Periodontology 2000, Vol. 64, 2014, 154–160
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Nutrigenomics: the role of nutrients in gene expression

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Improved understanding of the mechanism behind periodontal tissue destruction, the potential protective role of nutrients and the advent of modern genomic measurement tools have led to an increased interest in the association between nutrition and periodontal disease. The role that diet plays in the development and progression of dental caries has been well characterized in the literature, but the importance of nutrition as a predisposing factor for the development of periodontal diseases is less well defined. Gingivitis and periodontitis are prevalent forms of periodontal disease in humans (39) and are the result of inflammatory and immune responses to bacterial infections of the gingival tissues (18). Periodontitis represents a heterogeneous multifactorial group of infectious diseases with destructive inflammatory pathogenesis that lead to destruction of the supporting tissues of the teeth. If the inflammatory response fails to remove the causative pathogens, the prolonged release of neutrophils, proteolytic enzymes, proinflammatory mediators and reactive oxygen species occurs, which in turn destroy the periodontal attachment. Host-based risk factors such as genetic background of an individual, socio-economic status, smoking and dietary habits have all been suggested to alter the innate susceptibility of the host to periodontal disease (18, 25).

Recently it has been suggested that nutrition may be important in redressing the balance between microbial challenge and the host response because it has been implicated in a number of inflammatory diseases and conditions, including type 2 diabetes, cardiovascular disease, rheumatoid arthritis and inflammatory bowel disease, all of which have also been associated with periodontal disease (36). Diets high in saturated fats and sugars and low in fruit, vegetables and fiber are common risk factors associated with these chronic diseases (42).

The evidence, however, for a direct link between periodontal disease and nutrition comes mainly from observational cross-sectional studies, with a large proportion finding no significant association between the nutrient being analysed and markers of periodontal disease status (36). Nevertheless, a limited number of studies do give some indication of a relationship. Results from a prospective, observational study carried out over 14 years revealed that men with a high consumption of wholegrain were 23% less likely to develop periodontitis than were those who had the lowest consumption of wholegrain (22). Furthermore, three separate analyses of the US Third National Health and Nutrition Examination Survey (NHANES III) produced statistically significant associations between periodontitis and markers of increased body mass, leading the authors to conclude that obesity could be a potential risk factor for periodontal disease, especially in younger subjects (1, 27, 41).

It is well established that specific nutrients can modulate immune and inflammatory responses (10). Based on the pathology of periodontal disease the assumption is that these nutrients could modulate periodontal health. Increased production of reactive oxygen species raises requirements for the antioxidant nutrients involved in defence. Antioxidant vitamins (vitamins A, C and E) and trace elements (selenium, copper and zinc), known to be depleted during periods of inflammation (30), can counteract reactive oxygen species damage to cellular tissues and modulate immune-cell function through the regulation of redox-regulated transcription factors and ultimately affect the production of cytokines and prostaglandins (40). Moreover, selenium has further important redox functions, with selenium-dependent glutathione enzymes being involved in the reduction of damaging lipid and phospholipid hydroperoxides to harmless products. A rodent model of zinc

deficiency has shown an increased susceptibility to periodontal disease progression, as revealed by increased plaque and higher gingival index measurements (26). These vitamins and trace elements are also known to play a pivotal role in maintaining epithelial tissue integrity and structure, which is also relevant to periodontal health (6). Intake of n-3 polyunsaturated fatty acids, predominantly found in oily fish, increase the tissue concentrations of eicosapentaenoic acid and docosahexaenoic acid, which are known to downregulate inflammation (38). Studies of n-3 fatty acids in rodent models have demonstrated decreased levels of the major inflammatory mediators prostaglandin E_2, prostaglandin F_2 alpha, leukotriene B4 and platelet activating factor in gingival tissue, which are known to contribute to bone destruction in periodontal disease (17). Further analyses of these fatty acids suggest that n-3 polyunsaturated fatty acid metabolites may act as signals to prevent neutrophil-mediated tissue damage (37). A recent longitudinal study of periodontal disease markers in elderly patients revealed that those who consumed the lowest level of n-3 fatty acids (stratified for eicosapentaenoic acid and docosahexaenoic acid) had higher incidences of periodontal disease, suggesting an inverse relationship between dietary n-3 fatty acid intake and the progression of periodontal disease in older people (17).

The limited evidence linking the cause and prevention of periodontal disease, based on observational, cross-sectional or very small double-blind randomized supplementation trials, highlights the enormous scope for the use of nutritional genomics (nutrigenomics) to understand these links in more detail. Oral health scientists now have the opportunity to study nutrient–gene interactions and how diet affects the inflammatory mechanisms underlying severe periodontitis.

Nutrigenomics

The term genomics describes the process by which all genes present in the genome of a given species can be mapped, sequenced and characterized. Extensions of genomics, such as structural and/or comparative genomics, are also being used to characterize the genome in greater detail. Transcriptomics is the term used to describe the approach in which mRNA, and consequently gene expression, is analysed in a biological sample under certain conditions at a given point in time. Proteomics take this analysis further and aims to characterize all proteins in a biological sample at the functional level. Metabolomics is used to describe quantitative analysis of all metabolites in a biological system such as cell, tissue or biological fluid (blood, plasma and saliva). Nutrigenomics aims to reveal the relationship between nutrition and the genome and to provide the scientific basis for improved public health through dietary means. It is extremely likely that interactions between genotype and diet are important in determining the risk of the most common complex diseases, including periodontal disease. Numerous nutritional genetic studies where the outcome measure was markers of disease risk, most notably cardiovascular disease and cancer (2–5, 9, 12, 15, 20), provide proof of principle. The lower risk of prostate and breast cancers in areas such as Asia, where there is a habitual high intake of soy and isoflavone, are well documented. Recent meta-analysis data comparing western and Asian populations have estimated a reduction in prostate cancer risk of approximately 30% for men consuming high soy isoflavone diets (43). It has also been estimated that high intake of food rich in soy can be related to a reduction of approximately 15% in breast cancer risk for premenopausal women (3, 35). However, the association is not straightforward, and at least three long-term (up to 2 years) double-blind randomized controlled trials investigating isoflavone supplementation have reported no effect on markers of breast cancer risk, such as mammographic density (23). The inconsistencies in the results, and the failure to observe clear clinical outcomes, may be explained, in part, through a nutrigenomic approach. One such study has demonstrated the importance of interindividual variation in the genetic background of a population to assess the biological effect of the isoflavone. Interactions between dietary isoflavone intake, polymorphisms within the estrogen receptor-β gene and the risk of prostate cancer were examined in a case–control study performed in over 2000 Swedish men (14). This study reported that individuals homozygous or heterozygous for a particular single nucleotide polymorphism in the estrogen receptor gene, and who consumed higher amounts of phytoestrogen and isoflavone, had a significantly reduced prostate cancer risk (57% and 27%, respectively), whereas no association between soy diet and cancer risk was found in subjects who were homozygous for the wild-type allele, which was more frequently expressed in the population. A further example where nutrigenomics has helped to elucidate potential disease/diet interactions is given in a study relating to colon cancer risk, which revealed the interactive

effects of heterocyclic amines produced by cooking red meats and *N*-acetyl transferase 2 enzymes which metabolize these products. Carrying a variant of the *N*-acetyl transferase 2 gene led to a significantly increased risk of colon cancer in those who ate high amounts of well-cooked red meats (16), whereas in those with the genetic variants alone or dietary exposure alone there was no statistically significant increase in risk. In the context of cardiovascular disease, similar studies have been conducted. Plasma lipid concentrations are major modifiable risk factors for cardiovascular disease. The transcription factor, peroxisome proliferator activated receptor alpha, has been investigated in numerous studies. This transcription factor is activated by a number of exogenous and endogenous ligands, such as fibrates, fatty acids and their derivatives. Upon ligand binding, peroxisome proliferator activated receptor alpha translocates into the nucleus, heterodimerizes with a receptor and modulates gene transcription. Peroxisome proliferator activated receptor alpha is primarily expressed in tissues that actively oxidize fatty acids, including the liver and muscle. It is also expressed in vascular endothelium and smooth muscle, and in cells of the immune system. Fibrates, the synthetic ligands for peroxisome proliferator activated receptor alpha, modulate the expression of several key proteins involved in the metabolism of triglyceride-rich lipoproteins and high-density-lipoprotein cholesterol. Treatment with fibrates has been shown to lower the amount of plasma triglycerides, raise the amount of high-density-lipoprotein cholesterol and be preventative against cardiovascular disease (44). A polymorphism has been identified in the peroxisome proliferator activated receptor alpha gene in several populations, which appears to affect its transcriptional response to dietary fatty acid intake. In populations where intake of polyunsaturated fatty acids was low, subjects who carried the peroxisome proliferator activated receptor alpha gene polymorphism had higher plasma triglyceride levels, whereas, in those individuals who did not have this polymorphism, there was little association between polyunsaturated fatty acid intake and plasma triglyceride levels (44). These studies highlight the importance of understanding the interactions between genotype and diet in determining the risk of most common complex diseases. To date, however, there are no studies investigating the interaction between nutrigenomics and periodontal disease and thus the effect of dietary modification on periodontal disease progression may have been overlooked in previous observational studies.

Type 2 diabetes and periodontal disease: a possible role for nutrigenomics

Type 2 diabetes is a complex trait characterized by decreased insulin secretion and decreased insulin action at target tissues. A number of observational studies have reported an increased prevalence of periodontal disease in patients with type 2 diabetes and it is now widely accepted in the periodontal profession that type 2 diabetes is an important risk factor for periodontal disease (21, 22, 32). Knowledge of the molecular mechanisms and the underlying genetic background remain incomplete, although progress has been made in this area through recent genome-wide association studies (28, 29, 31, 45), and it is a formal possibility that common genetic risk factors may be partly responsible for cross-susceptibility between periodontal disease and type 2 diabetes and that these genetic risk factors may be modulated by diet.

To date, a small number of studies have investigated the genetic relationship between periodontal disease and type 2 diabetes (13, 34). A relatively small study looking for an association between interleukin-1 genotype and periodontal disease in diabetic patients identified, in diabetic patients of African-American origin, that the genotype *IL-1B-511* was significantly associated with periodontal disease (13). This study, limited by size, did not identify whether the patients were suffering from type 1 or type 2 diabetes. A more recent population-based study has confirmed this finding and identified a significant association between the variant genotypes of both interleukin-1A (interleukin-1A-889) and interleukin-1B (interleukin-1B+3954 and interleukin-1B-511) and periodontitis in subjects with type 2 diabetes, which was not seen in nondiabetic controls (34).

As an example of the potential of nutrigenomics tools for assessing the role of nutrition in periodontal disease, we describe work from our group investigating the role of the micronutrient zinc, a zinc transporter gene and the risk of developing type 2 diabetes.

Recent genome-wide association studies have identified a genetic-susceptibility locus for type 2 diabetes comprising a nonsynonymous single nucleotide polymorphism (C/T; rs13266634) in a beta cell-specific zinc-transporter gene. This zinc-transporter gene (*SLC30A8*, coding for ZnT8) may be important in insulin storage and release (29, 31, 45). In light of this new finding, the existing knowledge

that zinc has a specific role in beta cell function takes on new significance with respect to potential strategies to prevent or treat type 2 diabetes and potentially periodontal disease. Studies have revealed that the single nucleotide polymorphism is associated with an increased risk of type 2 diabetes, implicating zinc in the disease etiology (7, 29, 31, 45). Specifically, the risk of diabetes was increased for carriers of the C allele, which has a frequency of around 69% in the UK population (11). Subsequent studies have shown that the C allele is associated with decreased insulin secretion in nondiabetic individuals. In an insulin-secreting cell line model (INS-1E), *ZnT8* overexpression stimulated zinc accumulation and conferred enhanced glucose-stimulated insulin secretion at high (> 10 mM) extracellular glucose concentrations (8). Thus, there is a compelling argument that ZnT8-mediated zinc transport is important for normal beta cell function. The association of the *SLC30A8* major allele with an increased risk of type 2 diabetes (30, 32, 45), and the influence of this genotype on measures of beta cell function (19, 33), is consistent with zinc delivery to the insulin granule being compromised in carriers of this allele, but there is as yet no experimental evidence to support this assertion.

It was reported recently that small interfering RNA knockdown of *ZnT8* in a rat beta cell line model (INS-1E) resulted in a decrease in zinc transport activity of approximately 20% and that over-expression of the higher-risk C variant of *ZnT8* also reduced Zn transport activity by approximately 20% when compared with T variant-expressing cells (24). This study did not measure the effect of the knockdown on insulin secretion.

Using nutrigenomic techniques, we have been able to investigate the efficacy of zinc supplementation, modified by the *SLC30A8* genotype, to improve beta cell function in a cell-line model of diabetes. Using small interfering RNA knockdown, we created an insulin-secreting beta cell-line model that has reduced *ZnT8* mRNA levels (of approximately 50–60%) (Fig. 1). This *ZnT8* knockdown resulted in a 46% reduction in 2 mM glucose-stimulated insulin secretion [48.66 ± 7.67 μg/liter for small interfering RNA-treated beta cells compared with 90.34 ± 6.99 μg/liter for control beta cells (values are given as mean insulin secretion ± standard error of the mean); $n = 3$, $P < 0.01$ (Student's *t*–test)] (Fig. 2). These data indicate a role for ZnT8 in glucose-stimulated insulin secretion.

To investigate the potential impact of increased extracellular zinc on insulin secretion in this model, control or *ZnT8* small interfering RNA knockdown beta cells were incubated with a range of concentrations of extracellular zinc for 24 h before incubation with extracellular glucose (2 mM). In *ZnT8* small interfering RNA knockdown cells, insulin secretion was stimulated at increased concentrations of extracellular zinc and reached the level of control cells at

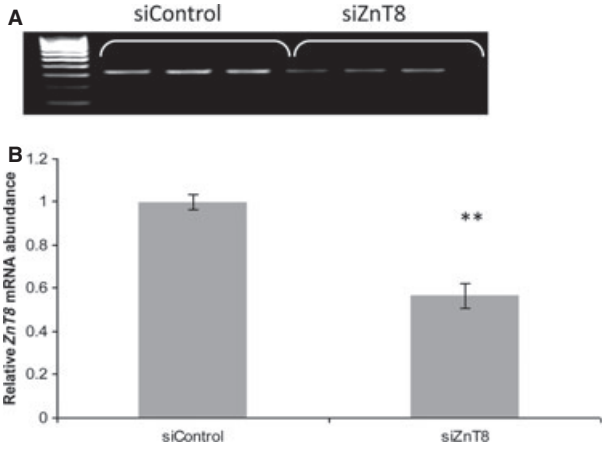

Fig. 1. Effect of ZnT8-specific small interfering RNA on *ZnT8* mRNA levels in cultured MIN6 cells. Cells were transfected transiently with specific mouse ZnT8 small interfering RNA or with scrambled control small interfering RNA at both 24 and 48 h after cell seeding. RNA was extracted 72 h after seeding, and RT-PCR was carried out for *ZnT8* and *ZnT5*. (A) Representative gel image showing *ZnT8* knockdown. (B) Densitometric analysis of band intensities. The results shown correspond to those obtained from cells used for glucose-stimulated insulin-secretion experiments (below and Fig. 2). Relative mRNA levels are normalized to a value of 1 for the control group and are presented as mean + standard error of the mean ($n = 3$ replicates); $^{**}P < 0.01$ (Student's *t*–test). This result was replicated in at least three independent experiments. No significant effect of knockdown was observed for ZnT5 in any experiment in this study (results not shown).

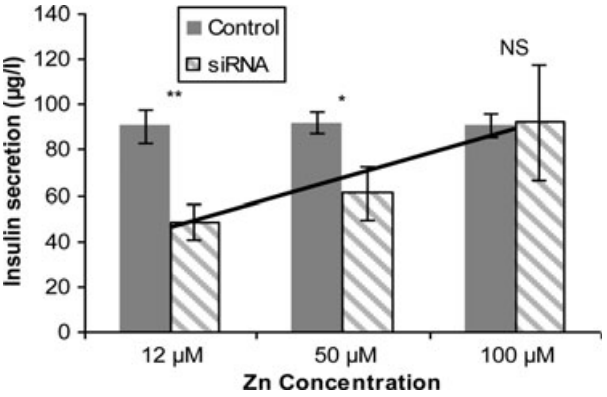

Fig. 2. Insulin secretion in 2 mM glucose-stimulated MIN6 cells at different extracellular Zn concentrations. Results are presented as mean values + standard error of the mean ($n = 3$ for each treatment). $^{**}P < 0.01$, $^{*}P < 0.05$, NS = not significantly different ($P = 0.9$) (one-way analysis of variance followed by least significant difference post-hoc analysis). Linear trend $P = 0.04$ (Spearman's rank correlation).

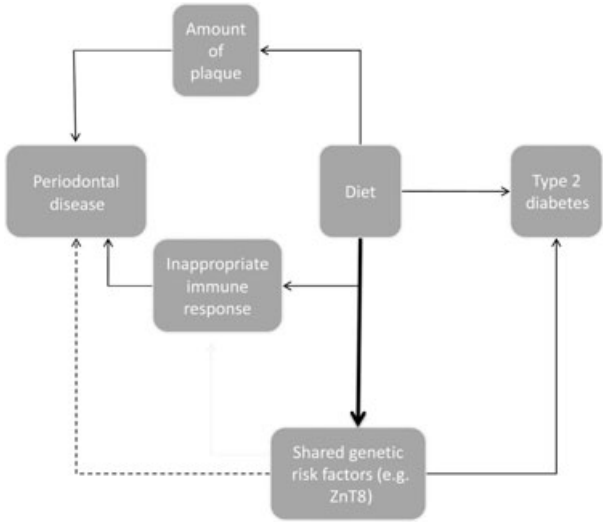

Fig. 3. Possible role for nutrient regulation of genes associated with periodontal disease. The proposed model demonstrates the known central role of diet in the etiology of periodontal disease. The putative shared genetic risk factors (e.g. *ZnT8*) between type 2 diabetes and periodontal disease indicate a possible modulating effect of diet.

the highest zinc concentration of 100 μM (Fig. 2). This important result demonstrates, for the first time, that increasing the extracellular zinc concentration has a positive effect on glucose-induced insulin secretion, indicating a potential benefit of zinc supplementation on susceptibility to type 2 diabetes in individuals carrying the risk allele.

These preliminary data, taken together with the established link between type 2 diabetes and periodontal disease, lead us to hypothesize that zinc supplementation may alter periodontal disease progression through changes in expression of the *ZnT8* transporter gene. Further studies investigating cross-susceptibility genes (including *ZnT8*) between periodontal disease, diabetes and modulation by diet, are required (Fig. 3).

The observed differences in the response of an individual to dietary modification can be attributed to differences in their genetic make-up, which emphasizes the importance of exploring the role of nutrient–gene interactions in the development of chronic diseases. Oral health scientists now have the opportunity to study nutrient–gene interactions and how diet affects the inflammatory mechanisms underlying severe periodontitis.

Acknowledgments

This work was supported by a Diabetes UK research grant RD08/0003819.

References

1. Al-Zahrani MS, Bissada NF, Borawskit EA. Obesity and periodontal disease in young, middle-aged, and older adults. *J Periodontol* 2003: **74**: 610–615.
2. Anderle P, Farmer P, Berger A, Roberts MA. Nutrigenomic approach to understanding the mechanisms by which dietary long-chain fatty acids induce gene signals and control mechanisms involved in carcinogenesis. *Nutrition* 2004: **20**: 103–108.
3. Bellenger J, Bellenger S, Clement L, Mandard S, Diot C, Poisson JP, Narce M. A new hypotensive polyunsaturated fatty acid dietary combination regulates oleic acid accumulation by suppression of stearoyl CoA desaturase 1 gene expression in the SHR model of genetic hypertension. *FASEB J* 2004: **18**: 773–775.
4. Calder PC, Davis J, Yaqoob P, Pala H, Thies F, Newsholme EA. Dietary fish oil suppresses human colon tumour growth in athymic mice. *Clin Sci (Lond)* 1994: **94**: 303–311.
5. Chang WL, Chapkin RS, Lupton JR. Fish oil blocks azoxymethane-induced rat colon tumorigenesis by increasing cell differentiation and apoptosis rather than decreasing cell proliferation. *J Nutr* 1998: **128**: 491–497.
6. Chapple IL. Role of free radicals and antioxidants in the pathogenesis of the inflammatory periodontal diseases. *Clin Mol Pathol* 1996: **49**: M247–M255.
7. Chimienti F, Devergnas S, Favier A, Seve M. Identification and cloning of a beta-cell-specific zinc transporter, ZnT-8, localized into insulin secretory granules. *Diabetes* 2004: **53**: 2330–2337.
8. Chimienti F, Devergnas S, Pattou F, Schuit F, Garcia-Cuenca R, Vandewalle B, Kerr-Conte J, Van Lommel L, Grunwald D, Favier A, Seve M. *In vivo* expression and functional characterization of the zinc transporter ZnT8 in glucose-induced insulin secretion. *J Cell Sci* 2006: **119**: 4199–4206.
9. Davidson LA, Nguyen DV, Hokanson RM, Callaway ES, Isett RB, Turner ND, Dougherty ER, Wang N, Lupton JR, Carroll RJ, Chapkin RS. Chemopreventive n-3 polyunsaturated fatty acids reprogram genetic signatures during colon cancer initiation and progression in the rat. *Cancer Res* 2004: **64**: 6797–6804.
10. Enwonwu CO, Ritchie CS. Nutrition and inflammatory markers. *J Am Dent Assoc* 2007: **138**: 70–73.
11. Frayling TM. Genome-wide association studies provide new insights into type 2 diabetes aetiology. *Nat Rev Genet* 2007: **8**: 657–662.
12. Gonzalez B, Iturralde M, Alava MA, Anel A, Pineiro A. Metabolism of n -9, n -6 and n -3 fatty acids in hepatoma Morris 7777 cells. Preferential accumulation of linoleic acid in cardiolipin. *Prostaglandins Leukot Essent Fatty Acids* 2000: **62**: 299–306.
13. Guzman S, Karima M, Wang HY, Van Dyke TE. Association between interleukin-1 genotype and periodontal disease in a diabetic population. *J Periodontol* 2003: **74**: 1183–1190.
14. Hedelin M, Balter KA, Chang ET, Bellocco R, Klint A, Johansson JE, Wiklund F, Thellenberg-Karlsson C, Adami HO, Gronberg H. Dietary intake of phytoestrogens, estrogen receptor-beta polymorphisms and the risk of prostate cancer. *Prostate* 2006: **66**: 1512–1520.
15. Ikeda I, Mitsui K, Imaizumi K. Effect of dietary linoleic, alpha-linolenic and arachidonic acids on lipid metabolism,

tissue fatty acid composition and eicosanoid production in rats. *J Nutr Sci Vitaminol (Tokyo)* 1996: **42**: 541–551.
16. Ishibe N, Sinha R, Hein DW, Kulldorff M, Strickland P, Fretland AJ, Chow WH, Kadlubar FF, Lang NP, Rothman N. Genetic polymorphisms in heterocyclic amine metabolism and risk of colorectal adenomas. *Pharmacogenetics* 2002: **12**: 145–150.
17. Iwasaki M, Yoshihara A, Moynihan P, Watanabe R, Taylor GW, Miyazaki H. Longitudinal relationship between dietary ω-3 fatty acids and periodontal disease. *Nutrition* 2010: **26**: 1105–1109.
18. Kinane DF. Causation and pathogenesis of periodontal disease. *Periodontol 2000* 2001: **25**: 8–20.
19. Kirchhoff K, Machicao F, Haupt A, Schäfer SA, Tschritter O, Staiger H, Stefan N, Häring HU, Fritsche A. Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion. *Diabetologia* 2008: **51**: 597–601.
20. Kramer JA, LeDeaux J, Butteiger D, Young T, Crankshaw C, Harlow H, Kier L, Bhat BG. Transcription profiling in rat liver in response to dietary docosahexaenoic acid implicates stearoyl-coenzyme a desaturase as a nutritional target for lipid lowering. *J Nutr* 2003: **133**: 57–66.
21. Mealey BL, Moritz AJ. Hormonal influences: effects of diabetes mellitus and endogenous female sex steroid hormones on the periodontium. *Periodontol 2000* 2003: **32**: 59–81.
22. Merchant AT, Pitiphat W, Franz M, Joshipura KJ. Whole-grain and fiber intakes and periodontitis risk in men. *Am J Clin Nutr* 2006: **83**: 1395–1400.
23. Messina M, McCaskill-Stevens W, Lampe JW. Addressing the soy and breast cancer relationship: review, commentary, and workshop proceedings. *J Natl Cancer Inst* 2006: **98**: 1275–1284.
24. Nicolson TJ, Bellomo EA, Wijesekara N, Loder MK, Baldwin JM, Gyulkhandanyan AV, Koshkin V, Tarasov AI, Carzaniga R, Kronenberger K, Taneja TK, da Silva Xavier G, Libert S, Froguel P, Scharfmann R, Stetsyuk V, Ravassard P, Parker H, Gribble FM, Reimann F, Sladek R, Hughes SJ, Johnson PR, Masseboeuf M, Burcelin R, Baldwin SA, Liu M, Lara-Lemus R, Arvan P, Schuit FC, Wheeler MB, Chimienti F, Rutter GA. Insulin storage and glucose homeostasis in mice null for the granule zinc transporter ZnT8 and studies of the type 2 diabetes-associated variants. *Diabetes* 2009: **58**: 2070–2083.
25. Nunn ME. Understanding the etiology of periodontitis: an overview of periodontal risk factors. *Periodontol 2000* 2003: **32**: 11–23.
26. Orbak R, Kara C, Ozbek E, Tezel A, Demir T. Effects of zinc deficiency on oral and periodontal diseases in rats. *J Periodontal Res* 2007: **42**: 138–143.
27. Reeves AF, Rees JM, Schiff M, Hujoel P. Total body weight and waist circumference associated with chronic periodontitis among adolescents in the United States. *Arch Pediatr Adolesc Med* 2006: **160**: 894–899.
28. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Boström K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Råstam L, Speliotes EK, Taskinen MR, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjögren M, Sterner M, Surti A, Svensson M, Svensson M, Tewhey R, Blumenstiel B, Parkin M, Defelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirn GW, Ma Q, Parikh H, Richardson D, Ricke D, Purcell S. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 2007: **316**: 1331–1336.
29. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 2007: **316**: 1341–1345.
30. Semba RD, Tang AM. Micronutrients and the pathogenesis of human immunodeficiency virus infection. *Br J Nutr* 1999: **81**: 181–189.
31. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, Balkau B, Heude B, Charpentier G, Hudson TJ, Montpetit A, Pshezhetsky AV, Prentki M, Posner BI, Balding DJ, Meyre D, Polychronakos C, Froguel P. A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 2007: **445**: 881–885.
32. Soop M, Duxbury H, Agwunobi AO, Gibson JM, Hopkins SJ, Childs C, Cooper RG, Maycock P, Little RA, Carlson GL. Euglycemic hyperinsulinemia augments the cytokine and endocrine responses to endotoxin in humans. *Am J Physiol Endocrinol Metab* 2002: **282**: E1276–E1285.
33. Staiger H, Machicao F, Stefan N, Tschritter O, Thamer C, Kantartzis K, Schäfer SA, Kirchhoff K, Fritsche A, Häring HU. Polymorphisms within novel risk loci for type 2 diabetes determine beta-cell function. *PLoS ONE* 2007: **2**: e832.
34. Struch F, Dau M, Schwahn C, Biffar B, Kocher T, Meisel P. Interleukin-1 gene polymorphism, diabetes, and periodontitis: results from the study of health in Pomerania (SHIP). *J Periodontol* 2008: **79**: 501–507.
35. Trock BJ, Hilakivi-Clarke L, Clarke R. Meta-analysis of soy intake and breast cancer risk. *J Natl Cancer Inst* 2006: **98**: 459–471.
36. van der Velden U, Kuzmanova D, Chapple IL. Micronutritional approaches to periodontal therapy. *J Clin Periodontol* 2011: **38**: 142–158.
37. Van Dyke TE, Serhan CN. Resolution of inflammation: a new paradigm for the pathogenesis of periodontal diseases. *J Dent Res* 2003: **82**: 82–90.
38. Vedin I, Cederholm T, Freund Levi Y, Basun H, Garlind A, Faxén Irving G, Jönhagen ME, Vessby B, Wahlund LO, Palmblad J. Effects of docosahexaenoic acid-rich n-3 fatty acid supplementation on cytokine release from blood mononuclear leukocytes: the OmegAD study. *Am J Clin Nutr* 2008: **87**: 1616–1622.
39. Williams RC. Understanding and managing periodontal diseases: a notable past, a promising future. *J Periodontol* 2008: **79**: 1552–1559.

40. Wintergerst ES, Maggini S, Hornig DH. Contribution of selected vitamins and trace elements to immune function. *Ann Nutr Metab* 2007: **51**: 301–323.
41. Wood N, Johnson RB, Streckfus CF. Comparison of body composition and periodontal disease using nutritional assessment techniques: Third National Health and Nutrition Examination Survey (NHANES III). *J Clin Periodontol* 2003: **30**: 321–327.
42. World Health Organization. *Diet nutrition and the prevention of chronic disease. WHO technical report series 916*. Geneva: WHO / FAO, 2003.
43. Yan L, Spitznagel EL. Meta-analysis of soy food and risk of prostate cancer in men. *Int J Cancer* 2005: **117**: 667–669.
44. Low YL, Tai ES. Understanding diet-gene interactions: lessons from studying nutrigenomics and cardiovascular disease. *Mutat Res* 2007: **622**: 7–13.
45. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, Timpson NJ, Perry JR, Rayner NW, Freathy RM, Barrett JC, Shields B, Morris AP, Ellard S, Groves CJ, Harries LW, Marchini JL, Owen KR, Knight B, Cardon LR, Walker M, Hitman GA, Morris AD, Doney AS, Wellcome Trust Case Control Consortium (WTCCC), McCarthy MI, Hattersley AT. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* 2007: **316**: 1336–1341.