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Symposium on 'Nutrition in the post-genomic era' Plenary session 1: Post-genomic opportunities for understanding nutrition

Post-genomic opportunities for understanding nutrition: the nutritionist's perspective

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Until 15–20 years ago, many nutritionists were mainly interested in the effects of diet on health-related variables such as blood pressure and serum cholesterol concentrations. Without doubt, these studies have made an important contribution to our current understanding of the relationship between diet and health. For many reasons, however, few researchers have tried to explain these effects at the molecular level. Nowadays, however, it seems that the picture has been reversed; much research is being directed towards studying the effects of dietary components at the molecular level. This type of research has been made possible by, among other factors, the implementation of techniques from the more fundamental sciences into nutrition research. Also, the availability of genome sequences has accelerated this shift of interest. The aims of these studies are to obtain detailed information on the molecular and metabolic responses of cells and tissues, or even the whole organism, to dietary components. In these studies, also, the interactions between diet and genetic background, and between diet and different physiological and pathological conditions need to be addressed. However, it is not only important to obtain information on mechanisms, but also on the functional consequences for the organism. One ultimate question, however, is whether this information can be used to develop tests that can form the basis of dietary advice for specific subpopulations. These challenging questions can only be tackled through an integrated approach that combines the expertise from various disciplines.

Résumé

Jusqu'il y a 15 ou 20 ans, des nombreux nutritionnistes étaient intéressés principalement dans les effets de l'alimentation sur des paramètres de santé comme la pression sanguine et les concentrations de cholestérol dans le sérum. Ces études ont signifié sans doute une contribution très important à la compréhension actuelle de la relation entre l'alimentation et la santé. Cependant, et pour de nombreuses raisons, il n'y a pas eu beaucoup de monde qui a essayé d'expliquer ces effets au niveau moléculaire. Toutefois, il paraîtra qu'aujourd'hui la tendance a était renversé; un grand nombre de travaux de recherche sont dédié a l'exploration des effets des components de l'alimentation à niveau moléculaire. Ce type de recherche a était fait possible grâce, entre autres facteurs, à l'implémentation de techniques des sciences plus fundamentaux à la recherche dans la nutrition. Ce aussi la disponibilité des séquences du génome qui a accéléré ce changement d'intérêt. Les objectives de ce projet sont l'obtention d'une information précis sur les réponses métaboliques et moléculaires des cellules et des tissues (ou même de l'organisme entier) aux components diététiques. Dans ces études, les interactions entre l'alimentation et l'héritage génétique, ainsi qu'entre l'alimentation et des conditions physiologiques et psychologiques doivent aussi être adressées. Cependant, il n'est pas seulement important l'obtention d'information sur les mécanismes, mais aussi sur les conséquences fonctionnelles pour l'organisme. Une dernière

Abbreviations: ABC, ATP-binding cassette sterol transporter; HMGCoA, hydroxymethylglutaryl-CoA.
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question se pose sur l'usage de cette information pour le développement de tests capable de prescrire des conseils diététiques aux subpopulations spécifiques. Ces questions stimulantes peuvent juste être abordées à travers d'une approximation intégrée combinant l'expertise de plusieurs disciplines.

Diet: Genetics: Human studies

Before the 1980s nutritionists mainly investigated the effects of diet on health-related variables such as blood pressure and serum cholesterol concentrations, and these studies contributed greatly to our current understanding of the relationship between diet and health. A well-known example is the research carried out in the 1950s by Keys and co-workers. In these well-controlled experiments the effects of dietary fatty acids on plasma total cholesterol concentrations were examined. Groups of physically-healthy men were fed diets that differed widely in the amount of fat and in dietary fatty acid composition. At the end of the studies an empirical formula was derived that could be used for a group of subjects to predict changes in plasma cholesterol concentrations from changes in dietary fatty acid composition (Keys *et al*. 1965*b*). These and many other studies have led to population-based strategies to promote good health through healthy diets. However, Keys *et al*. (1965*a*) also recognised at that time that '…responsiveness to change in the diet tends to be related to the intrinsic characteristics of the individual'. This statement implies that not all individuals may benefit to the same extent from dietary recommendations, and that there is a need to identify subgroups that will profit more from dietary changes than others. To some extent, this approach has already been adopted. Dietary recommendations can, for example, be age and gender specific to account for variations in nutritional requirements. Nowadays, however, a new concept is emerging, accelerated by the application of techniques from the more fundamental sciences to nutrition research and by knowledge of genome sequences. Much research is being directed towards studying the effects of dietary components at the molecular level, in order to obtain detailed information on mechanisms. The findings may ultimately contribute to the formulation of dietary recommendations for well-defined smaller subgroups of the population.

Diet–gene interactions

In its simplest form diet interacts with genes, which may subsequently affect gene transcription, protein formation, metabolic pathways and ultimately function. However, dietary components may also interact directly with protein formation or activity, e.g. by interacting with post-transcriptional and post-translational events. This simple straightforward example already demonstrates that, as for many other environmental factors, studies on effects of diet on processes that play a role in health and disease may be very complex.

In general, the response to dietary changes varies between individuals. Decreasing dietary cholesterol intake, for example, will cause a substantial change in LDL-cholesterol in some subjects, while changes in other subjects will be very small. These differences in response are reproducible and hyper- and hyporesponders to dietary cholesterol intake can be identified (Katan *et al*. 1986). This finding demonstrates that individual characteristics such as gender, age, BMI, physical activity, and also genetic background, may determine dietary responsiveness. Identification of these characteristics, including genetic polymorphisms (genomics), should make it possible to identify subjects within a population who are more likely to be responsive to a particular dietary manipulation than other subjects.

Relatively little is known about how nutrients affect gene transcription (transcriptomics) and protein formation (proteomics). Nevertheless, there is an increasing number of examples of nutrients that affect gene transcription and subsequently mRNA and active protein levels. Fatty acids, for example, are natural ligands for the peroxisome proliferator-activated receptor transcription factors. Thus, the fatty acid composition of the diet may change Δ6-desaturase mRNA expression, which is mediated by the transcription factor peroxisome proliferator-activated receptor α (Cho *et al*. 1999). A high dietary cholesterol intake increases the expression of the intestinal ATP-binding cassette sterol transporter (ABC) A1, a process that is initiated by oxysterols activating the nuclear hormone receptor liver X receptor and consequently ABCA1, ABCG5 and ABCG8 transcription (Repa *et al*. 2002). Not only ABCA1 mRNA, but also ABCA1 protein concentrations increase, suggesting not only a higher transcription but also an increased translation. In addition to effects on liver X receptors, a high dietary cholesterol intake also lowers expression of the LDL receptor through interaction with the transcription factor sterol response element-binding protein–2 (Brown & Goldstein, 1997).

Information on gene expression or protein formation does not necessarily give information on enzyme activity, nor does it allow the quantification of metabolic events *in vivo* (metabolomics). An increase, for example, in the synthesis of hydroxymethylglutaryl-CoA (HMGCoA) reductase does not necessarily mean that cholesterol synthesis is also increased. To address such questions, validated markers for metabolic processes are needed. Also, stable-isotope technology can be useful in obtaining quantitative information in human subjects, such as *in vivo* rates of synthesis, degradation, turnover, and fluxes between cells and tissues.

The example of plant stanols

Numerous studies have shown that plant stanols effectively lower LDL-cholesterol concentrations (Law, 2000). For absorption by the enterocyte, cholesterol needs to be incorporated into micelles. Plant stanols can then displace

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cholesterol from mixed micelles (Ikeda *et al*. 1989), due to its structural similarity with cholesterol. This replacement causes a reduction in micellar cholesterol concentrations, which results in a lowered absorption of cholesterol of both dietary and biliary origin. The precise mechanism of cholesterol absorption is not known, but the existence of a specific cholesterol transporter in the intestinal mucosa has been suggested.

In a recent study we examined the effects of a pine wood-based (%, w/w; 92 sitostanol, 8 campestanol) and a vegetable oil-based (%, w/w; 68 sitostanol, 32 campestanol) stanol ester mixture on the plasma lipoprotein profile (the biomarker) of 112 healthy non-hypercholesterolaemic volunteers. For 4 weeks subjects received a control margarine without added plant stanol esters. For the next eight weeks, subjects were then randomised over three different groups. The first group continued with the control margarine, while the second group received products enriched with plant stanols from either pine wood (4·0 g/d) or vegetable oil (3·8 g/d). Serum LDL-cholesterol decreased with 12·8 (SD 11·2) % in the pine wood group and with 14·6 (SD 8·0) % in the vegetable oil group. Thus, this study showed that vegetable oil- and pine wood-based plant stanol esters, which have a different sitostanol:campestanol value, effectively lowered LDL-cholesterol (Plat & Mensink, 2000). Responses between individuals varied widely. As this variation may have been caused by differences in genetic background, we have analysed the presence of a gene–diet interaction (genomics) between plant stanol ester consumption and several genes involved in lipid metabolism: apolipoprotein A-IV; scavenger receptor-BI; HMGCoA reductase; cholesteryl ester transfer protein; apolipoprotein E. No significant relationships between any of the polymorphisms and the response to plant stanol ester consumption could be demonstrated (Plat & Mensink, 2002*a*). It should be emphasised, however, that genotyping was carried out after the study had ended. Thus, subjects were not selected on the basis of their genotypes. This approach decreases the statistical power of the design, because the number of subjects with a certain polymorphism can be low. To study gene–diet or gene-gene interactions, it might therefore be preferable to select subjects on the basis of their genotypes before the start of the study, or include larger numbers of subjects in order to increase the numbers of less-frequent genotypes.

To examine the effects of the decreased cholesterol absorption on other aspects of cholesterol metabolism, we looked at mRNA levels of HMGCoA reductase and of the LDL receptor (transcriptomics). In man the liver plays a central role in lipid metabolism, but for obvious reasons is not easily accessible for investigation. However, mRNA levels of the LDL receptor and HMGCoA-reductase in human mononuclear blood cells and liver correlate positively (Powell & Kroon, 1994). This finding suggests that effects of diet, drugs, diseases, etc. on expression of these two genes can be investigated in mononuclear blood cells. Indeed, we found that after feeding plant stanol esters mRNA levels of both genes increased, although only the change in LDL receptor mRNA reached statistical significance (Plat & Mensink, 2002*b*). Using flow cytometry it was demonstrated that LDL receptor protein expression on the surface of mononuclear blood cells was also increased, suggesting that the increased mRNA levels indeed resulted in increased protein levels (proteomics). These effects were evident in the monocytes and T lymphocytes, but not in the B lymphocytes. Cholesterol-standardised lathosterol concentrations, which correlate with HMGCoA reductase activity (Björkhem *et al*. 1987), increased (metabolomics). This observation seems to contradict the findings for mRNA levels in the mononuclear blood cells, which did not change significantly. Thus HMGCoA-reductase activity is not only regulated at the transciptional level, but also at the post-translational level. Alternatively, it is possible that in this respect mononuclear blood cells are not a valid biomarker for HMGCoA reductase mRNA in the liver.

In this study, the serum lipoprotein profile was used as a biomarker for atherosclerotic risk. The atherosclerotic process itself, however, was not examined in human subjects, but in transgenic apolipoprotein E3-Leiden mice. It was found that adding plant stanyl esters to their diets reduced cholesterol concentrations in the atherogenic lipoproteins by about 48 %, as well as atherosclerotic lesion development (Volger *et al*. 2001). Although these animals develop human-like atherosclerotic plaques (Gijbels *et al*. 1999), the question remains, of course, as to what extent these results can be extrapolated to the human situation.

In this example of dietary plant stanol esters we analysed the effects or interactions at the level of single genes, two different mRNA, one protein and several metabolites. Techniques such as DNA and protein arrays can also be used together to obtain information on the expression and formation of many genes and proteins respectively. In this way, information on the expression profiles of numerous known and unknown genes can be obtained in different tissues at various stages of a disease. However, these techniques are as yet not used on a routine basis, while further development and validation of experimental models and biomarkers is needed.

Conclusions

Without doubt, it is essential that we increase our understanding at the molecular level of the interactions between diet, health and disease. The opportunities to study such interactions have greatly improved during recent decades by the introduction and application of techniques from other disciplines However, we should also realise that these techniques are as yet not in widespread use and need to be developed further. Also, the aetiologies of multifactorial disorders such as atherosclerosis, diabetes, cancer and obesity are complex and cannot be explained by one single gene or one single dietary factor. Validation and further development of experimental models and biomarkers are therefore urgent issues to address. Also, aspects of data interpretation (bioinformatics) and biomodelling need attention. It is also important, however, to obtain information on functional consequences for the organism. One ultimate question, however, is whether this information can be used to develop tests on which to base dietary advice for specific subpopulations or even for an individual. These challenging issues can only be addressed by using an integrated approach, combining expertise from various disciplines.

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