Cellular Defense System Gene Expression Profiling of Human Whole Blood: Opportunities to Predict Health Benefits in Response to Diet[1,2]

Janice E. Drew*

Metabolic Health, Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, Scotland, United Kingdom

ABSTRACT

Diet is a critical factor in the maintenance of human cellular defense systems, immunity, inflammation, redox regulation, metabolism, and DNA repair that ensure optimal health and reduce disease risk. Assessment of dietary modulation of cellular defense systems in humans has been limited due to difficulties in accessing target tissues. Notably, peripheral blood gene expression profiles associated with nonhematologic disease are detectable. Coupled with recent innovations in gene expression technologies, gene expression profiling of human blood to determine predictive markers associated with health status and dietary modulation is now a feasible prospect for nutrition scientists. This review focuses on cellular defense system gene expression profiling of human whole blood and the opportunities this presents, using recent technological advances, to predict health status and benefits conferred by diet. *Adv. Nutr. 3: 499–505, 2012.*

Introduction

Humans require cellular defense systems to defend against environmental stresses ranging from bacteria, fungi and viruses to those associated with aging, diet and lifestyle. Failure to defend against environmental stresses during our lifetime leads to a number of chronic diseases and resulting morbidities such as cardiovascular disease, hypertension, metabolic syndrome, diabetes, neurodegenerative disorders, and cancers (1–10). Environmental stresses also promote unhealthy aging and increased risk of the aforementioned chronic diseases and associated morbidities (4,11). Control and regulation of our cellular defense systems are thus intimately linked to health status, disease risk, and healthy aging.

Diet is recognized as a critical factor in maintaining cellular defenses. However, mechanistic information on the impact of diet and specific dietary components on maintaining or restoring optimal cellular defense systems and associated health benefits has been problematic. It has been difficult to obtain this information from the necessary human intervention studies due to the inherent problems in accessing target tissues and observed interindividual responses to nutrition. Recent technological advances in gene expression technologies have presented new opportunities to characterize gene expression changes that can be used as predictive markers of health or disease status (12–16). This approach yields information on gene expression changes in target tissues and organs, but it has also been demonstrated that gene expression changes in peripheral blood, as a consequence of nonhematologic disease, can be detected (17–22). After parallel technological advances in gene expression profiling of blood samples (23–29), it is now feasible to interrogate cellular defense system components in human blood samples. Consequently, gene expression signatures predicting health benefits in humans in response to diet are a viable prospect (30,31). This review focuses on modulation of cellular defense system gene expression in response to specific dietary components in human whole blood and developments in blood profiling to predict health status and health benefits of diet.

Cellular defense systems

Humans have 5 key interlinked cellular defense systems: the immune system (32), the inflammatory response (33), redox regulation (1,34), metabolism (2,7,35), and DNA repair (3,4). All 5 cellular defense systems are required to combat diverse stressors and maintain a healthy and functional physiology and reduce disease risk in humans (1–7). These defense systems operate at the molecular level within cells

[1] Supported by the Scottish Government's Rural and Environment Science and Analytical Services Division.

[2] Author disclosure: J. E. Drew, no conflicts of interest.

* To whom correspondence should be addressed. E-mail: j.drew@abdn.ac.uk.

and tissues to maintain appropriate functioning of cellular processes and prevent stress-related changes that lead to deteriorating health and increased risk of chronic diseases. Control and regulation of these cellular defense systems are interlinked, often using a common network of gene products and signaling pathways to direct cellular defense and responses to the environment. Diet is a critical factor in the maintenance of these cellular defense systems and one that can be manipulated to ensure health benefits, preventing physiological deterioration and the associated ill health and disease (36–42).

Strategies for monitoring cellular defense systems in human blood

It is problematic to assess the impact of diet on these key cellular defense systems in biologically relevant tissues and organs in humans. However, using technological advances, it is now feasible to assess the status of cellular defense systems and responses to dietary factors by gene expression profiling blood samples extracted from the systemic circulation (22,31,38,39,41,43–45). The systemic circulation pervades all cells and tissues. Consequently, changes in the cellular defense systems in cells and tissues can be monitored in blood samples via both the effect on gene transcription induced by secretion of organ-specific factors and the associated changes in the transcriptome associated with blood cell types (31) present in the systemic circulation as a consequence of altered production of blood cells from the bone marrow and thymus. The well-characterized CD markers provide a means of identifying changes in human blood cell types (31) and provide a means of monitoring responses to diet.

Monitoring cellular defense systems in blood to predict health status and responses to diet has presented considerable challenges for researchers. Studies revealed difficulties in reliable detection of RNA transcripts that may be influenced by blood collection and processing methods, sample handling, RNA extraction protocol, and the choice of technological platforms used to assess transcript levels (46–48). Difficulties in obtaining reliable gene expression data proved to be largely due to induction of gene expression due to phlebotomy in whole-blood analysis or the alternative approach involving purification of mononuclear cells from whole blood. Purification methods led to changes in gene transcript levels as a consequence of messenger RNA (mRNA) degradation and gene expression changes during processing of live cells (46–48). Rainen et al. (46) identified RNA degradation and initiation of gene expression in response to whole-blood collection by conventional methods in EDTA tubes followed by an organic extraction of RNA. Spiker et al. (47) reported observations of marked changes in basal gene expression levels in cell populations in response to isolation from whole-blood samples.

Fortunately, technological advances in gene expression profiling of blood extracted from the systemic circulation now present opportunities for interrogation of human whole-blood samples, giving rise to the new field of transcriptional bloodomics (26,30,31). Commercially available systems, such as the PAXgene Blood RNA kit (PreAnalytiX) and the Tempus Blood RNA kit (Applied Biosystems) have revolutionized transcript profiling of human whole-blood samples. These systems permit ease of collection of blood samples directly from donors. The blood RNA profiles are stabilized within the collection tubes using proprietary solutions. Collection and storage at room temperature before frozen storage facilitate the use in human intervention studies because samples can be collected for later RNA extraction. The whole-blood RNA extracted using these commercial systems has proved to be of sufficient yield and quality for gene expression profiling studies (23,25,27–29,49). Extending room temperature storage to the maximum recommended range before freezing has been demonstrated to increase yield and is a useful strategy when sample volumes are limited (25,27). However, it has been noted that differences in the handling of PAXgene blood collections, such as room temperature storage before freezing applied for practical purposes in clinical studies, can contribute to differences in gene expression. However, this was considered to be minimal (0.09% of total variation of measured by Affymetrix arrays) (23). Notably different commercial products for whole-blood collection have also been observed to have an effect on the measurement of differential gene expression of immune activated targets in peripheral blood (29). This highlights the importance of standardization of procedures for collection and processing of blood samples for gene-expression profiling. Application of standardized procedures has permitted satisfactory gene-expression profiles with appropriate pairwise correlations between gene expression profiles using Affymetrix GeneChips (23,25,28) and real-time PCR (27). Further technological advances have permitted increased sensitivity of measuring gene expression in whole-blood samples by depleting abundant globin RNA transcripts (28,49,50). Ryu et al. (50) reported 75% recovery of high-purity mRNA after globin reduction, with an additional 6861 mRNA transcripts detectable in whole blood from volunteers consuming a zinc-depleted diet compared with whole-blood total RNA not subjected to globin reduction. However, it was noted by Liu et al. (49) that processing steps to remove globin transcripts can reduce the quantity and quality of RNA and that transcript profiles may deviate from those of untreated control samples. Novel multiplex gene expression technologies, such as the GenomeLab System (Beckman) now permit assay of multiple targets using nanogram quantities of total RNA (12,51,52). Studies in our lab have established that in combination with the PAXgene system, multiple components of cellular defense pathways in human whole-blood samples can be monitored using the GenomeLab system (52), permitting development of strategies to assess dietary modulation. The technological advances in human blood gene expression profiling discussed previously have led to further exploitation of whole human blood, a readily accessible biological sample, to assess cellular defense system gene expression in response to specific dietary components (discussed later). Plant-based foods, micronutrients, bioactive phytochemicals, high-energy diets, and dietary supplements are notable in this respect and have been subject

to intense investigation to identify the conferred health benefits on cellular defense systems (22,36,39,42,53–61). Differential gene expression in blood has thus been used in recent studies to characterize the impact of a diverse range of dietary factors (22,38–41,44,45,62). The influence of specific dietary components associated with influences on cellular defense systems are discussed further in the following.

Micronutrients and cellular defense system gene expression in blood

Various micronutrients have long been associated with health status and links to cellular defense systems, including vitamins, minerals, amino acids, and fatty acids (37,56,63–65). The importance of dietary antioxidant vitamins, such as vitamins C and E, in maintaining redox balance is well-known, but vitamins are also linked to homeostatic regulation of cellular defenses linked to immunity, inflammation, and DNA repair (37,56,64). A vitamin C–rich diet is reported to activate a number of oxidative stress–responsive genes in lymphocytes (antioxidant protein 1 homolog, catalase, cytochrome b-245, copper chaperone for superoxide dismutase, oxidation resistance 1, and oxidative stress responsive 1, peroxiredoxin 4, prostaglandin endoperoxide synthase, cyclooxygenase, and soluble superoxide dismutase 1 (64). Reactive species and oxidative stress in tissues can disrupt the flux of reducing molecules in the blood system, resulting in activation of Nrf2, with antioxidant response elements in the promoter region of redox responsive genes (5). Reports of limited induction of Nrf2 by vitamins (66) imply that there may be additional mechanisms involved in the activation of redox genes in blood cells. Nrf2 induction of antioxidant response element–responsive genes is linked with DNA repair and inflammatory and immune processes (5,67). Vitamin C–rich diets have been linked to induction of Nudix (nucleoside diphosphate–linked moiety X)-type motif (*NUDT1*) that encodes for 7,8-dihydro-8-oxoguanie triphosphatase, an enzyme preventing nucleotide misincorporation caused by oxidative DNA damage (67). Dietary vitamin C is also linked to expression of the glutathione peroxidase gene family important in regulating redox balance (67). Likewise, dietary selenium is an important mineral in regulating selenium-containing glutathione peroxidases. Selenium, together with other dietary minerals, zinc, iron, and copper are required for production of many of the enzymes needed for functional cellular defense systems (68). For example, dietary sources of selenium are essential for the maintenance of selenoproteins, which are essential for regulating redox balance, inflammatory responses, and immunity (65). Micronutrients arginine, glutamine, branched chain amino acids, and [n-3] polyunsaturated fatty acids are implicated in the maintenance of healthy immune and inflammatory defenses (69–72). The [n-3] polyunsaturated fatty acids are found in large amounts in fish oils and have been reported to alter peripheral mononuclear blood cell gene expression of signaling pathways controlled by the inflammatory mediator, nuclear transcription factor κB (73). Strategies for enhancing immunity and reducing inflammation using micronutrients have been applied in elderly subjects and cancer patients (37,54). Modulation of immune defense cells and inflammatory mediators appears to be a key factor in immunonutrition (54,69–72,74). Increased arginine and [n-3] fatty acids have been reported to enhance activity of natural killer cells and helper T cells in blood (74). Glutamine has been reported to be an important micronutrient for immune cell functioning of monocytes, lymphocytes, and neutrophils (70).

Notably, studies have also identified potential negative impacts of dietary micronutrients on cellular defense systems (37,54,63,68,75,76). Excess dietary selenium can lead to toxicity (63). Increased mortality from all cancers in smokers with intake of vitamins [multivitamins alone, RR = 1.13 (95% CI: 1.05–1.23) or in combination with vitamin A, C, or E (RR = 1.16 (95% CI: 1.06–1.26)] has been reported (77). Although clinical trials have indicated reduced risk of infection, fewer days on a ventilator, and decreased length of intensive care unit and hospital stay with use of enteral immune-modulating diets, patients with severe sepsis, shock, and organ failure could be disadvantaged by immunonutrition strategies (37). Individuals with systemic inflammatory response syndrome may experience adverse effects on cellular defenses in response to immune-modulating micronutrients such as arginine and unsaturated fatty acids (37). Consequently, an excess of micronutrients can be detrimental, leading to impaired cellular defense and development of disease (63) and increased severity (37,54).

Phytochemicals and cellular defense system gene expression in blood

Phytochemicals present in plant-based foods have long been associated with bioactivity in humans, particularly sulfur-containing and phenolic compounds. Sulfur-containing compounds, isothiocyanates, and dithiolethiones derived from cruciferous vegetables, and diallyl sulfides coming from the allium family plants such as garlic and onion are reported to influence cellular defense systems through mechanisms linked to redox balance and DNA stability (40,78). Phenolic compounds in the human diet, such as flavonoids, are among the most studied for their bioactive properties. However, the vast array of phytochemicals in the human diet and extensive metabolism and interindividual responses have led to conflicting reports of the effects of phytochemicals on cellular defenses (79). Recent studies have focused on dietary interventions with phytochemical-rich foods (38,39,43,45,59). A flavonoid-rich diet, including soyabeans and derivatives, onions, green tea, and bilberry juice was associated with increased expression of DNA repair and decreased metabolic pathway gene expression in whole blood and a parallel decrease in antimutagenicity measured in urine (38). Flavonoid-mediated detoxification processes have also been linked to the *CYP* gene family and regulation of the aryl hydrocarbon receptor (80). The aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator are transcription factors that regulate *CYP* genes that detoxify mutagenic metabolites that cause

DNA damage. Antioxidant-rich diets have also been reported to alter expression of DNA repair and aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator genes in blood (39), concomitant with increased plasma antioxidant markers. Despite the potential for a beneficial effect of increased DNA repair and reduced DNA damage indicated by these studies, other reports have implicated consumption of flavonoids with strand breaks in DNA (81).

Isoflavonoids are a class of flavonoid phenolic compounds that include a number of bioactive compounds, such as phytoestrogens, and are produced by pea or bean family. Soy foods contain considerable amounts of isoflavones and have been linked to altered gene expression of cellular defense genes in peripheral mononuclear blood cells (43). Consumption of isoflavones in soy foods (at least 50 mg/d) was associated with altered blood cell gene expression in metabolic pathways linked to insulin signaling, immune and inflammatory processes (FC receptors, inducible cyclooxygenase, calmodulin, and adducin) (43). However, these studies were performed on a population that mostly was unable to produce the isoflavone metabolite equol from diadzein. Isoflavones are also attributed to cardiovascular protection through targeting of the redox-sensitive Nrf2-Keap1 defense pathway (60). Indeed many phytochemicals have been reported to have antioxidant properties. Phytochemical-rich diets containing soft fruits, food plants of the *Brassica* family, green tea, herbs, nuts, seeds, and dark chocolate considerably increased bioavailable phenolic compounds and were correlated with increased antioxidant biomarkers in plasma and altered regulation of cellular defense genes involved in DNA repair and metabolic pathways (39). Consumption of the increased phenolic compounds in tomato and green tea extracts has been linked to altered expression of inflammatory genes in peripheral mononuclear blood cells (45).

Deduction of the direct effects of phenolic compounds on regulating cellular defense system genes in human blood is problematic in human intervention studies using phytochemical enriched diets. The food components rich in phytochemicals are also associated with increased consumption of many of the micronutrients that have also been correlated with altered regulation of cellular defenses (38,39,45). Further studies to identify phenolic metabolites present in whole-blood samples, together with in vitro mechanistic studies, are necessary to determine the specific effects on cellular defense gene regulation.

Macronutrients and cellular defense system gene expression in blood

Macronutrients have a profound effect on cellular defense systems, giving rise to alterations in the blood transcriptome providing predictive indicators of health status (22,41, 43,45). Dietary carbohydrate and protein intake is correlated with changes in the blood leukocyte transcriptome. Differential expression of genes associated with glycogen metabolism and protein biosynthesis were measured by Affymetrix microarray analysis in response to consumption of either a high-carbohydrate or high-protein breakfast (44). Differential gene expression in response to both the high-carbohydrate or high-protein breakfasts showed similarities in regulation and consisted of 141 genes, including a number of immune-related transcripts (44). It was also noted that there were considerably more differentially expressed genes in leukocytes in response to consumption of the high-protein compared with high-carbohydrate breakfast. It was noted in this study that consumption of the high-protein breakfast induced a number of factors influencing satiety. However, despite inclusion in the microarray chip, the related gene transcripts were not detected on microarray analysis of leukocytes (44). This highlights issues to be considered in blood profiling of cellular defense system gene expression in blood and the choice of sample-processing procedure.

Consumption of energy-dense foods and the resulting obesity, now a worldwide epidemic (82), is characterized by aberrant metabolic regulation. Individuals who are obese exhibit altered metabolism in biologically relevant tissues such as adipose and muscle (41,83,84). These changes are characterized by distinct metabolic changes in peripheral blood. Aberrant blood profiles of hormones, peptides, and lipids measured in obese individuals are linked to the development of diabetes (85), heart disease (86), and cancer (8). Ghosh et al. (22) reported increased expression of genes, such as carbonic anhydrase, ferrochelatase, synuclein, and glycophorin B, associated with erythrocytes and reticulocytes, supporting previous observations of higher red blood cell counts in obese individuals (87).

Blood gene expression profiles associated with obesity have identified components of metabolic regulation linked to other cellular defense systems (22,76,88,89). Ghosh et al. (22) reported a decrease in expression of transcripts linked to immune responses. This may be linked to reports of aberrant leptin signaling in obese individuals via activation of leptin receptors expressed by natural killer cells (90). High-energy diets leading to obesity are associated with oxidative stress (89) and inflammation (76,88,91). Low-fat diets (11% of energy from fat compared with control diet of 27% energy from fat) were been linked to cellular defense gene expression changes in blood cells after an 8-week intervention (43). *NAMPT* showed the greatest fold reduction in response to decreased dietary fat in this study and plays a role in redox reactions in addition to regulating cellular metabolism and possibly insulin secretion (43). A number of transcripts associated with inflammation and immune responses linked to phagocytosis have been reported to be regulated in response to dietary fat (43).

Energy restriction has been reported to restore metabolic health (92). This effect may be a consequence of nutrient-sensing pathway down-regulation of the metabolic rate. Studies in model organisms indicate that energy restriction has a important effect on increasing life span. This is attributed to influencing genes, such as the sirtuins, that are known to be involved in DNA repair (93). Human homologs of genes regulated by energy restriction and associated with increased life span in model systems, such as *SIR-2* are known to regulate DNA repair factors (94,95).

Dietary supplements and cellular defense system gene expression in blood

Evidence of beneficial effects of specific dietary components on cellular defenses has led to the production of dietary extracts in an attempt to elicit health benefits. A mixture of 5 bioactive phytochemicals (ashwagandha, *Bacopa* extract, green tea extract, silymarin, and curcumin) (61), has been reported to reduce plasma markers of oxidative stress (67) and regulate a number of gene markers associated with atherosclerosis, colon cancer, and Alzheimer's disease (5).

Nutritional supplements, such as cysteine-rich proteins, whey or keratin, and papaya extract (96–98), have been reported to improve antioxidant status and alleviate oxidative stress. Marotta et al. (96) measured considerably increased expression of Nrf2-regulated glutathione peroxidase, superoxide dismutase, and catalase genes in leukocytes. The base excision repair gene 8-oxoguanine glycosylase (hOGG1) was also up-regulated in response to consumption of papaya extract (96). Dietary supplementation with fermented papaya extract (6 g/d for 6 mo) was also shown to down-regulate the inflammatory mediator TNF-α in isolated monocytes (98).

Conclusion

Human blood and the dynamic cell population that it contains may serve as a useful biological indicator to determine predictive signatures indicating health status with respect to cellular defense systems and the impact of exposure to diet. Gene predictors linked to cellular defense system signaling pathways and associated with a healthy phenotype are being identified and could potentially be used to predict health status, prepathological changes linked to diet and lifestyle factors, and, crucially, responses to dietary changes to optimize diet and lifestyle for health. Use of blood transcriptomic analysis to determine predictive gene signatures will require further functional and biological validation to generate confidence in prediction of health status. However, this approach is already proving feasible in commercial molecular diagnostics with clinically useful information being obtained from application of assays based on gene expression patterns in blood cells. Assessment of multiple components of cellular defense systems in human blood samples could ultimately permit monitoring and survey of the impact of diet and lifestyle factors to generate evidence for effective translation of research on food, drink, and health.

Acknowledgments

The author is grateful for support from the Scottish Government's Rural and Environment Science and Analytical Services Division. The sole author had responsibility for all parts of the manuscript.

Literature Cited

1. Landis GN, Tower J. Superoxide dismutase evolution and life span regulation. Mech Ageing Dev. 2005;126:365–79.
2. Biddie SC, Conway-Campbell BL, Lightman SL. Dynamic regulation of glucocorticoid signalling in health and disease. Rheumatology (Oxford). 2012;51(3):403–12.
3. Borgesius NZ, de Waard MC, van der Pluijm I, Omrani A, Zondag GC, van der Horst GT, Melton DW, Hoeijmakers JH, Jaarsma D, Elgersma Y. Accelerated age-related cognitive decline and neurodegeneration, caused by deficient DNA repair. J Neurosci. 2011;31:12543–53.
4. Diderich K, Alanazi M, Hoeijmakers JH. Premature aging and cancer in nucleotide excision repair-disorders. DNA Repair (Amst). 2011;10:772–80.
5. Hybertson BM, Gao B, Bose SK, McCord JM. Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation. Mol Aspects Med. 2011;32:234–46.
6. Maher SG, Reynolds JV. Basic concepts of inflammation and its role in carcinogenesis. Recent Results Cancer Res. 2011;185:1–34.
7. Tamashiro KL, Sakai RR, Shively CA, Karatsoreos IN, Reagan LP. Chronic stress, metabolism, and metabolic syndrome. Stress. 2011;14:468–74.
8. World Cancer Research Fund/American Institute for Cancer Research. Food Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington, DC: AICR; 2007.
9. Reis JP, Loria CM, Sorlie PD, Park Y, Hollenbeck A, Schatzkin A. Lifestyle factors and risk for new-onset diabetes: a population-based cohort study. Ann Intern Med. 2011;155:292–9.
10. Zelber-Sagi S, Ratziu V, Oren R. Nutrition and physical activity in NAFLD: An overview of the epidemiological evidence. World J Gastroenterol. 2011;17:3377–89.
11. Arbeev KG, Ukraintseva SV, Akushevich I, Kulminski AM, Arbeeva LS, Akushevich L, Culminskaya IV, Yashin AI. Age trajectories of physiological indices in relation to healthy life course. Mech Ageing Dev. 2011;132:93–102.
12. Drew JE, Mayer C-D, Farquharson AJ, Young P, Barrera LN. Custom design of a GeXP multiplexed assay used to assess expression profiles of inflammatory gene targets in colon normal, polyp and tumour tissue. J Mol Diagn. 2011;13:233–42.
13. Baehner FL, Lee M, Demeure MJ, Bussey KJ, Kiefer JA, Barrett MT. Genomic signatures of cancer: basis for individualized risk assessment, selective staging and therapy. J Surg Oncol. 2011;103:563–73.
14. Rotunno M, Hu N, Su H, Wang C, Goldstein AM, Bergen AW, Consonni D, Pesatori AC, Bertazzi PA, Wacholder S, et al. A gene expression signature from peripheral whole blood for stage I lung adenocarcinoma. Cancer Prev Res (Phila). 2011;4:1599–608.
15. Trevino V, Tadesse MG, Vannucci M, Al-Shahrour F, Antczak P, Durant S, Bikfalvi A, Dopazo J, Campbell MJ, Falciani F. Analysis of normal-tumour tissue interaction in tumours: prediction of prostate cancer features from the molecular profile of adjacent normal cells. PLoS ONE. 2011;6:e16492.
16. Van Laar RK. Design and multiseries validation of a web-based gene expression assay for predicting breast cancer recurrence and patient survival. J Mol Diagn. 2011;13:297–304.
17. Batliwalla FM, Li W, Ritchlin CT, Xiao X, Brenner M, Laragione T, Shao T, Durham R, Kemshetti S, Schwarz E, et al. Microarray analyses of peripheral blood cells identifies unique gene expression signature in psoriatic arthritis. Mol Med. 2005;11:21–9.
18. Batliwalla FM, Baechler EC, Xiao X, Li W, Balasubramanian S, Khalili H, Damle A, Ortmann WA, Perrone A, Kantor AB, et al. Peripheral blood gene expression profiling in rheumatoid arthritis. Genes Immun. 2005;6:388–97.
19. Glatt SJ, Everall IP, Kremen WS, Corbeil J, Sásik R, Khanlou N, Han M, Liew CC, Tsuang MT. Comparative gene expression analysis of blood and brain provides concurrent validation of SELENBP1 up-regulation in schizophrenia. Proc Natl Acad Sci U S A. 2005;102:15533–8.
20. Edelman LB, Toia G, Geman D, Zhang W, Price ND. Two-transcript gene expression classifiers in the diagnosis and prognosis of human diseases. BMC Genomics. 2009;10:583.
21. Aarøe J, Lindahl T, Dumeaux V, Saebø S, Tobin D, Hagen N, Skaane P, Lönneborg A, Sharma P, Børresen-Dale AL. Gene expression profiling of peripheral blood cells for early detection of breast cancer. Breast Cancer Res. 2010;12:R7.
22. Ghosh S, Dent R, Harper ME, Gorman SA, Stuart JS, McPherson R. Gene expression profiling in whole blood identifies distinct biological pathways associated with obesity. BMC Med Genomics. 2010;3:56.

23. Thach DC, Lin B, Walter E, Kruzelock R, Rowley RK, Tibbetts C, Stenger DA. Assessment of two methods for handling blood in collection tubes with RNA stabilizing agent for surveillance of gene expression profiles with high density microarrays. J Immunol Methods. 2003; 283:269–79.
24. Feezor RJ, Baker HV, Mindrinos M, Hayden D, Tannahill CL, Brownstein BH, Fay A, MacMillan S, Laramie J, Xiao W, et al. Inflammation and Host Response to Injury, Large-Scale Collaborative Research Program. Whole blood and leukocyte RNA isolation for gene expression analyses. Physiol Genomics. 2004;19:247–54.
25. Wang J, Robinson JF, Khan HM, Carter DE, McKinney J, Miskie BA, Hegele RA. Optimizing RNA extraction yield from whole blood for microarray gene expression analysis. Clin Biochem. 2004;37:741–4.
26. Zheng Z, Luo Y, McMaster GK. Sensitive and quantitative measurement of gene expression directly from a small amount of whole blood. Clin Chem. 2006;52:1294–302.
27. Carrol ED, Salway F, Pepper SD, Saunders E, Mankhambo LA, Ollier WE, Hart CA, Day P. Successful downstream application of the Paxgene Blood RNA system from small blood samples in paediatric patients for quantitative PCR analysis. BMC Immunol. 2007;8:20.
28. Kruhøffer M, Dyrskjøt L, Voss T, Lindberg RL, Wyrich R, Thykjaer T, Orntoft TF. Isolation of microarray-grade total RNA, microRNA, and DNA from a single PAXgene blood RNA tube. J Mol Diagn. 2007;9: 452–8.
29. Asare AL, Kolchinsky SA, Gao Z, Wang R, Raddassi K, Bourcier K, Seyfert-Margolis V. Differential gene expression profiles are dependent upon method of peripheral blood collection and RNA isolation. BMC Genomics. 2008;9:474.
30. Mohr S, Liew CC. The peripheral-blood transcriptome: new insights into disease and risk assessment. Trends Mol Med. 2007;13:422–32.
31. Watkins NA, Gusnanto A, de Bono B, De S, Miranda-Saavedra D, Hardie DL, Angenent WG, Attwood AP, Ellis PD, Erber W, et al. A HaemAtlas: characterizing gene expression in differentiated human blood cells. Blood. 2009;113:e1–9.
32. Yano T, Kurata S. Intracellular recognition of pathogens and autophagy as an innate immune host defense. J Biochem. 2011;150:143–9.
33. Crane MJ, Hokeness-Antonelli KL, Salazar-Mather TP. Regulation of inflammatory monocyte/macrophage recruitment from the bone marrow during murine cytomegalovirus infection: role for type I interferons in localized induction of CCR2 ligands. J Immunol. 2009;183:2810–7.
34. Lee J-M, Johnson JA. An important role of Nrf2-ARE pathway in the cellular defense mechanism. J Biochem Mol Biol. 2004;37:139–43.
35. Tamashiro KL. Metabolic syndrome: links to social stress and socioeconomic status. Ann N Y Acad Sci. 2011;1231:46–55.
36. Lesgards JF, Durand P, Lassarre M, Stocker P, Lesgards G, Lanteaume A, Prost M, Lehucher-Michel M-P. Assessment of lifestyle effects on the overall antioxidant capacity of healthy subjects. Environ Health Perspect. 2002;110:479–86.
37. Mitchell BL, Ulrich CM, McTiernan A. Supplementation with vitamins or minerals and immune function: can the elderly benefit? Nutr Res. 2003;23:1117–39.
38. Guarrera S, Sacerdote C, Fiorini L, Marsala R, Polidoro S, Gamberini S, Saletta F, Malaveille C, Talaska G, Vineis P, et al. Expression of DNA repair and metabolic genes in response to a flavonoid-rich diet. Br J Nutr. 2007;98:525–33.
39. Bøhn SK, Myhrstad MC, Thoresen M, Holden M, Karlsen A, Tunheim SH, Erlund I, Svendsen M, Seljeflot I, Moskaug JO, et al. Blood cell gene expression associated with cellular stress defense is modulated by antioxidant-rich food in a randomised controlled clinical trial of male smokers. BMC Med. 2010;8:54.
40. Zhao CR, Gao ZH, Qu XJ. Nrf2-ARE signaling pathway and natural products for cancer chemoprevention. Cancer Epidemiol. 2010;34:523–33.
41. Brattbakk HR, Arbo I, Aagaard S, Lindseth I, de Soysa AK, Langaas M, Kulseng B, Lindberg F, Johansen B. Balanced caloric macronutrient composition downregulates immunological gene expression in human blood cells-adipose tissue diverges. OMICS. Epub 2011 Jun 16.
42. de Mello VD, Schwab U, Kolehmainen M, Koenig W, Siloaho M, Poutanen K, Mykkänen H, Uusitupa M. A diet high in fatty fish, bilberries and wholegrain products improves markers of endothelial function and inflammation in individuals with impaired glucose metabolism in a randomised controlled trial: The Sysdimet study. Diabetologia. 2011;54:2755–67.
43. Wang J, Siegmund K, Tseng CC, Lee AS, Wu AH. Soy food supplementation, dietary fat reduction and peripheral blood gene expression in postmenopausal women - a randomized, controlled trial. Mol Nutr Food Res. 2011;55 Suppl 2:S264–77.
44. van Erk MJ, Blom WA, van Ommen B, Hendriks HF. High-protein and high-carbohydrate breakfasts differentially change the transcriptome of human blood cells. Am J Clin Nutr. 2006;84:1233–41.
45. Bakker GC, van Erk MJ, Pellis L, Wopereis S, Rubingh CM, Cnubben NH, Kooistra T, van Ommen B, Hendriks HF. An anti-inflammatory dietary mix modulates inflammation and oxidative and metabolic stress in overweight men: a nutrigenomics approach. Am J Clin Nutr. 2010; 91:1044–59.
46. Rainen L, Oelmueller U, Jurgensen S, Wyrich R, Ballas C, Schram J, Herdman C, Bankaitis-Davis D, Nicholls N, Trollinger D, et al. Stabilization of mRNA expression in whole blood samples. Clin Chem. 2002;48:1883–90.
47. Spijker S, van de Leemput JC, Hoekstra C, Boomsma DI, Smit AB. Profiling gene expression in whole blood samples following an *in vitro* challenge. Twin Res. 2004;7:564–70.
48. Pahl A. Gene expression profiling using RNA extracted from whole blood: technologies and clinical applications. Expert Rev Mol Diagn. 2005;5:43–52.
49. Liu J, Walter E, Stenger D, Thach D. Effects of globin mRNA reduction methods on gene expression profiles from whole blood. J Mol Diagn. 2006;8:551–8.
50. Ryu MS, Langkamp-Henken B, Chang SM, Shankar MN, Cousins RJ. Genomic analysis, cytokine expression, and microRNA profiling reveal biomarkers of human dietary zinc depletion and homeostasis. Proc Natl Acad Sci U S A. 2011;108:20970–5.
51. Farquharson AJ, Steele RJ, Carey FA, Drew JE. Novel multiplex method to assess insulin, leptin and adiponectin regulation of inflammatory cytokines associated with colon cancer. Mol Biol Rep. 2012;39:5727–36.
52. Drew JE, Farquharson A. J Novel multiplex technology platform to assess gene expression signatures in blood: predicting health benefits in response to diet. Proc Nutr Soc, in press.
53. Ciolino HP, Daschner PJ, Yeh GC. Dietary flavonols quercetin and kaempferol are ligands of the aryl hydrocarbon receptor that affect CYP1A1 transcription differentially. Biochem J. 1999;340:715–22.
54. Suchner U, Kuhn KS, Furst P. The scientific basis of immunonutrition. Proc Nutr Soc. 2000;59:553–63.
55. Giudice A, Montella M. Activation of the Nrf2-ARE signaling pathway: a promising strategy in cancer prevention. Bioessays. 2006;28:169–81.
56. Wannamethee SG, Lowe GD, Rumley A, Bruckdorfer KR, Whincup PH. Associations of vitamin C status, fruit and vegetable intakes, and markers of inflammation and hemostasis. Am J Clin Nutr. 2006;83:567–74.
57. Weimann A, Braga M, Harsanyi L, Laviano A, Ljungqvist O, Soeters P; DGEM (German Society for Nutritional Medicine), Jauch KW, Kemen M, Hiesmayr JM, Horbach T, Kuse ER,Vestweber KH; ESPEN (European Society for Parenteral and Enteral Nutrition). ESPEN guidelines on enteral nutrition: surgery including organ transplantation. Clin Nutr. 2006;25:224–44.
58. Surh YJ, Kundu JK, Na HK. Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. Planta Med. 2008; 74:1526–39.
59. Holt EM, Steffen LM, Moran A, Basu S, Steinberger J, Ross JA, Hong CP, Sinaiko AR. Fruit and vegetable consumption and its relation to markers of inflammation and oxidative stress in adolescents. J Am Diet Assoc. 2009;109:414–21.
60. Mann GE, Bonacasa B, Ishii T, Siow RC. Targeting the redox sensitive Nrf2-Keap1 defense pathway in cardiovascular disease: protection afforded by dietary isoflavones. Curr Opin Pharmacol. 2009;9:139–45.
61. Velmurugan K, Alam J, McCord JM, Pugazhenthi S. Synergistic induction of heme oxygenase-1 by the components of the antioxidant supplement Protandim. Free Radic Biol Med. 2009;46:430–40.

62. Astley SB, Elliott RM, Archer DB, Southon S. Evidence that dietary supplementation with carotenoids and carotenoid-rich foods modulates the DNA damage: repair balance in human lymphocytes. Br J Nutr. 2004;91:63–72.
63. Yang GQ, Wang SZ, Zhou RH, Sun SZ. Endemic selenium intoxication of humans in China. Am J Clin Nutr. 1983;37:872–81.
64. Alleva R, Di Donato F, Strafella E, Staffolani S, Nocchi L, Borghi B, Pignotti E, Santarelli L, Tomasetti M. Effect of ascorbic acid-rich diet on in vivo-induced oxidative stress. Br J Nutr. 2011;Sep 16:1–10.
65. Huang Z, Rose AH, Hoffmann PR. The role of selenium in inflammation and immunity: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal. 2012;16:705–43.
66. Wagner AE, Ernst I, Iori R, Desel C, Rimbach G. Sulforaphane but not ascorbigen, indole-3-carbinole and ascorbic acid activates the transcription factor Nrf2 and induces phase-2 and antioxidant enzymes in human keratinocytes in culture. Exp Dermatol. 2010;19:137–44.
67. Nelson SK, Bose SK, Grunwald GK, Myhill P, McCord JM. The induction of human superoxide dismutase and catalase in vivo: a fundamentally new approach to antioxidant therapy. Free Radic Biol Med. 2006;40:341–7.
68. Vural H, Demirin H, Kara Y, Eren I, Delibas N. Alterations of plasma magnesium, copper, zinc, iron and selenium concentrations and some related erythrocyte antioxidant enzyme activities in patients with Alzheimer's disease. J Trace Elem Med Biol. 2010;24:169–73.
69. Barnes PJ, Karin M. Nuclear factor-kB. A pivotal transcription factor in chronic inflammatory diseases. N Engl J Med. 1997;336:1066–71.
70. Andrews FJ, Griffiths RD. Glutamine: essential for immune nutrition in the critically ill. Br J Nutr. 2002;87:S3–8.
71. Calder PC. Dietary modification of inflammation with lipids. Proc Nutr Soc. 2002;61:345–58.
72. Suchner U, Heyland DK, Peter K. Immune-modulatory actions of arginine in the critically ill. Br J Nutr. 2002;87:S121–32.
73. Bouwens M, van de Rest O, Dellschaft N, Bromhaar MG, de Groot LC, Geleijnse JM, Müller M, Afman LA. Fish-oil supplementation induces antiinflammatory gene expression profiles in human blood mononuclear cells. Am J Clin Nutr. 2009;90:415–24.
74. Maruyama T, Mimura K, Izawa S, Shiba S, Watanabe M, Kawaguchi Y, Fujii H, Kono K. Immunonutritional diet modulates natural killer cell activation and Th17 cell distribution in patients with gastric and esophageal cancer. Nutrition. 2011;27:146–52.
75. Martí A, Marcos A, Martínez JA. Obesity and immune function relationships. Obes Rev. 2001;2:131–40.
76. Monteiro R, Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. Mediators Inflamm. 2010;2010. p ii:289645.
77. Watkins ML, Erickson JD, Thun MJ, Mulinare J, Heath CW. Multivitamin use and mortality in a large prospective study. Am J Epidemiol. 2000;152:149–62.
78. Brauer HA, Libby TE, Mitchell BL, Li L, Chen C, Randolph TW, Yasui YY, Lampe JW, Lampe PD. Cruciferous vegetable supplementation in a controlled diet study alters the serum peptidome in a GSTM1-genotype dependent manner. Nutr J. 2011;10:11.
79. Traka MH, Mithen RF. Plant science and human nutrition: challenges in assessing health-promoting properties of phytochemicals. Plant Cell. 2011;23:2483–97.
80. Murray M. Altered CYP expression and function in response to dietary factors: potential roles in disease pathogenesis. Curr Drug Metab. 2006; 7:67–81.
81. Strick R, Strissel PL, Borgers S, Smith SL, Rowley JD. Dietary bioflavonoids induce cleavage in the *MLL* gene and may contribute to infant leukemia. Proc Natl Acad Sci U S A. 2000;97:4790–5.
82. World Health Organisation. Nutrition and the Prevention of Chronic Diseases Diet, nutrition and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation. WHO technical report series; 916. Geneva, Switzerland: WHO; 2003.
83. Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, Miyazaki Y, Kohane I, Costello M, Saccone R, et al. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. Proc Natl Acad Sci U S A. 2003;100:8466–71.
84. Pérez-Pérez R, García-Santos E, Ortega-Delgado FJ, López JA, Camafeita E, Ricart W, Fernández-Real JM, Peral B. Attenuated metabolism is a hallmark of obesity as revealed by comparative proteomic analysis of human omental adipose tissue. J Proteomics. 2012;75:783–95.
85. Ohlson L-O, Larsson B, Svardsudd K. The influence of body fat distribution on the incidence of diabetes mellitus. 13.5 Years of follow-up of the participants in the study of men born in 1913. Diabetes. 1985;34: 1055–8.
86. Kannel WB, Cupples LA, Ramaswami R, Stokes J, Kreger BE, Higgins M. Regional obesity and risk of cardiovascular disease; the Framingham Study. J Clin Epidemiol. 1991;44:183–90.
87. Kalofoutis A, Jullien G, Koutselinis A, Miras C. Erythrocyte 2,3-Diphosphoglycerate as related to diabetes and obesity. Clin Chem. 1975;21:1414–5.
88. Hofmann MA, Schiekofer S,Kanitz M, Klevesath MS, Joswig M, Lee V, Morcos M, Tritschler H, Ziegler R, Wahl P, et al. Insufficient glycemic control increases nuclear factor B binding activity in peripheral blood mononuclear cells isolated from patients with type 1 diabetes. Diabetes Care. 1998;21:1310–6.
89. Molnár D, Decsi T, Koletzko B. Reduced antioxidant status in obese children with multimetabolic syndrome. Int J Obes Relat Metab Disord. 2004;28:1197–202.
90. Wrann CD, Laue T, Hübner L, Kuhlmann S, Jacobs R, Goudeva L, Nave H. Short-term and long-term leptin exposure differentially affects human natural killer cell immune functions. Am J Physiol Endocrinol Metab. 2012;302:E108–16.
91. Wellen KE, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. J Clin Invest. 2003;112:1785–8.
92. Spindler SR. Rapid and reversible induction of the longevity, anticancer and genomic effects of caloric restriction. Mech Ageing Dev. 2005;126: 960–6.
93. Tissenbaum HA, Guarente L. Increased dosage of a sir-2 gene extends life span in Caenorhabditis elegans. Nature. 2001;410:227–30.
94. Cohen HY, Miller C, Bitterman KJ, Wall NR, Hekking B, Kessler B, Howitz KT, Gorospe M, de Cabo R, Sinclair DA. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science. 2004;305:390–2.
95. Fenech M, Bonassi S. The effect of age, gender, diet and lifestyle on DNA damage measured using micronucleus frequency in human peripheral blood lymphocytes. Mutagenesis. 2011;26:43–9.
96. Marotta F, Koike K, Lorenzetti A, Jain S, Signorelli P, Metugriachuk Y, Mantello P, Locorotondo N. Regulating redox balance gene expression in healthy individuals by nutraceuticals: a pilot study. Rejuvenation Res. 2010;13:175–8.
97. McPherson RA, Hardy G. Clinical and nutritional benefits of cysteine-enriched protein supplements. Curr Opin Clin Nutr Metab Care. 2011; 14:562–8.
98. Marotta F, Chui DH, Jain S, Polimeni A, Koike K, Zhou L, Lorenzetti A, Shimizu H, Yang H. Effect of a fermented nutraceutical on thioredoxin level and TNF-alpha signalling in cirrhotic patients. J Biol Regul Homeost Agents. 2011;25:37–45.