Proceedings of the Nutrition Society (2013), **72**, 348–351 doi:10.1017/S0029665113001304
 First published online 28 May 2013

The Summer meeting of the Nutrition Society hosted by the Irish Section was held at Queen's University, Belfast on 16–19 July 2012

Conference on 'Translating nutrition: integrating research, practice and policy' Symposium 2: Intervention study design and personalised nutrition

Use of nutrigenomics endpoints in dietary interventions

Henk F. J. Hendriks

TNO (Netherlands Organization of Applied Scientific Research), Utrechtseweg 48, 3704 HE Zeist, P.O. Box 360, 3700 AJ Zeist, The Netherlands

In this paper, the nutrigenomics approach is discussed as a research tool to study the physiological effects of nutrition and consequently how nutrition affects health and disease (endpoints). Nutrigenomics is the study of the effects of foods and food constituents on gene expression; the analyses include analysis of mRNA, proteins and metabolites. Nutrigenomics may be useful in dealing with the challenges that nutrition research is facing; by integrating the description of numerous active genes and metabolic pathways stronger evidence and new biomarkers for subtle nutritional effects may be obtained. Also, a new definition of disease and health may be needed. The use of tests challenging homoeostasis is being proposed to help define health. Challenge tests may be able to demonstrate in a better way subtle beneficial effects of nutrition on health. The paper describes some basic concepts relevant to nutrition research as well as some of the possibilities that are offered by nutrigenomics technology. Some of its applications are described.

Nutrigenomics: Challenge testing: Nutrition intervention: Anti-inflammatory

Endpoints and biomarkers are needed in nutrition research to show efficacy of nutrition on health and disease. The term endpoint originates from medicine where clinical trials investigate the effects of an intervention (taking a drug or any other treatment) on a disease-related outcome studied in a patient population. An endpoint typically is a characteristic of the disease. The term biomarker is often used to refer to a protein measured in blood whose concentration reflects the severity or the presence of some disease state. More generally, a biomarker is anything that can be used as an indicator of a particular disease state or some other physiological state of an organism. In nutrition research, endpoints are being investigated that reflect physiological effects. Since the term biomarker is a general term and can be applied in many different settings, various biomarker definitions have been put forward: the ILSI PASSCLAIM project for instance has made a distinction between biomarkers of nutrition exposure, biomarkers of nutrition status and biomarkers of disease[1]. The latter is, however, too narrow a definition, because nutrition research does not focus on disease treatment but rather on prevention of disease and optimisation of health.

In this paper, we introduce a new conceptual approach on how to study the effects of nutrition on health, namely by combining nutrigenomics technology with challenge testing. Such an approach may ultimately lead to developing new biomarkers for health[2,3].

Biomarkers for health

Showing a beneficial physiological effect of nutrition is essentially different from evaluating drug efficacy and safety. In general, the effects of nutrition are subtle and the relation to a disease, if relevant, will occur only after a long-term exposure. In addition, the target population is typically not consisting of patients where intervention leads to a reduction of disease symptoms; in nutrition trials usually, apparently healthy people are being used. These may consist of a representative sample of the general

Abbreviations: AIDM, anti-inflammatory dietary mix; CRP, C-reactive protein.

Corresponding author: Henk F. J. Hendriks, email henk.hendriks@tno.nl

population varying in their health status, their susceptibility to the intervention and subsequently in their benefit to health. In addition, nutrition consists of a mixture of active ingredients, rather than a one compound drug, designed to target one specific receptor or process. In cases, like for instance, evaluating the effects of fruit and vegetables, combination of various naturally occurring compounds may induce effects rather than one specific compound. Such mixtures may result in various interactions working through multiple mechanisms active in various organs. Therefore, studying the effects of nutrition on health is essentially different.

The definition of health was originally based on the concept of disease; health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity[4]. More recently, health was defined more in biological terms, where health was described as the ability to adapt to one's environment[4,5]. As such, health is not a fixed entity, but a state that can vary with the circumstances that people are situated in. Important in the new approach for nutrition research is the possibility to test an organism's ability to adapt or its resilience. Such an approach may make better use of complex biomarkers. As such, it may be important to take into account various processes that contribute to an organism's ability to adapt[6,7].

Complex biomarkers can be studied in a better manner by nutrigenomics technology. Nutrigenomics has been developed as the application of high-throughput analytical tools (genetic, gene expression and metabolic) in nutrition research. The term 'high throughput tools' in nutrigenomics, refers to tools that enable thousands to millions of screening tests to be conducted at a single time. When such high throughput screening is applied in nutrition research, it allows the examination of how nutrients affect the activity of, in principle, all genes, proteins and metabolites. Nutrigenomics involves the characterisation of all gene products and the physiological functions and interactions of these products[8].

Nutrigenomics, however, has its limitations; it is an emerging science still in its beginning stages. The tools to study protein expression and metabolite production have not been developed to the point to enable efficient and reliable measurements. Also, results obtained at various levels need to be integrated to produce results that are meaningful and validated. These technologies are still in the process of development[6].

Studying complex nutrition effects

The ability of nutrigenomics technology to describe in a better way the complex effects of nutrition on multiple physiological processes was tested by studying a specific mixture of compounds, called an anti-inflammatory dietary mix (AIDM)[9]. The mixture was designed with the aim to target various metabolic pathways reported to affect low-grade inflammation. Low-grade inflammation in individuals who are overweight is thought to be one of the mediating processes in metabolic disease development. Several studies support a link between oxidative stress and inflammation in atherogenesis[10]. Adipose tissue is crucial for inflammatory status associated with obesity, primarily due to macrophage infiltration[11] and subsequent secretion of both pro- and anti-inflammatory adipokines[12]. Reduced adiponectin levels and increased C-reactive protein (CRP) levels are associated with inflammatory status and CVD and type-2 diabetes disease risk[13,14]. The established inflammatory marker high sensitivity-CRP originates from the liver and adiponectin from adipose tissue. The Mediterranean diet contains several compounds that have been associated with an anti-inflammatory effect and a reduction of CVD and type-2 diabetes[15]. These compounds consist of high contents of antioxidant polyphenols, vitamins, long chain unsaturated fatty acids and carotenoids.

The nutrition intervention was set up as a cross-over study using men with a higher than normal body mass index and having a somewhat higher inflammatory state as evaluated by their increased high sensitivity-CRP levels. The compounds were selected based on their described specific anti-inflammatory effect; each compound targeted a specific pathway in the inflammatory process. The mixture of compounds, referred to as AIDM, was based on Mediterranean diet and contained active ingredients like resveratrol, PUFA contained in fish oil, antioxidant vitamins and extracts of tomato and green tea. The effects on classical single biomarkers for inflammation, such as high sensitivity-CRP, were analysed. Also, AIDM effects were investigated in more detail by large-scale analysis of gene expression, proteins and metabolites in blood, urine and adipose tissue biopsies.

The highest scoring network in the analysis without gene expression data illustrates the effects of AIDM on inflammation (immune response), oxidative stress (production of reactive oxygen species) and lipid metabolism (quantity of lipid). The network indicates a central role for transcription factor NF-κB in the effects of AIDM. A more detailed biological interpretation of the data has been described in Bakker et al.[9].

We substantiated the effects further by testing their efficacy on endpoints in two animal models. First, an established model for cholesterol-induced atherosclerosis was used; ApoE*3 Leiden transgenic mice[16,17], displaying a human-like lipoprotein profile, were fed a high cholesterol diet inducing atherosclerotic plaques that resemble human plaques in morphology and cellular composition. Atherosclerosis development was studied in the aortic root using histological evaluation and quantification. Secondly, AIDM was evaluated in an inflammation model, male human-CRP transgenic mice[18]. The effects of AIDM on basal and IL-1β-stimulated CRP expression were investigated.

AIDM reduced cytokine-induced human CRP and fibrinogen expression in human-CRP transgenic mice after a 6-week treatment. AIDM also strongly reduced plasma cholesterol (by 43% within 2 weeks), TAG (by 41% after 2 weeks), and serum amyloid A concentrations compared with placebo. More importantly, long-term treatment (16 weeks) of ApoE*3-Leiden mice with AIDM markedly reduced the development of atherosclerosis by 96% compared with placebo. The effect on atherosclerosis was paralleled by a reduced expression of vascular

https://doi.org/10.1017/S0029665113001304 Published online by Cambridge University Press

inflammation markers and adhesion molecules intercellular adhesion molecule-1 and E-selectin. This meant that dietary supplementation of AIDM improves lipid and inflammatory risk factors of CVD and strongly reduces atherosclerotic lesion development in transgenic mice[19].

Challenging homoeostasis

An emerging concept for assessing individual health and susceptibility to disease is the use of perturbations or challenges to homoeostasis. This means that biomarkers responding to physiological challenges may show a very different response in one healthy individual *v.* another, more susceptible, individual. The best known example of a perturbation test is the oral glucose tolerance test[20], which monitors the ability of the body to respond to glucose intake. The oral glucose tolerance test is used as one of the key surrogate endpoints for diagnosing type 2 diabetes and was used to assess effects of nutrition in laboratory animals.

As an analogy, a postprandial challenge test was used to quantify the postprandial response of multiple metabolic processes by metabolomics and proteomics technology[21]. In the same human study described earlier[9], the postprandial challenge consisted of a standardised 500 ml dairy shake containing 59, 30 and 12 energy percent of lipids, carbohydrates and protein, respectively. At several time points after the challenge, blood samples were collected and analysed using GC–MS metabolic profiling (145 plasma metabolites), multiplex proteomics (seventy-nine plasma proteins) and a series of clinical chemistry analyses including liver enzymes, creatinine, albumin, insulin, glucose, cholesterol and TAG among others. Multiple processes related to metabolism, oxidation and inflammation reacted to the postprandial challenge, as demonstrated by changes of 106 metabolites, thirty-one proteins and five clinical chemistry parameters. This same postprandial challenge was applied in the AIDM dietary intervention. Of the 231 quantified parameters, thirty-one had different responses over time between treated and control groups, revealing differences in amino acid metabolism, oxidative stress, inflammation and endocrine metabolism. The results showed that acute, short-term metabolic responses to the postprandial challenge were different in subjects on the supplement mix as compared with the controls. The postprandial challenge showed metabolic changes that differed between the two treatments, which were not observed in non-perturbed conditions. Thus, a metabolomics-based quantification of a standardised perturbation provides more (differential) information on metabolic changes due to the treatment than such a quantification under homoeostatic conditions[21].

Emerging technologies in biological research provide an enormous wealth of data. At the same time, researchers are challenged to interpret this data in an integrated and meaningful manner. Multiple parameters describe processes and interactions between processes. We have developed an analysis and visualisation method, named the 'health space', which projects subjects' health status in a multidimensional space, based on predefined multivariate parameterisation of the axes. This allows researchers to analyse responses according to the underlying biological processes, defining parameterisation in a biologically meaningful manner. We applied this method to the AIDM nutrition intervention. Selection of the 'overarching processes' oxidation, metabolism and inflammation was adopted from the human study as was the selection of significant affected parameters and the grouping of these molecules in the three processes[22].

The plasma clinical chemistry, metabolomics and proteomics results of a human nutritional intervention study were visualised in a three-dimensional space, where the three axes represented the 'overarching processes' oxidation, metabolism and inflammation. The method is based on the construction of three Partial Least Squares Discriminant Analysis models, one for each of the above processes. In order to compare the scores per process, the scores of the three models were subsequently scaled around zero (the average of the AIDM group) and one (average of the placebo group). The difference between 0 and 1 was used to separate relatively healthy subjects from less healthy subjects, assuming that people become healthier using the anti-inflammatory mix. The 'health space' showed that several subgroups responded differently. One subgroup reacted mainly by modulating its metabolic stress profile, while a second subgroup showed a specific inflammatory and oxidative response to treatment. Therefore, this approach referred to as the 'health space' model allows visualisation of multiple results and to interpret them.

Conclusion

Application of nutrigenomics techniques (large-scale profiling of genes, proteins and metabolites) showed that an AIDM was able to influence processes of inflammation, oxidative stress and metabolism in human subjects. These changes were observed prior to changes in accepted biomarkers. The use of metabolic, protein and gene profiling strongly facilitated accurate and detailed quantification and description of the molecular processes involved. The studies presented suggested that integrated measures describing complete pathways may enable the description of subtle changes induced by nutrition over a short period of time. Challenge tests may further increase sensitivity to show subtle effects of nutrition on health relevant processes in human subjects.

Acknowledgements

There is no specific funding associated with this paper. The contributions of all TNO (co-)authors, who contributed to a better understanding of nutrigenomics endpoints in nutrition research, are gratefully acknowledged. The author declares no conflict of interest.

References

1. Aggett PJ, Antoine JM, Asp NG *et al.* (2005) PASSCLAIM, process for the assessment of scientific support for claims on foods. *Eur J Nutr* **44**, S1.

2. van Ommen B, Keijer J, Kleemann R *et al.* (2008) The challenges for molecular nutrition research 2: quantification of the nutritional phenotype. *Genes Nutr* **3**, 51–59.
3. van Ommen B, Keijer J, Heil SG *et al.* (2009) Challenging homeostasis to define biomarkers for nutrition related health. *Mol Nutr Food Res* **53**, 795–804.
4. WHO (2006) Constitution of the World Health Organization. http://www.who.int/governance/eb/who_constitution_en.pdf
5. Huber M, Knottnerus JA, Green L *et al.* (2011) How should we define health? *Br Med J* **343**, d4163. doi:10.1136/bmj.d4163.
6. Afman LA & Müller M (2012) Human nutrigenomics of gene regulation by dietary fatty acids. *Prog Lipid Res.* **51**, 63–70.
7. van Dijk SJ, Mensink M, Esser D *et al.* (2012) Responses to high-fat challenges varying in fat type in subjects with different metabolic risk phenotypes: a randomized trial. *PLoS ONE* **7**, e41388. doi:10.1371/journal.pone.0041388.
8. Kaput J, Ordovas JM, Ferguson L *et al.* (2005) The case for strategic international alliances to harness nutritional genomics for public and personal health. *Br J Nutr* **94**, 623–632.
9. Bakker GCM, van Erk MJ, Pellis L *et al.* (2010) An anti-inflammatory dietary mix modulates inflammation and oxidative and metabolic stress in overweight men: a nutrigenomics approach. *Am J Clin Nutr* **91**, 1044–1059.
10. Ross R (1999) Atherosclerosis – an inflammatory disease. *N Eng J Med* **340**, 115–126.
11. Hotamisligil GS (2006) Inflammation and metabolic disorders. *Nature* **444**, 860–867.
12. Ronti T, Lupattelli G & Mannarino E (2006) The endocrine function of adipose tissue: an update. *Clin Endocrinol (Oxf)* **64**, 355–365.
13. Schulze MB, Rimm EB, Shai I *et al.* (2004) Relationship between adiponectin and glycemic control, blood lipids, and inflammatory markers in men with type 2 diabetes. *Diabetes Care* **27**, 1680–1687.
14. Danesh J, Wheeler JG, Hirschfield GM *et al.* (2004) C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Eng J Med* **350**, 1387–1397.
15. Esposito K, Marfella R, Ciotola M *et al.* (2004) Effect of a Mediterranean-style diet on endothelial cell dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA, J Am Med Assoc* **292**, 1440–1446.
16. Havekes LM, van Vlijmen BJ, Jong MC *et al.* (1997) Use of transgenic mice in lipoprotein metabolism and atherosclerosis research. *Prostaglandins Leukot Essent Fatty Acids* **57**, 463–466.
17. Lardenoye JH, Delsing DJ, de Vries MR *et al.* (2000) Accelerated atherosclerosis by placement of a perivascular cuff and a cholesterol-rich diet in ApoE*3-Leiden transgenic mice. *Circ Res.* **87**, 248–253.
18. Kleemann R, Verschuren L, de Rooij BJ *et al.* (2004) Evidence for anti-inflammatory activity of statins and PPARalpha activators in human C-reactive protein transgenic mice *in vivo* and in cultured human hepatocytes *in vitro*. *Blood* **103**, 4188–4194.
19. Verschuren L, Wielinga PY, van Duyvenvoorde W *et al.* (2011) A dietary mixture containing fish oil, resveratrol, lycopene, catechins, and vitamins E and C reduces atherosclerosisin transgenic mice. *J Nutr* **141**, 863–869.
20. Wopereis S, Rubingh CM, van Erk MJ *et al.* (2009) Metabolic profiling of the response to an oral glucose tolerance test detects subtle metabolic changes. *PLoS ONE* **4**, 143–149.
21. Pellis L, van Erk MJ, van Ommen B *et al.* (2012) Plasma metabolomics and proteomics profiling after a postprandial challenge reveal subtle diet effects on human metabolic status. *Metabolomics* **8**, 347–359.
22. Bouwman J, Vogels JTWE, Wopereis S *et al.* (2012) Visualization and identification of health space, based on personalized molecular phenotype and treatment response to relevant underlying biological processes. *BMC Med Genomics* **5**, 1–9.

https://doi.org/10.1017/S0029665113001304 Published online by Cambridge University Press