chapter fifteen

Studies of adverse effects of food and nutrition in humans

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15.1 Introduction

Studies in humans are indispensable for assessing and evaluating risks following the intake of food. The screening of substances for toxicity can be carried out in experimental animals. Extrapolation of the results to humans, however, is difficult. In laboratory

experiments, the animals are locked up and the investigator regulates the exposure conditions (usually exposure to a single substance). In addition, the genetic background of experimental animals is often the same, as inbred strains are used. Except for the exposure, most conditions are maintained constant.

One of the problems with extrapolation of animal data to humans is that species can differ greatly in their sensitivity to a toxic substance. Further, in animal experiments, the exposure levels are often relatively high in order to detect possible effects following the exposure. On intake of food, humans are exposed to various combinations of substances and their biological effects can differ from what is expected on the basis of the effects of the individual components. Therefore, studies in humans are needed for the assessment of toxicological risks from the intake of foodstuffs. Studies in humans require a specific methodology; humans cannot be locked up for years, keeping all conditions but one constant; exposure of humans to carcinogenic substances is forbidden. Yet, relationships between exposure and adverse health effects have to be studied in humans.

Sometimes, humans expose themselves voluntarily to all kinds of harmful substances. In such cases, associations between exposure and adverse effects and diseases can be studied. An essential difference between human and animal studies is that humans, in observational studies, choose their own exposure. This may raise the problem that exposure is also related to other factors which may be important in relation to the disease. Thus, exposed and non-exposed subjects may differ in other factors, playing a role in causing disorders. An example is the observation that lung cancer occurs more frequently in people who drink alcohol than in those who do not. This can be attributed to the fact that among alcohol consumers the percentage of cigarette smokers is higher than in the group of alcohol abstainers.

This chapter is an introduction to the use of epidemiological methods in general, and to the use of epidemiology in studying associations between food intake and adverse health effects in particular. Section 15.2 introduces epidemiological methods. This is followed by a section on nutritional epidemiology in which pitfalls, possibilities, and limitations of nutritional methods are described. In order to circumvent the difficulties connected with studying nutrition, recently methods of identification of biological markers for the intake of particular food components are being developed. This will be dealt with in Section 15.4. Section 15.5 looks at the role of nutrition in the risk of cancer.

15.2 Epidemiology

15.2.1 Introduction

Epidemiology can be defined as the science that studies the occurrence and determinants of diseases in human populations. This section introduces the basic principles of epidemiology. It will enable the reader to evaluate critically the results of epidemiological research.

In epidemiology, the term "relationship" (between exposure and disease) is used if a disease is causally related to exposure. If causality has not (yet) been proven, the term "association" applies. Since a single study can never prove causality, the term association is generally appropriate. Associations between exposure and adverse health effects can be studied at different levels. Often, three steps can be distinguished in the etiology of a disease:

lifestyle habits	→	*physiological variables*	→	*disease*
nutrition		blood pressure, weight		cardiovascular
smoking		serum cholesterol concentration		diseases, cancer
physical activity		serum vitamin concentration		

Table 15.1 Cross-tabulation of subjects according to exposure and disease state

		Disease		
		Yes	No	Total
Exposure	yes	a	b	a + b
	no	c	d	c + d
Total		a + c	b + d	a + b + c + d

In studying associations between nutrition and elevated blood pressure for example, food intake is the determinant or input variable (also called exposure variable) and elevated blood pressure is the (adverse) effect or outcome variable. In studies on possible relationships between blood pressure and coronary heart disease, blood pressure is the input or exposure variable and coronary heart disease is the outcome variable. Thus, a variable can be input variable in one study, and outcome variable in another one. Changes in physiological variables are sometimes referred to as adverse health effects, because they can be risk factors to diseases, e.g., elevated blood pressure to coronary heart disease.

The different ways to evaluate associations between exposure and disease are diagrammatically summarized in Table 15.1. Study designs and disease frequency parameters which can be related to the distinctions made in this diagram are discussed in the following sections. First, the diagram shows the simplest way in which a population can be divided with respect to exposure (yes/no) and disease (yes/no). One possibility is to select exposed (a + b) and unexposed individuals (c + d), followed by comparison of the number of diseased persons in the exposed (a) with the number of diseased persons in the unexposed (c). Another possibility is to select diseased (a + c) and non-diseased persons (b + d) and to compare the number of exposed persons among the diseased and the non-diseased (a vs. b).

15.2.2 Disease frequency parameters

For the description and quantification of the occurrence of a disease in a population, there are two important parameters: incidence and prevalence.

Incidence is defined as the number of new cases which arise during a specific period of time. An example is the yearly cancer incidence: the number of persons who during a year were diagnosed to have cancer for the first time. The significance of this parameter becomes clear if it is related to the number of inhabitants. Therefore, the *incidence rate* is often used. It is defined as the incidence divided by the number of persons at risk:

$$\text{incidence rate} = \frac{\text{number of new cases arising in a given period of time}}{\text{total number of persons at risk of the disease}} \text{ (per unit of time)}$$

The yearly cancer incidence rate can be expressed in terms of the number of new cancer cases during a year per 100,000 inhabitants. For example, if the incidence of cancer in country A is the same as that in country B, and country A has more inhabitants (persons at risk), the incidence rate is lower in country A. If a disease only affects a particular subpopulation, e.g., men, in the case of prostate cancer, the incidence is related to that subpopulation.

Prevalence is defined as the number of cases that are present in the population at a given point of time:

$$\text{prevalence} = \frac{\text{number of cases in a population at a given period of time}}{\text{total number of individuals in the population}}$$

As the equation shows, prevalence is dimensionless. Incidence and prevalence are related to each other. In a steady-state population (i.e., if the number of new cases equals the number of cases which disappear), the relationship between prevalence and incidence is given by:

$$P = I \times D$$

where P = prevalence, I = incidence rate and D = duration of the disease. This means that the prevalence is determined by the duration of the disease if the incidence rates of two diseases are equal.

15.2.3 *Effect parameters*

In epidemiological studies, biological effects are measured by comparing the occurrence of the disease of one subpopulation with that of another differing in exposure conditions. The differences in occurrence of a disease can be expressed in absolute or relative terms.

Differences in incidence rate between exposed and unexposed populations are *absolute* effects. They are calculated by subtracting the incidence rate in the unexposed group (I_0) from the incidence rate in the exposed group (I_1). The difference $I_1 - I_0$ is referred to as *rate difference*. The incidence rate in the unexposed group can be interpreted as the baseline incidence rate, and only the incidence rate exceeding this figure is due to the exposure. Therefore, the rate difference is also known as *attributable rate*. A difference in incidence rate of 0 means that the disease is not related to exposure ($I_1 = I_0$).

Relative effects are expressed in terms of the quotient I_1/I_0 which is called the rate ratio or relative risk (RR). Calculation of RR using the data given in Table 15.1 results in $(a/a + b)/(c/c + d)$. A relative risk of 1 indicates that the disease is not related to the exposure ($I_1 = I_0$).

The incidence can be estimated in a cohort study, but not in a case-control study (see Section 15.2.4.2 for an explanation). This is due to the fact that in a case-control study, cases and controls are selected at the same time. In such studies a measure can be calculated that is a good approximation of the relative risk: the so-called *odds ratio* (*OR*). This measure compares the ratio exposed/unexposed among the diseased with the ratio exposed/unexposed among the controls: $(a/c)/(b/d) = ad/bc$.

Intermezzo

In epidemiological studies, it is frequently observed that the relative risk (RR) in older age groups is lower than that in younger age groups. This is illustrated by the following example from the so-called Framingham Study (Figure 15.1). Diabetes is a risk factor for the development of cardiovascular diseases. The RR of coronary heart disease for diabetics is 2.7 in the age group of 45 to 54 years and 2.1 in the age group of 65 to 74 years. However, this does not mean that diabetes is a less important risk factor in the elderly. The absolute rate difference in the age group of 45 to 54 is 20, and 30 in the age group of 65 to 74. Since the rate in non-diabetics of the older age group is higher than that in the younger age group, the lower RR (2.1 vs. 2.7) leads to a larger rate difference.

The relative risk can be used to calculate another effect parameter. With respect to public health, it can also be important to know which proportion of diseased persons

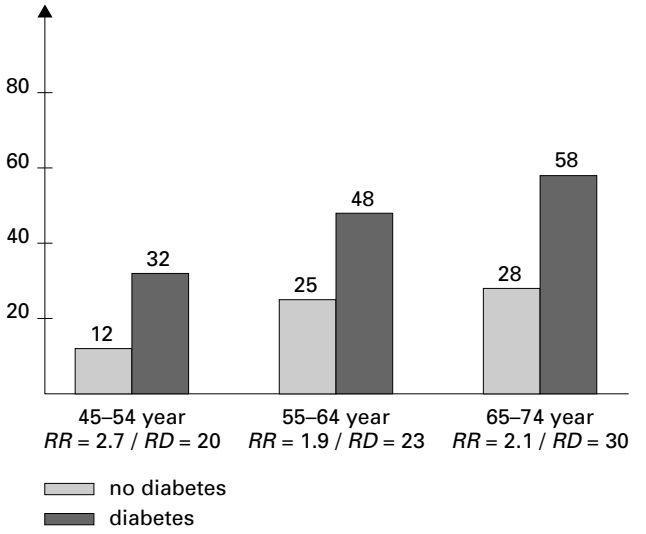

Figure 15.1 Annual cardiovascular disease incidence per 1000 individuals. Source: Kannel and McGee, 1979.

(cases) can be attributed to exposure, the so-called *attributable proportion* (*AP*). This proportion (AP_e) is obtained by dividing the rate difference by the rate among the exposed:

$$AP_e = \frac{I_1 - I_0}{I_1}$$

This equation can be converted to a function of the relative risk:

$$AP_t = 1 - (1/RR) = (RR - 1)/RR$$

There is also a parameter for the proportion of cases in the total population which can be attributed to exposure. The total population can be divided in a proportion unexposed individuals (P_0) and a proportion exposed individuals (P_1). The incidence rate in the total population (I_t) can be calculated from $I_t = P_0I_0 + P_1I_1$. The attributable proportion among the total population (AP_t) is defined as $(I_t - I_0)/I_t$. Substitution of $P_0I_0 + P_1I_1$ for I_t results in:

$$AP_t = \frac{P_1(RR - 1)}{P_1(RR - 1) + 1}$$

Intermezzo

Suppose the *RR* of liver cancer due to exposure to factor X is 2.0 and that of liver cancer due to exposure to factor Y is 10. To calculate which factor leads to the highest AP_t it is important to note that the formula for AP_t contains the *RR* for a particular exposure as well as the prevalence of the effect under investigation. Without information on the prevalences it is impossible to calculate AP_t. If 60% of the population is exposed to factor X and 0.5% to factor Y the calculation runs as follows:

AP_t for factor X is $0.6(2-1)/0.6(2-1)+1 = 0.38$
AP_t for factor Y is $0.005(10-1)/0.005(10-1)+1 = 0.04$

This example illustrates that RR only gives information on the strength of the association, and not on the contribution of the exposure to the public health risk for the total population.

15.2.4 Types of epidemiological studies

15.2.4.1 Experimental studies

In *experimental studies* the exposure conditions are chosen by the investigator, as in animal studies. If patients are the subjects, this type of study is often referred to as a *clinical trial*. For ethical reasons, exposure is bound to certain restrictions of which the most important one is that examining potentially toxic substances in humans is prohibited. This implies that potentially adverse effects of food components can only be investigated in *non-experimental studies*. For example, studying the beneficial effect of adding vitamin A to the diet of smokers in relation to the incidence of lung cancer would be permitted. In contrast, the effect of PCBs in mother's milk on the health of babies can only be evaluated in a non-experimental study design.

In experimental studies, two groups of subjects are compared with regard to the outcome variable: subjects exposed to the substance under investigation (intervention group), and subjects not exposed (control group). An essential condition of this type of study (referred to as an *intervention study*) is that the exposure is randomly distributed over the subjects. Maintaining all conditions constant except for the exposure has to be achieved by randomization of the study subjects, as lifestyle and genetic background differ greatly from one person to another. If, in an intervention study on the effect of vitamin C intake on lung cancer, the average number of cigarettes smoked by the intervention group is much lower than that of the control group, the incidence of lung cancer can be expected to be much lower in the intervention group, apart from the effect of vitamin C intake. This underlines the need for randomization of the exposure.

If possible, the study should be double-blind. This means that the investigator as well as the study subjects do not know whether they are in the intervention group or the control group. At the end of the study, the information on who received the substance under investigation and who did not is added to the information already available and the data obtained. In this way, the observations are not influenced by the investigator or the respondent.

15.2.4.2 Observational studies (non-experimental studies)

In *observational studies*, the exposure is "chosen" by the subjects themselves. The investigator confines him/herself to observing the subjects and to collecting data on their exposure and disease, without interfering with their way of life. In the following, four types of observational studies will be discussed.

The various types of epidemiological studies are summarized in Table 15.2. The rank order from weak suggestions to strong evidence of a causal relation in the studies would be ecological studies, cross-sectional studies, case-control studies, cohort studies, and finally randomized controlled trials.

15.2.4.2.1 Cross-sectional studies. In *cross-sectional studies*, data on exposure as well as biological effects are collected at the same time. This kind of study is often used to describe the prevalence of certain exposures or diseases in a population. From an etiological point of view, an essential disadvantage of these studies is the problem of discerning effect from cause. For example, if the total cholesterol serum level is observed to be lower in persons with cancer, this does not allow the conclusion that a low cholesterol serum level causes

cancer. It may just as well be that the opposite is true: cancer causes a low cholesterol serum level. In the case of an association between intake of saturated fatty acids and cholesterol serum levels, however, it is more likely that consumption influences the cholesterol serum levels, than the other way round. Knowledge of biological pathways is necessary for making valid inferences.

15.2.4.2.2 Follow-up studies (cohort studies). In a *follow-up study*, the subjects (also referred to as the cohort) are followed for some time (follow-up period). At the start of the study (also called the baseline), the cohort consists of people who are free of the disease under investigation and differ in exposure conditions. To begin with, all persons are examined and information on variables of interest is collected. During the course of the study the occurrence of diseases is recorded. From this, the incidence of the disease in the study population can be calculated. Based on these data, inferences on the association between exposure and occurrence of diseases can be drawn. An important advantage of a cohort study is that exposure is measured before the disease has set in. The appropriate follow-up period depends on the associations which are studied. In the case of salmonellosis following the consumption of raw eggs, a follow-up period of only a few days is sufficient. But, to study the associations between diet and specific types of cancer, a follow-up period of years or decades is necessary. Because the majority of follow-up studies concern chronic diseases, the follow-up period is usually long. Consequently, results are only available after many years. Further, for the assessment of associations between exposure and disease, it is necessary for the number of cases which manifest themselves during the follow-up period to be sufficiently large. This means that the cohort approach is not suitable for studying rare diseases. In order to assess the occurrence of diseases in the cohort in a reliable way, it is of great importance to keep track of all study subjects, and to prevent loss during follow-up as much as possible. Another advantage of follow-up studies is that a large number of both exposures and outcomes can be studied. At baseline, a large number of parameters are usually measured in all study subjects. For a cohort study on a chronic disease, for example, these parameters may include lifestyle factors such as diet, physical activity, smoking habits, and biological variables such as blood pressure, serum cholesterol concentration, height, and weight. Recent developments in the design of cohort studies include storage of biological material such as serum and white or red blood cells at –20°C or –70°C. This can be very useful if during the follow-up period new hypotheses arise about the role of variables which have not been measured at baseline. In this way additional baseline information on the study subjects can still be obtained, for example, after 10 years of follow-up.

There are two special types of cohort studies. For a study on a particular effect of an industrial chemical, a cohort can be selected from groups of industrial workers who have been exposed to the chemical. Such cohorts are referred to as *special cohorts*. The prevalence of adverse health effects in such a cohort can then be compared with that among workers in the same industry who have not been exposed, or compared with adverse health effects in the general population.

Because a cohort has to be followed for many years after exposure has been measured, a *retrospective cohort study* is sometimes carried out. This means that a cohort is selected that has been exposed in the past. The investigator then has to establish the appearance of adverse health effects for all individuals of that cohort at the time of the actual study.

15.2.4.2.3 Case-control studies. While in a cohort study exposure is determined at baseline and the occurrence of diseases is followed after the exposure, a *case-control study* starts with identification of diseased subjects and then collects information on exposure in the past. In a case-control study, cases of a particular disease are selected and the patient's

exposure in the past is compared with that of controls. This type of study is suitable for studying rare diseases. The numbers of subjects needed here are small compared to those needed in cohort studies. Since the cases are selected without knowing the size of the source population at risk from which they arose, no information on the incidence rate of the disease in the population is obtained in case-control studies. Consequently, the relative risk cannot be calculated. It is approximated by the so-called odds ratio (see Section 15.2.3). The advantage of this study design is that exposure and disease are both measured at the same time, and therefore one does not have to wait as long for results as in the cohort design. In this type of study, however, valid assessment of exposure may be a problem, since exposure in the past is measured after the disease has occurred. The disease may have affected recollection of the exposure by the subject. For instance, the occurrence of the disease may be a stimulus to search for an explanation, leading to a more accurate recollection of exposure. This may be the case for a woman who has given birth to a malformed baby, and who starts thinking about exposures during her pregnancy that may have caused the malformation. Mothers of healthy babies may not have such a stimulus. Therefore, on comparing exposure in complicated pregnancies with exposure in uncomplicated pregnancies, an artificial difference may be observed due to differences in recollection. Also, the disease may lead to denial of the exposure: people with lung cancer may underestimate the role of smoking in the past. For diseases with a long latency period and which influence the factor under investigation, information on exposure in the distant past is needed. This may well be impossible.

15.2.4.2.4 Ecological studies. In this type of study the unit of observation is not the individual but a group of people in a particular environment, such as workers in a factory or inhabitants of a city or a country. *Ecological studies* can be useful if information on individuals is not available; exposure is then an overall measure for the population under investigation. For example, nutritional data are sometimes only available per country as food balance sheets. The outcome variable under investigation in ecological studies is often mortality. For example, the mortality level due to cardiovascular diseases in different countries correlates well with the average saturated fat consumption per capita in those countries. This association has been supported by the results of intervention studies.

A well-known phenomenon occurring in this type of study is the so-called ecological fallacy. On comparing countries, it may be found that the higher the average level of a risk factor A for a country, the higher the mean level of mortality due to disease B, while within each country (based on individual measurements of A and B) risk factor A is negatively associated with disease B.

15.2.5 Precision and validity

Measurements are important in epidemiology. That refers to the input variables (determinants/exposure) as well as the outcome variables (adverse health effect/disease/mortality). For instance, in the case of a study on the relationship between magnesium intake and blood pressure, the investigator wants to know the blood pressure and magnesium intake of each subject. The measurements of these parameters are estimates of the "true" blood pressure and the "true" magnesium intake. These true values, however, are not known, and are therefore hypothetical. An estimate of a true value always contains some measurement error. This error may be random or systematic. Random errors can occur if the observer is not very accurate or the measuring device is not very easy to read. This results in values that are sometimes too low and sometimes too high. However, on average the over- and underestimation compensate each other, resulting in a group mean that is close to the true group mean.

Table 15.2 Summary of possibilities and limitations of epidemiological studies

	Experimental study	Cross-sectional study	Follow-up study	Case-control study	Ecological study
Possibilities	Strong indication of causal relation	Estimation of prevalence of exposure or disease	A large number of exposures and diseases can be studied Exposure is determined before onset of the disease Estimation of the incidence of a disease	A large number of exposures can be studied The number of study subjects may be relatively small Suitable for rare diseases	Can be used when information is only available on an aggregated level
Limitations	Only beneficial effects can be studied Only a small number of study subjects can be used for logistic reasons	Distinction between cause and effect is difficult	During the follow-up period the investigators must keep track of all study subjects Expensive (time and money) Only suitable for frequently occurring diseases	Exposure is determined after onset of the disease; reporting of exposure by the respondents might be affected by the disease	Ecological fallacy

A systematic error implies that all measurements are too low or too high. This can be the case if the measuring device is not properly calibrated. Apart from measurement errors, there is likely to be biological variability, as a result of which repeated measurements yield not exactly the same values. Biological variation can also be random (e.g., fluctuating around an average value) or systematic (e.g., height is largest at the beginning of the day and decreases slightly during the day).

Because of measurement errors and biological variability, the average value of repeated measurements usually gives a better estimate of the true value than the result of a single measurement.

Precision, also referred to as reproducibility, implies that there are no random errors in the measurements. The reproducibility of a measurement is high if there is good concordance between repeated measurements.

The *validity* of a measurement concerns the concordance between the value of a measurement and the true value, in other words: do you measure what you want to measure? A high reproducibility is a prerequisite for validity, but does not automatically imply validity.

Suppose a person weighs 65 kg. Balance A yields a value of 70.2 kg for each measurement, and balance B gives 65.3 , 65.6, and 65.7 kg on the first, second, and third measurement, respectively. In this case, balance A has a high reproducibility, but is not very valid. Balance B is reasonably precise and yields a valid estimate of the "true" weight.

With regard to the validity of the results of epidemiological studies, a distinction is made between internal and external validity. *Internal validity* is the validity of the inferences drawn for the population under investigation, while *external validity* refers to the ability to generalize of the results beyond the study population. It will be clear that if there is no internal validity, there can be no external validity either. In general, internal validity can be influenced by three types of bias: selection bias, information bias, and confounding. However, the distinction between these three is not always strict.

Selection bias can be defined as the fact that the effect measured is perverted due to the selection of the study subjects. This means that the association between exposure and disease in the study population differs from the association in the total population. Case-control studies are especially sensitive to selection bias. If subjects are systematically excluded from or included in the case or control group, the comparison of these groups can give biased results. Since cases are often recruited from hospitals, controls are sometimes also selected from the same hospitals. Since hospitalized persons are likely to differ from the general population, this may influence the study results. For example, if lung cancer cases are compared with controls which have been recruited from a hospital, the smoking habits between the two groups might not differ very much because smoking prevalence among hospitalized persons is higher than in the general population. This is due to the fact that smoking is a risk factor of a large number of diseases. Therefore, in the study design, special attention should be paid to the selection of controls. Often, several control groups are used to estimate the consequences of the choice of the source population of controls. Other source populations of controls that are used in addition to hospital controls are neighborhood controls (to control for socio-economic differences between cases and controls) or a random population sample, in order to compare the exposure in the cases with that in the general population.

Information bias is the term for errors in the necessary information, leading to errors in the classification of subjects. If the errors in the necessary information (e.g., in exposure measurement) are not related to the state of disease, the misclassification is called random or non-differential. This is the case if equal proportions of subjects in the groups which are compared, are classified incorrectly with respect to exposure or disease. *Random misclassification* dilutes the true difference and therefore always changes the observed effect

towards the null hypothesis (i.e., no relationship between exposure and disease). If the measurement error in the exposure is related to the disease, the misclassification is called differential. *Differential misclassification* has more serious consequences. It can lead to either underestimation or overestimation of the effect.

A type of information bias that is of importance in case-control studies is *recall bias*, which means that cases differ from controls in the recollection of exposure. An example is that after giving birth to a malformed baby, mothers start thinking about potential causes for this malformation during their pregnancy (see Section 15.2.4.2). A way to solve this problem may be the selection of a control group of which the memory has also been activated. In a case-control study on congenital heart disease, for example, a control group can be selected with other congenital diseases. It should be noted that the congenital heart disease studied and the congenital disease in the control group should not have a common determinant.

The third type of bias is *confounding*, one of the most important concepts in epidemiology. Confounding can be defined as the combined effect of the factor under investigation and other (confounding) factors. An illustrative example of confounding is the finding that lung cancer is associated with alcohol consumption. However, this finding is caused by the fact that smoking is associated with alcohol consumption, and lung cancer is associated with smoking. In the association between alcohol consumption and lung cancer, smoking is called the confounding factor (the confounder). A factor can only be a confounder if the occurrence of the disease as well as the exposure under investigation is associated with it. There is an essential difference between confounding and information or selection bias. If information on the confounder is collected during the study, it can be adjusted for in the statistical analyses. Sometimes, however, an unknown or not measured confounder is present. Such a confounder cannot be adjusted for in the statistical analyses, and gives rise to biased study results.

External validity determines whether the results can be generalized beyond the study population. Internal validity is a prerequisite for external validity. If an association is not validly assessed for the population under investigation, it cannot be generalized to other populations. For external validity, a judgment must be made on the plausibility that the effect observed in the study population can be generalized. In this context, questions can be asked such as: Do associations found in men also apply to women?, Are associations found in young people also valid for elderly people?, and Are the results of an American study also applicable to the Dutch population?.

15.2.6 Causality

In epidemiological studies, associations of disease(s) with exposure may be found. This does not necessarily mean that the exposure caused the disease(s). The English statistician Hill introduced a number of criteria which should be met before inferences about causality can be made. Although only one of these criteria is imperative for a factor to be causal, all of them are briefly discussed below:

1. *Strength of an association.* Weak associations are more likely to be attributed to confounding than strong associations. On the other hand, weak associations certainly do not exclude causality. Particularly in nutritional research, the majority of the associations between food intake and adverse health effects can be classified as weak (meaning a relative risk of about 1.5 to 2.0);
2. *Consistency.* If an association is causal, it must be possible to observe this association in different populations under different circumstances. However, it is also possible that a factor causes a disease under one circumstance but not under another;

3. *Specificity.* This criterion means that the cause should lead to a specific effect. This can be easily proven to be wrong. For example, smoking causes not only lung cancer, but also several other lung diseases and ischemic heart diseases;
4. *Temporality.* This requires exposure always to precede the effect in order to be causal;
5. *Biological gradient.* In a number of cases, indeed, a dose–response relationship is found. Sometimes, however, all exposure levels measured are high enough to cause the disease. In that case, no dose–response relationship is observed;
6. *Plausibility.* The association should be biologically plausible. A problem with this criterion is that sometimes associations are found before the underlying biological mechanisms are elucidated;
7. *Coherence.* According to this criterion, associations are not incompatible with what is known about the etiology of the disease. It is closely related to plausibility;
8. *Experimental evidence.* An association should be confirmed in a controlled laboratory (animal) experiment. This cannot be done, however, if the toxicity of the substance under investigation in laboratory animals is extremely low;
9. *Analogy.* This means that if a substance causes a particular effect, a structurally related substance may cause the same effect.

In fact, only one of of these criteria is a "conditio sine qua non" to prove causality. The criterion of temporality should always be met: the cause must precede the effect! However, it is difficult to prove causality. In practice, this can only be achieved if information of a number of scientific disciplines is integrated. Sometimes, an association is indicated by epidemiological studies, and subsequently the mechanism is investigated in experimental animals or laboratory experiments. It can also be the other way around: an effect shown in experimental animals or laboratory experiments is confirmed in epidemiological studies.

15.3 Nutritional epidemiology

In the last few decades, the interest in the role of diet in the etiology of diseases has increased strongly. For the identification of the role of nutritional factors in the etiology of diseases, the methodology of food consumption measurement is of particular importance. Measuring individual food intake is difficult. In epidemiological studies, a number of methods are available to measure food intake. They will be dealt with briefly.

15.3.1 Methods for measuring food intake

15.3.1.1 Record method

The record method is used to obtain detailed information on food intake during a limited number of days, usually 1 to 7 days. During that period the subjects write down everything they eat, and measure the quantities. A problem with this method is that people tend to forget to write things down, or change their eating habits due to the fact that they have to write down everything they eat. A record method for 2 days cannot be used to obtain information on the usual diet of the study subjects. Due to the large day-to-day variability in the intake of foods, a 2-day period is too short to obtain a valid estimate of the usual food intake. If information on food consumption at the individual level is needed, the record method has to be repeated several times during a certain period of time. However, the 2-day record method can give a good estimate of food consumption at the group level, because then a large number of 2-day records is averaged to estimate the mean intake by the group.

15.3.1.2 Interview method

Two frequently used interview methods are the *24-hour recall method* and the dietary history method. In the 24-hour recall method, a complete description of the total food intake during the 24 hours preceding the interview is requested. As with the 2-day record method, a single 24-hour recall does not give a good estimate of food consumption by individuals, because of the large day-to-day variation in food intake. With the *dietary history method*, respondents are asked about their usual food intake during a specific period of time, usually the 2 to 4 weeks preceding the interview. This method gives a better indication of the usual dietary intake by individuals. Since a dietary history interview takes about 1 to 2 hours, this method cannot be applied in studies in which many thousands of people participate.

15.3.1.3 Food frequency method

If one wants to obtain dietary information from study subjects in a large-scale study, there is a need for a relatively quick and simple method. For this purpose, food frequency questionnaires have been developed. These questionnaires ask about the usual intake frequency (and sometimes also the quantities) of a limited number of food products. Only products which contribute substantially to the intake of the nutrients of interest are selected. A disadvantage of this method is that no information on total food consumption is obtained. Since food consumption patterns differ widely from one population to another, a new food frequency list has to be designed and validated for every study.

15.3.2 Calculation of nutrient intake from food intake

Once an estimate of the food intake has been made, associations between food intake and biological variables, diseases, or mortality can be studied. Information on the composition of the diet of an individual can be obtained from chemical analyses. Nutrients and other substances the investigator is interested in (e.g., contaminants) can be identified. However, this is usually expensive and laborious. Therefore, food tables are used which contain the average nutrient content of a number of frequently consumed foods. From these food tables, nutrient intake can be calculated. However, calculating nutrient intake from food intake introduces a source of error in the estimate of the true nutrient intake because the nutrient content of a particular food varies with the type of product, mode of cultivation, storage conditions, processing, and preparation.

Furthermore, no information on additives, contaminants, natural toxins, or products formed during preparation of foods can be obtained from the food tables. If one is interested in contaminants or natural toxins, for instance, special chemical analyses of foods have to be carried out. Particularly in the case of contaminants, the variability is high. One apple, for example, may have been sprayed with pesticides, whereas another may not. Therefore, it is not possible to give unequivocal averages for the amount of these substances in food tables.

15.3.3 Analysis of dietary data

Associations between food consumption on the one hand, and a biological variable or a disease on the other, can be studied on the basis of data on food as well as on nutrient intake.

Studies on food intake have the advantage that their results can be easily translated into preventive actions. In order to get insight into the etiology of a disease, it is important to know which food component(s) is (are) responsible for the effect. For example, a protective effect of the consumption of fruits and vegetables against lung, stomach, and

colon cancer has been reported. For the prevention of those cancers, this can lead to the recommendation to eat more fruits and vegetables. However, the question remains which substances are responsible for the association. Possibly antioxidants, such as β-carotene and vitamin C play an essential role. Also, non-nutritive components with anticarcinogenic properties such as indoles, phenols, and flavones, may play a role.

When associations between dietary intake and diseases are studied, it should be borne in mind that the intake levels of many nutrients are strongly related to each other. For instance, a diet with a relatively high fat content will automatically have a relatively low carbohydrate content (see Chapter 12). This may lead to the problem that it is hard to distinguish the effect of a high fat intake from the effect of a low carbohydrate intake.

15.4 Application of biomarkers in epidemiology

15.4.1 Introduction

As described in the preceding section, measuring food intake is difficult, and in a number of cases almost impossible. An alternative would be to do it indirectly by measuring nutrient intake after consumption has taken place. For example, instead of estimating vitamin intake by measuring food consumption, the vitamin blood concentration can be used as an indicator of vitamin intake. The vitamin blood concentration is then called a *biomarker* for vitamin intake. The interest in biomarkers has increased greatly in the last few years, although they are not always the right solution. They have their limitations, as will become evident later in this section. Broadly, three categories of biomarkers are distinguished: markers of exposure, markers of effect, and markers of susceptibility. However, the distinction is not always strict. In this section, the use of biomarkers as a substitute for food intake (biomarkers of exposure) will be discussed.

In a number of cases, the biomarkers provide a more valid and precise estimate of food intake than food consumption methods. This is especially true for nutrients or contaminants of which the concentration in food may vary widely as a result of activities such as cultivation, storage, etc. (see Section 15.3.2). Errors as made by respondents in reporting their intake are prevented. Further, the use of biomarkers can provide information on micronutrients, contaminants, or substances formed during processing of foods. Another advantage is that biomarkers can be analyzed in retrospect, in frozen blood samples. However, it should be noted that if, for example, measured in serum, biomarkers do not only reflect interindividual differences in intake, but also in absorption, metabolism, and bioavailability. Since the human body keeps the concentration of many substances constant (homeostasis), levels measured in the body may not always reflect actual intake. Therefore, a requirement for a biomarker of intake is that there is a good relationship between the level of intake and the level of the biomarker. Biomarkers are most valuable if they reflect long-term intake. In that way, the biomarker is a good estimate of the usual intake that can be used for ranking individuals with respect to intake level. Not for all food components are suitable biomarkers available. A well-known example is the fact that the serum cholesterol concentration is a very poor marker of dietary cholesterol intake. On the other hand, the blood concentration of vitamin E is a fairly good indicator of dietary vitamin E intake.

15.4.2 Examples of biomarkers of dietary intake

As far as macronutrients are concerned, a well-known biomarker for protein intake is the 24-hour nitrogen (N) excretion. If subjects are in N balance, daily urine N excretion is strongly related to daily N intake. Also for a number of micronutrients, i.e., vitamins, biomarkers are available. In the case of vitamin E, the plasma concentration is well related

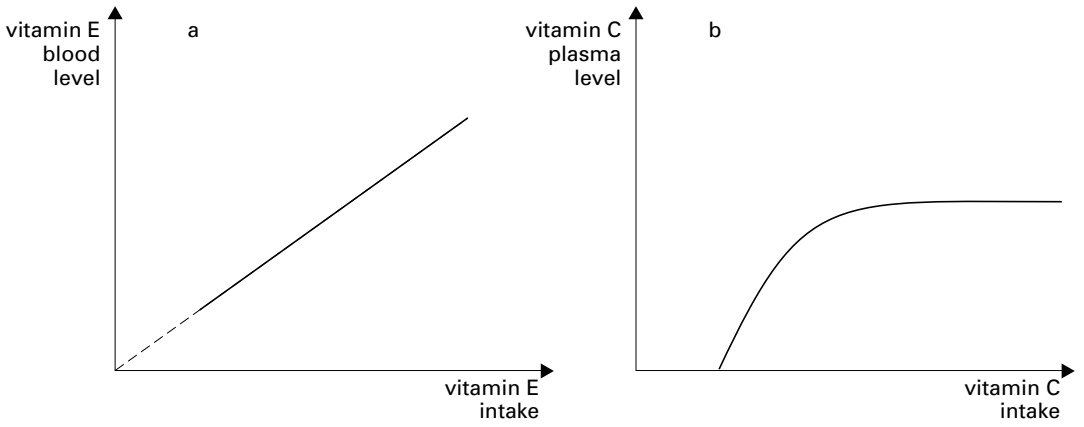

Figure 8.2 Blood/plasma level-intake curves for vitamin E (a) and vitamin C (b).

to intake. The relationship between dietary vitamin C intake and plasma vitamin C concentration is more complex. At high intake levels, plasma vitamin C levels reach a maximum (Figure 15.2).

Another example concerns selenium. The selenium concentration in toenails reflects long-term selenium intake. Biomarkers are also used for exposure to naturally occurring toxins. For example, on exposure to aflatoxins, the carcinogenic products of the fungus *Aspergillus flavus*, aflatoxin B1-albumin adducts can be measured in serum.

In comparison to the number of nutrients and other substances in foods, the number of biomarkers for intake is still small. Therefore, further development of the application of biomarkers in nutritional epidemiology is needed. When validated, the use of biomarkers can contribute substantially to nutritional epidemiology. In the future, a dietary questionnaire or interview in combination with the use of biomarkers may appear to be an adequate way to measure exposure. For some nutrients or substances, a questionnaire may provide reliable data, while for others the measurement of biochemical parameters may be a better or the only way to obtain reliable information.

15.5 Dietary factors and the risk of cancer

At present, much is known about the role of food intake in the etiology of cardiovascular diseases. The relationship between the intake of saturated fatty acids and the occurrence of ischemic heart diseases, for example, is now generally recognized. However, the role of food components in the induction of various types of cancer is less clear, although it is beyond doubt that dietary factors do play an important role. The types of cancer frequently occurring differ from one country to another. In Japan, cancer of the stomach occurs more often than in the US or Europe, while the incidence of breast and colon cancer is higher in the US and Europe than in Japan. The fact that in Japanese people who migrated to the US, the incidence of stomach cancer decreased whereas the incidence of breast and colon cancer increased, suggests that lifestyle and environmental factors are important.

As far as the role of dietary factors in the etiology of cancer is concerned, laypersons mostly think that contaminants and additives are the main risk factors. A well-known publication in which the contribution of dietary factors to the occurrence of cancer has been estimated is *The causes of cancer* written by Doll and Peto (1981). According to their estimates, the effects of contaminants and additives on the occurrence of cancer range from a decrease of 5% (due to a protective effect of antioxidants) to an increase of 1 to 2%.

Table 15.3 Summarizing of the conclusions about associations between food components and cancer, based on literature data

Food component	Association[1]	Type of cancer
Fat	+	Colon, breast
	(+)	Prostate, pancreas
Alcohol	+	Mouth, throat, esophagus
Vitamin A and β-carotene	–	Lung, bladder
	(–)	Prostate
Nitrate, nitrite	+	Stomach
Vitamins C and E	–	Stomach
Products of pyrolysis	Recently, a number of these products have been found to be highly mutagenic and/or carcinogenic	

[1]+: higher incidence of tumors is associated with higher intake of dietary factor.
(+): higher incidence of tumors is probably associated with higher intake of dietary factor.
–: lower incidence of tumors is associated with higher intake of dietary factor.
(–): lower incidence of tumors is probably associated with higher intake of dietary factor.

Epidemiological studies on the role of contaminants and additives in the induction of cancer are very cumbersome. Usually, exposure is very low and identification of exposed subjects is very difficult. Sometimes, there are large differences in effect between studies in experimental animals to which relatively high, single doses are given for a relatively short period of time, and human studies in which very low doses are ingested during long periods. An example is the long dispute about the safety of saccharin, a non-caloric sweetener. Saccharin has been used since its discovery in 1879. Studies carried out in the 1960s and 70s in rodents showed that high doses of saccharin caused bladder cancer. As a result of this finding a ban on the use of saccharin was proposed in some countries. To investigate potential effects on humans, different types of epidemiological studies were carried out. In descriptive studies, trends in the use of saccharin were compared with the occurrence of bladder cancer. In other studies, the incidence of bladder cancer in diabetics (from whom a rather large consumption of artificial sweeteners could be expected), was compared with that in non-diabetics. In case-control studies, bladder cancer patients and controls were compared for the use of saccharin. In a cohort study, the incidence of bladder cancer in saccharin users was compared with that in unexposed groups. The results of the various studies led to the conclusion that there is no increased risk of bladder cancer for humans from the use of saccharin. The composition of the diet with regard to macro- and micronutrients is of more importance for the occurrence of cancer than the intake of additives. Based on a large number of studies on micro- and macronutrients, Doll and Peto estimated that probably about 35% of all cancers are caused by an unbalanced nutrient content of the diet (with a confidence interval of 10 to 70%).

In 1986 the Dutch Nutrition Council reported that despite all the research that had been carried out, no definite conclusions could be drawn on the role of the different food components in the induction of cancer. Based on literature data, only general conclusions were presented about associations between dietary factors and several types of cancer. A few of these are listed in Table 15.3.

15.6 Summary

This chapter dealt with the basic principles of epidemiology, the ways in which epidemiological methods can be used for the assessment of food intake, the significance of the use

of biomarkers for nutritional epidemiology, and the importance of epidemiology for the examination of the role of dietary factors in the risk of cancer.

Disease frequency can be expressed in terms of incidence rate or prevalence. Effects of exposure can be expressed in absolute terms, as incidence rate difference, or in relative terms, as relative risk or odds ratio. The proportion of the diseased which can be attributed to exposure, the attributable proportion, can be calculated for exposed individuals as well as for total populations. Epidemiological studies can be experimental (clinical trials/intervention studies) or non-experimental (cross-sectional studies, follow-up or cohort studies, case-control studies, and ecological studies). Further, the concepts precision and validity, bias and confounding were introduced, and a number of criteria concerning causality were briefly discussed.

Food intake can be measured by using a record method, an interview method, or a food frequency method. Sometimes, the use of a so-called biological marker (biomarker) can give a more valid and precise estimate of the intake.

During the last decades, results of epidemiological studies have contributed substantially to the insight that dietary factors play an important role in the etiology of cancer. Nutritional imbalance of the diet with regard to macronutrients appeared to be the major cause. The risks due to the intake of food contaminants and food additives are minimal.

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