minimize errors through careful study design and standardization of the protocol. It is important to recognize two sorts of errors; random and systematic. If systematic errors are different between groups then a biased estimate of effect will be obtained.

For a measure to be valid it must measure the relevant exposure accurately. There is no absolutely true measure of dietary intake. Therefore, when a measure is being validated it is being compared with another measure, and the relative validity of the observed measure to the relative standard is assessed (Fig. 1). Clayton (1994) has suggested that these studies should be called calibration rather than validation studies.

For a validation study to be appropriate both the measure of relative validity and the observed measure must be related to the relevant exposure, an assumption which is rarely tested in validation studies. It does not matter how accurate a measure of relative validity may appear to be, if it is not an appropriate measure of the relevant exposure it will not be of any value in checking on the validity of the observed measure. The optimal way to express results from validation studies is not agreed; some workers use correlation coefficients to assess the degree of association, others prefer plots of data as recommended by Bland & Altman (1986). The advantage of Bland–Altman plots is that it is possible to observe whether the differences between the two measures are consistent across the range of intakes (Thompson *et al.* 1994). A correlation coefficient is a convenient numerical summary of the overall association between two variables and is simpler to present.

Less consideration has been given to the way that results of validation studies are used. Many studies have presumed that statistically significant correlation coefficients indicate that one measure is a good proxy for another. Correlation coefficients of about 0·6 would generally be considered good for such studies. Clayton & Gill (1991) have shown that the percentage of individuals who could be classified as part of the correct third of the distribution of 'true' exposure using the measured exposure would be about 64% when there was a correlation of 0·6 between true and measured exposure. The effect of this misclassification on the observed estimate of effect (relative risk) will be to reduce the observed risk. For example, with one-third of subjects being misclassified and a true relative risk of 3, the observed relative risk will be about 2. For most major chronic disease the observed relative risks are about 2–3, suggesting that using existing methods and without correction for measurement error potentially important risks may be missed. It is possible to use the data from validation studies to correct the estimate of effect for measurement error, although this is seldom used (Willett *et al.* 1992).

Differential recall bias and subject participation may account for some of the differences found in the results obtained from case–control studies and cohort studies. For example, case–control studies where diet is recalled from some time in the past have suggested that there is an increased risk of breast cancer associated with higher fat intake (Howe *et al.* 1990), whereas in cohort studies where diet is recorded prospectively there is no relationship (Willett *et al.* 1992). Giovannucci *et al.* (1993) have recently shown that a retrospective assessment of fat intake, in women with breast cancer compared with women without breast cancer, was higher than that of a prospectively assessed intake. Giovannucci *et al.* (1993) suggested, therefore, that the effect of fat intake on breast cancer found in case–control studies was likely to be due to differential misclassification of the exposure in cases, rather than being an indication of a real difference.

EXAMPLES OF COLLABORATION

Diet and heart disease

The development of our understanding about the relationship between diet and heart disease is a good example of the way that the field and the laboratory come together. International geographical (ecological) studies have suggested that countries with higher fat intakes also have higher rates of heart disease. Different types of studies have suggested that the risk of heart disease was related to the circulating levels of cholesterol

and that serum cholesterol was related to dietary intakes of fat (Caggiula *et al.* 1983). This view has been refined as different fractions of circulating cholesterol have been identified which suggested that cholesterol carried on LDL is a risk factor and that higher levels of HDL-cholesterol were protective. This fat hypothesis, however, has never been able to explain satisfactorily the variation in rates of heart disease within a country. For example, in England Cade *et al.* (1988) showed that there was relatively little difference in fat intake around the courrtry despite substantial differences in rates of heart disease.

More recently the protective role of dietary antioxidants has been shown to be related to risk of heart disease (Duthie *et al.* 1989). At about this time there was a growing appreciation (or reawakening) of the whole-body integrated approach to metabolic stress. The notion that the maintenance of optimal function depends on a balance between supply and demand for nutrients and that the biological effect of this balance is also related to the availability of other potentially rate-limiting substrates and cofactors. From laboratory-based research it was becoming clearer that measuring dietary anti-oxidant intake without measuring the demand related to free-radical load would not give an appropriate indication of the balance between supply and demand and, therefore, would not indicate functional availability.

Work on diet and smoking has helped to clarify this notion. The diets of smokers are different from those of non-smokers in many ways including the availability of antioxidant nutrients. Smoking also provides an acute inflammatory stimulus, as well as producing free radicals directly, both of which place extra metabolic demands on the body. LDL has been shown to be modified to oxidized LDL when free-radical load exceeds the antioxidant defences of the host (Duthie *et al.* 1989). It has also been suggested that the effect of an imbalance between antioxidant defences and free-radical load may relate to higher levels of oxidized (modified) LDL, which has been shown to lead to the formation of foam cells and atheroma. Smokers not only have lower antioxidant defences and higher demands, they also have higher LDL levels and, therefore, are more likely to have higher levels of oxidized LDL. It is now apparent that the risk of heart disease is related to neither fat nor antioxidant intake alone, but to a balance between the two and the net effect of this balance is influenced by other factors such as smoking. Recent animal experimental studies by Grimble and his colleagues (Grimble, 1992; Clamp *et al.* 1993; Mulroney & Grimble, 1993) show that changing the type and amount of fat in the diet can have significant effects on the production of cytokines and acute-phase proteins such as albumin and fibrinogen, which have been shown to be risk factors for heart disease (Fig. 2).

Experience in this area shows that progress will only be made when the field and the laboratory cooperate in identifying and refining an understanding of the mechanisms involved in the causation of heart disease within a model which embraces the complexity of diet.

Development of research in fetal origins of adult disease

Fig. 3 sets out the development of research on the fetal origins of adult disease. Professor Barker has already described much of this work to the Society (Barker, 1992). What I want to emphasize here is the evolution of the research programme and the intimate nature of the relationship between the field and the laboratory which now exists in this work. The research began by an exploration of the geographic association between
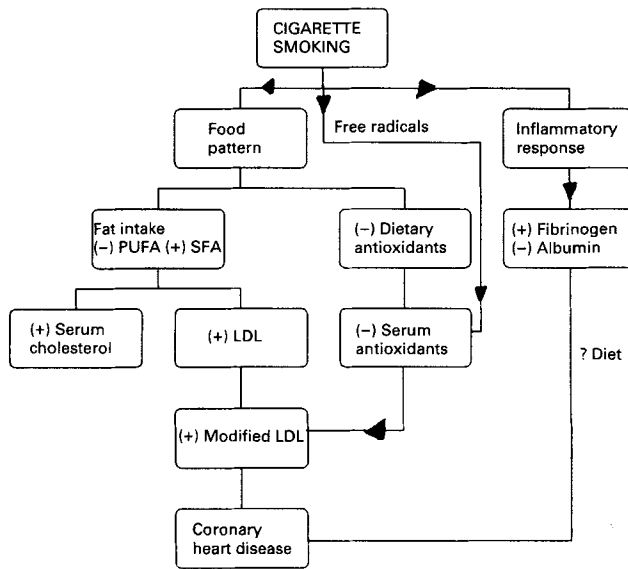
Fig. 2. The relationship between diet, smoking and heart disease. PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
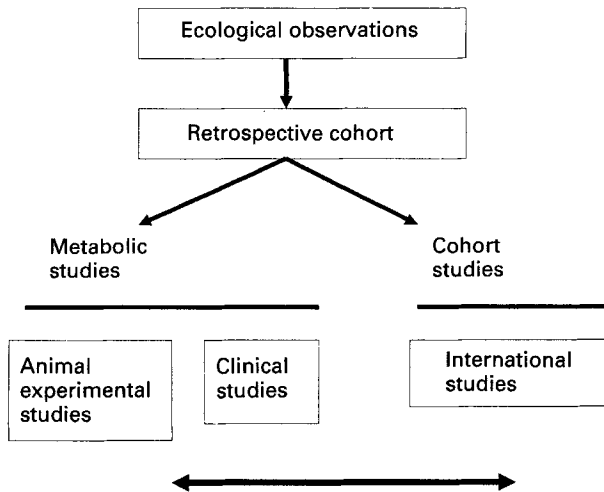

Fig. 3. Development of research on the fetal origins of adult disease.

current adult death rates in the UK and patterns of infant and maternal mortality 50 years ago in the same geographic location. These ecological studies suggested that there was a close association between the current adult death rates and past infant mortality. The weaknesses of ecological studies are well known (Hiller & McMichael, 1991), particularly in the way that other confounding factors may lead to spurious associations. Nevertheless, these studies lead to an hypothesis which could subsequently be tested by more rigorous scientific methods. Because of the long lead time, a prospective cohort

study could not be mounted on the basis of the evidence that was available and, therefore, a search was undertaken of possible sources of dietary data from the past. Around the turn of the century health visitors began to visit infants and systematically to record birth weight and growth data during the first year of life. These records contained sufficient information for the children subsequently to be followed up as adults some 50 years later to determine whether those who were born small and grew poorly in the first year of life were at higher risk of heart disease. Although the birth-weight data were collected 50 years ago, they were collected prospectively, before the outcome was known, so they could in no way have been biased by the outcome, a major strength of cohort studies over cross-sectional or case–control designs. These findings provided further support for the hypothesis and subsequently led to a series of more detailed studies to unravel further the nature of the relationship. Since this first retrospective cohort study, numerous other retrospective cohort studies, following up children and adults who are still alive, have shown that risk factors for chronic disease such as high blood pressure and diabetes are related to impaired fetal development. It is at this stage that the field and the laboratory came together. These studies suggested a cause–effect relationship, but were all observational and the mechanisms by which the measures were related was not clear. Independently, Jackson (1991) and others, working on protein turnover in Jamaica, showed that there was an increase in 5-oxoproline excretion during pregnancy, an index of glycine insufficiency. There are also changes in protein turnover during pregnancy which differ between Jamaica and the UK and may explain differences in rates of hypertension between the two populations (McClelland *et al.* 1994). Animal experimental studies reported elsewhere (Langley & Jackson, 1994) have subsequently shown that modification of maternal protein intake during pregnancy has a substantial effect on the blood pressure and other known risk factors for chronic disease in the offspring.

As a result of the metabolic studies more relevant measures of exposure which link to suggested mechanisms can now be applied to the observational field-based studies to further our understanding of the causal mechanisms.

Vegetarian diets

There is now good evidence that people who follow a vegetarian diet have lower age-specific risk for most chronic disease. For example, Chang-Claude *et al.* (1992) in an 11-year follow-up study have recently shown that German vegetarians have lower all causes mortality than the general population at the same age. McMichael (1992), in an editorial related to this paper, suggested that the adoption of a vegetarian diet would not only be good for the health of the individual, but a reduction in meat production would lead to a more ecologically sustainable global food system. McMichael focused on meat consumption as the key element of diet, although in the Chang-Claude *et al.* (1992) study diet was not actually measured. Meat intake may be an important aspect of the lower risk of disease among vegetarians, but it is clear that vegetarian diets differ in many ways other than in the level of animal protein in the diet (Margetts & Jackson, 1993). Vegetarians in the UK, however, do not tend to replace the protein lost by not eating meat with protein from other sources, they simply eat less protein (Jackson & Margetts, 1993). A recent comparison of the total amount and major sources of protein in the diet of populations from around the world shows that most of the difference in intake is due
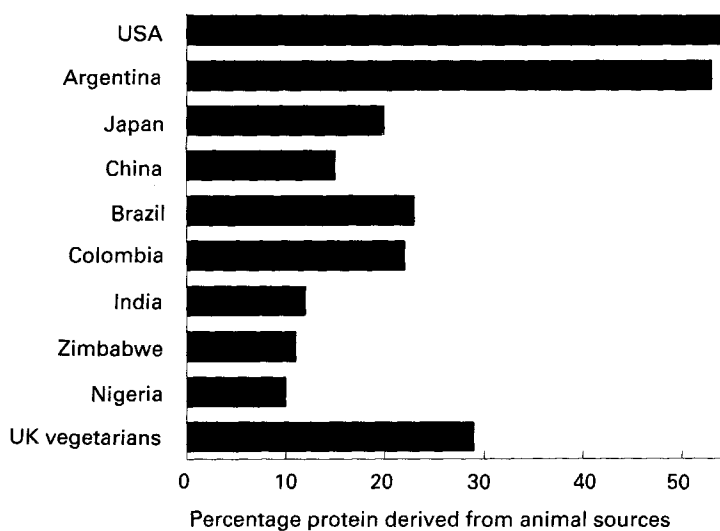
Fig. 4. The percentage dietary protein derived from animal sources in several countries (data derived from Jackson & Margetts, 1993).

to the level of meat in the diet, with non-meat sources of protein being surprisingly constant (Jackson & Margetts, 1993). UK vegetarians eat about the same amount of protein (and thus non-meat protein) as many populations in developing countries.

In parallel with these field-based studies has been a debate about the amino acid requirements coming from laboratory-based metabolic studies. Using labelled C to trace the fate of dietary amino acids, Young & Pellett (1990) have suggested that essential amino acid requirements will not be satisfied unless at least 30% of the total protein intake is derived from animal sources. The only populations who are likely to meet this are those in the developed world eating meat (Fig. 4). UK vegetarians and people following current dietary guidelines are likely to risk not meeting their amino acid requirements and, if Young & Pellett (1990) are correct, should perhaps be eating more animal products, not fewer!

In the most recent results from the National Food Survey (Ministry of Agriculture, Fisheries and Food, 1993) there has been a small decline in the percentage energy derived from fats over the last 10 years, but there has also been a decline in the intake of protein (Fig. 5) and, if Young & Pellett (1990) are correct, the population are at increasing risk of not meeting their requirements for amino acids.

How is it possible to reconcile the apparent contradictions between the evidence suggesting a long-term benefit of following a vegetarian diet with the apparent shorter-term risk of not meeting amino acid requirements? Recently Jackson (1993) suggested that an important part of the pool of available protein may be overlooked in conventional balance studies. In a series of metabolic studies Jackson and his colleagues have shown that N can be salvaged from the large bowel and contribute to the amino acid pool. Studies in vegetarians suggest that they can salvage about 50% of their urea-N, thereby effectively supplementing the available N from the diet. This salvage appears to be greater in vegetarians than in omnivores. Studies not measuring this salvage, therefore, are likely to underestimate the net available N. In field studies, where the
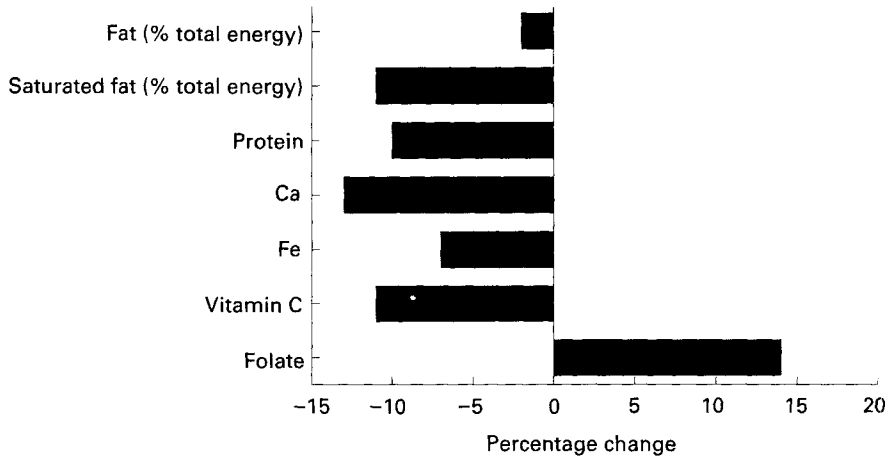
Fig. 5. Change in consumption of selected nutrients over 10 years, 1982–1992 (data from the National Food Survey, derived from Ministry of Agriculture, Fisheries and Food, 1993).

relevant exposure is the amino acid level available in the body pool, measuring dietary intake and urinary output alone is unlikely to give an accurate measure of the relevant exposure.

SUMMARY

The three examples presented illustrate the dynamic way in which the field and the laboratory interact. Both types of study rely on sound research design and clear articulation of the relevant exposure. In all studies it is essential to consider the effect that measurement error will have on the estimate of the effect of the exposure on the outcome.

Epidemiological studies rely on metabolic research to refine the methods and measures of nutritional exposures used in studies of cause–effect relationships. Metabolic studies rely on population studies to determine whether the experimental evidence they reveal is of public health importance. The Nutrition Society provides a vital forum within which this exchange between the field and the laboratory will continue.

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