Vitamin D and Oxidation-Induced DNA Damage: is there a connection?

Wang EW[1], Collins[2] AR, Pang MYC[3], Siu PM[1], Lai CKY[4], Woo J[5], Benzie IFF[1*]

[1]Department of Health Technology and Informatics, The Hong Kong Polytechnic University;

[2]Department of Nutrition, The University of Oslo; [3]Department of Rehabilitation Sciences and

[4]School of Nursing, The Hong Kong Polytechnic University; [5]Department of Medicine and

Therapeutics, The Chinese University of Hong Kong,

*Corresponding author: Professor Iris Benzie, Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Kowloon, Hong Kong;

email htbenzie@polyu.edu.hk tel +852 34008572 fax +852 23624365

Key words

Vitamin D; DNA damage; comet assay; oxidative stress; cancer; aging

Acknowledgments

This work was financially supported by the Hong Kong Research Grants Council General Research Fund Grant B-Q39Q and by The Hong Kong Polytechnic University.

Abstract

Introduction: Oxidation-induced DNA damage can cause mutations, phenotypic changes and apoptosis. Agents that oppose such damage offer potential therapies for health promotion. Vitamin D reportedly lowered DNA damage in type 2 diabetic mice, and higher DNA damage was seen in mononuclear cells of severely asthmatic patients who were vitamin D deficient. We hypothesized that lower vitamin D status associates with higher oxidation-induced DNA damage. Vitamin D deficiency is highly prevalent worldwide, and if found to associate with DNA damage this relationship would be of high potential importance and impact.

Method: Oxidation-induced DNA damage in peripheral lymphocytes of 121 young (18-26y) adults was measured using the FPG-assisted comet assay. Vitamin D status was assessed by plasma 25(OH)D concentrations, measured by LC-MS/MS. Correlational analysis was performed between plasma 25(OH)D and DNA damage. Differences in DNA damage across tertiles of 25(OH)D were explored using ANOVA. DNA damage scores in those with plasma 25(OH)D <50 nmol and ≥50 nmol/l were compared using unpaired t-test.

Results: Mean(SD) DNA damage (as %DNA in comet tail) and plasma 25(OH)D were, respectively, 18.58(3.39)% and 44.7(13.03) nmol/l. No significant correlation was seen between 25(OH)D and DNA damage ($r=-0.0824$; $p>0.05$). No significant difference was seen across 25(OH)D tertiles: mean(SD) %DNA in comet tail/25(OH)D nmol/l values in lowest, middle and highest tertiles were, respectively, 18.64(3.30)/31.6(4.4), 18.90(3.98)/42.9(3.5), 18.19(2.84)/59.9(8.5), or across the binary divide: 18.73(3.63)% in <50nmol/l group vs. 18.27(2.84)% in the ≥50 nmol/l group.

Conclusion: Results do not support the hypothesized inverse association between vitamin D status and oxidation-induced DNA damage.

Introduction

Genomic stability is vital for health, and agents or processes, such as oxidative stress, that increase DNA damage or inhibit its repair induce telomere shortening, mutations, phenotypic changes and apoptosis, with consequent functional decline, aging, carcinogenesis and metabolic disease [1-3]. Agents, such as some phytochemicals, that oppose DNA damage or promote its repair, offer potential therapeutic strategies for disease prevention and the promotion of healthy aging [1-6]. Vitamin D is best known for its effects on bone health, but is reported to have pleiotropic effects, and epidemiological studies show increased risk of some types of cancer and various other non-communicable diseases (NCDs) in association with low vitamin D status (assessed by measuring plasma or serum 25(OH)D concentration) [7-12]. Deficiency of vitamin D is present when plasma 25(OH)D <50 nmol/l, and levels <30 nmol/l have been linked strongly to mortality from cancer and all causes [8]. However, the relationship between vitamin D and non-skeletal disease and mortality are not yet clear, and there is no agreement on what level of 25(OH)D is optimal for health [12-16]. For bone health, 50 nmol/l and above is proposed as sufficient [15] but this is disputed [16], and levels of 75-100 nmol/l may be desirable, based on observations in relation to risk of mortality and NCDs, with risk increasing below <75 nmol/l [8,9,14,16]. While vitamin D can be obtained from some foods, and there are various reports on recommended dietary intakes [14-17, by far the major determinant of vitamin D status in healthy subjects is synthesis of pre-vitamin D3 in sun-exposed skin, and 10-15 min exposure of arms and legs to solar ultraviolet radiation per day is sufficient to avoid deficiency [18,19]. However, due to avoidance of sun and the largely indoor modern lifestyle, there is very high prevalence of vitamin D deficiency worldwide [20]. Indeed, vitamin D deficiency is so common as to be endemic.

Deficiency of vitamin D has been linked to increased oxidative stress, and treatment with vitamin D is reported to lower oxidative stress in cell culture and animal studies [21-24]. Vitamin D supplementation improved biomarkers of oxidative stress in a group of 20 deficient patients suffering from major depressive disorder [25]. Oxidative stress induces damage to DNA, and supplementation with active vitamin D (1,25(OH)D; calcitriol) was reported to lower DNA damage (measured using the alkaline comet assay) in pancreatic and liver cells of type 2 diabetic mice [26]. Human studies of vitamin D and DNA damage are scarce. A study of 2161 women reported a weak but statistically significant correlation ($r=0.07$; $p<0.001$) between serum 25(OH)D and lymphocytic telomere length [27]. A study of 23 subjects with a history of adenomatous colorectal polyps who were given 800 IU/d supplemental vitamin D for 6 months showed a significant decrease in a biomarker of oxidation-induced DNA damage (8-hydroxy-2'-deoxyguanosine (8-OHdG)) in normal appearing colorectal mucosal cells [28]. Elevated DNA damage (measured using the alkaline comet assay) was seen in peripheral blood mononuclear cells of severe asthmatic patients who were deficient in vitamin D, and damage was ameliorated by supplemental vitamin D (7.5mg by injection on two separate days) [29]. DNA damage and vitamin D inter-relationship was examined also using the cytokinesis-block micronucleus cytome (CBMN-cyt) assay [30]. Using this assay, lymphocytes from 207 subjects aged 25-60 years were tested for chromosome breakage and loss, double-strand breaks, telomere end fusions and micronuclei frequency. The authors reported seeing no overall relationship between plasma 25(OH)D and DNA damage, but reported that there were weak modulating effects of vitamin D on micronucleus formation after exposure to solar radiation [30]. This suggests that those with vitamin D deficiency may be more susceptible to DNA damage when exposed to damaging agents such as ultraviolet light or oxidative stress.

As yet, no clear role for vitamin D in genoprotection or development of cancer and other NCDs has been established [7-13,31] There are many factors that contribute to DNA damage, including diet, lifestyle and repair capacity [32]. Still, the reported association of vitamin D with oxidative stress and DNA damage is intriguing and worthy of further study. There have been no studies to date on the relationship between vitamin D status and oxidation-induced DNA damage in young adults. Studying still-healthy young subjects removes the confounding effects of advanced age and pre-existing disease. A relationship, if found, would be of interest and value because of the high prevalence of vitamin D deficiency and the potentially profound effect of DNA damage on risk of future disease. This forms the rationale for this study.

Materials and methods

This was an observational study of oxidation-induced DNA damage in peripheral lymphocytes from 121 (44 males and 77 females) volunteers and its association with plasma 25(OH)D. The volunteers were young (18-26 years), non-obese, did not smoke, and none took regular food or vitamin supplements. All volunteers reported being in good general health, none had been hospitalized within the previous 12 months or had required medical treatment within the previous 6 months. The study was approved by the Human Subjects Ethics Review Committee of The Hong Kong Polytechnic University, all procedures involving human subjects complied with the Declaration of Helsinki, and all volunteers gave their written informed consent. Subject demographics are given in Table 1.

Fasting venous blood samples were collected into heparinized blood collection tubes, and peripheral lymphocytes were harvested and cryopreserved within 1 hour, following an

established procedure using density gradient centrifugation-assisted separation described previously [33,34]. Cells were thawed, once only, for measurement of oxidation-induced damage to DNA using the Formamidopyrimidine DNA glycosylase (FPG)-assisted comet assay and following well established and validated protocols [33,34]. The same investigator performed all comet assay work in order to minimize analytical variation. In brief, washed cells were embedded low melting point agarose layered onto microscope slides. Embedded cells were lysed and membranes and histones were removed by immersing slides in high salt and detergent solution pH 10, after which slides were washed and a buffered solution of FPG (Trevigen, Gaithersburg, MD, US) or buffer alone was layered onto the gels. The enzyme creates single strand breaks at oxidation-induce lesions in DNA. During a subsequent unwinding step, single strand breaks (pre-existing and newly created by the action of FPG) cause areas of local 'relaxation' in the tightly wound DNA, and electrophoresis at high pH then pulls relaxed loops of DNA from the nucleoid head in the direction of the anode, forming a comet 'tail'. Comets are visualized by a fluorescent stain (in this case SYBR Green Life Technologies, Carlsbad, CA, USA), and scored using a fluorescent microscope and computerized image analysis system. We used a Leica CTR6500 fluorescent microscope and LAS-AF scanning software (Leica Microsystems Ltd. Wetzlar, Germany) and the Comet Assay IV Lite scoring system (Perceptive Instruments Ltd. Bury St Edmunds, UK).

For each sample, 50 nucleoids were scored in each of two gels treated with FPG, and also in each of two gels treated, in parallel, with buffer. The difference between the average DNA score (as %DNA in comet tail) of A) 100 buffer-treated nucleoids (a measure of pre-existing single strand breaks) and B) the 100 FPG-treated nucleoids (a measure of pre-existing plus single strand

breaks created by the action of FPG at oxidation-induced lesions) of each subject's sample was taken as the measure of oxidation-induced DNA damage.

Plasma 25(OH)D concentration was measured, also by a single investigator, using gradient LC-MS/MS [35]. Mobile phase A was 70.5% v/v methanol and 29.5% v/v aqueous ammonium acetate (4 mmol/l); mobile phase B was 95% v/v methanol and 5% v/v aqueous ammonium acetate (23.6 mmol/l), both mobile phase A and B were at pH 5.25. The gradient mobile phase was programmed as follows: 1-17 min, 100% mobile phase A; 17.1-25 min, 100% mobile phase B; 25.1-30 min, 100% mobile phase A. The eluate at 12.5-18.5 min went to the mass spectrometer for analysis, and the rest went to waste. The internal standard was 3-epi-25-Hydroxyvitamin D_3. (Sigma) and a 3PLUS® Multilevel serum calibrator set 25-OH-Vitamin D3/D2 (Chromsystems, Gräfelfing, Germany), was used as calibrator. Lyophil control sample for 25-OH-Vitamin D3/D2, Levels I & II (Recipe, München, Germany) were used. The HPLC system was from Agilent (Agilent 1100 Series, quaternary pump, G1311A and microvacuum degasser, G1379A, Agilent Technologies Inc., Waldbronn,Germany). A non-thermostatted well-plate autosampler (G1313A) was used. The mass spectrometer linked to the HPLC system was a 3200 QTRAP mass spectrometer from Applied Biosystems/MDS Sciex (MDS Inc., Concord, ON, Canada).

To investigate association between 25(OH)D and %DNA in comet tail, three approaches were taken. Correlation may be linear, and Pearson's correlational analysis was performed between plasma 25(OH)D and %DNA in comet tail results to investigate this. There may be non-linear or 'threshold' affects, and so differences in DNA damage across tertiles of 25(OH)D were explored

(using ANOVA), and DNA damage in those who were found to be deficient in vitamin D (plasma 25(OH)D <50 nmol) was compared to those with 25(OH)D ≥50 nmol/l (using the unpaired t-test). A p value >0.05 was regarded as statistically significant.

Results

Descriptive statistics of lymphocytic DNA damage and plasma 25(OH)D results are shown in Table 2. The range of 25(OH) was wide (<20 to >80 nmol/l), but most (82/121; 68%) of the young adults studied were found to be deficient in vitamin D (plasma 25(OH) <50 nmol/l), and only two of our young subjects had levels >75 nmol/l. This echoes the very high prevalence of vitamin D deficiency previously reported [20]. The range of oxidation-induced DNA seen was also quite wide (9.8-30.0%), but no significant overall association was found between plasma 25(OH)D concentration and oxidation-induced DNA damage : r=-0.0824; p>0.05. There was no difference seen in oxidation-induced DNA damage across the tertiles of plasma 25(OH)D (Table 3), or across the 50 nmol/l binary divide (Table 4).

Tables 1-4 near here

Discussion

A potentially important relationship between vitamin D status and DNA damage has been postulated due to epidemiological findings of increased risk and mortality from cancer and other NCDs in relation to low plasma 25(OH) concentration, and to results of cell culture and animal studies linking 25(OH)D, oxidative stress, and DNA damage [8-12,21-26]. Human studies of vitamin D and DNA damage are scarce and data are conflicting [27-31]. Telomeres are shorted by oxidative stress, and telomere length was reported to be inversely correlated with plasma

25(OH)D in women, though the association was weak [27]. No overall association was seen in an Australian study when vitamin D status and lymphocytic DNA damage was investigated in a group of 207 adults aged 25-60 years, however, the authors reported that there was some evidence suggesting higher susceptibility to solar UV-induced DNA damage to internal tissues when vitamin D is low [30]. We could find only two published studies of vitamin D in association with DNA damage measured using the alkaline comet assay, neither of which used an enzyme-assisted version. One was an animal study [26] that showed decreased lymphocytic DNA damage in pancreas and liver cells of diabetic mice given 1,25(OH)D (calcitriol; the active form of vitamin D) by peritoneal injection for 15 days. Results reported lower ($p<0.05$) comet tail length in the diabetic mice treated with 1,25(OH)D compared to diabetic mice with no 1,25(OH)D treatment (tail length average ~20 μm compared to ~30 μm, n=8/group). However, there was no information on the baseline vitamin D status of the animals, and the physiological relevance of injection of very high doses of calcitriol is unclear. The alkaline comet assay was used also in a study of DNA damage in peripheral lymphocytes from 8 severely asthmatic vitamin D deficient subjects who were given two very large doses (7.5mg) of 25(OH)D3 by intramuscular injection [29]. DNA damage was reported to be markedly lower in this group compared to 8 vitamin D deficient subjects, also with severe asthma, who received no vitamin D supplement. It is noted that vitamin D deficiency in that study was defined as ≤30 ng/ml (≤75 nmol/l), and no plasma 25(OH)D data were shown from either baseline or after supplementation [29]. Therefore, it is not possible to assess the degree of severity of vitamin D deficiency, or the plasma 25(OH)D response to supplementation. It is noted that, while plasma 25(OH)D concentrations of 75-100 nmol/l are recommended by some, and that oral intake of up to 4000 IU/day is accepted as safe, very high plasma 25(OH) levels and excessively high

supplementation with vitamin D should be avoided, as there are some reports of adverse effects [8,9,13,16,17].

The comet assay is a well validated and widely used measure of 'global' DNA damage. In human studies the most commonly used cells are peripheral lymphocytes. The use of lesion-specific enzymes markedly increases its sensitivity and specificity, and %DNA in the comet tail is linearly related to single strand breaks in DNA [33,34]. In this current study we used the FPG-assisted comet assay. This enzyme creates single strand breaks in DNA at (mainly) oxidation-induced lesions, thereby revealing them for measurement in the comet assay. Peripheral lymphocytes harvested from fasting blood collected from 121 healthy adults aged 18-26 years were tested, and results were related to fasting plasma 25(OH)D concentrations measured by a sensitive and specific LC-MS/MS method. Results showed no overall correlation, no differences were seen across tertiles of 25(OH)D, and no difference was seen in those with plasma 25(OH)D <50 nmol/l (generally agreed to reflect deficiency) and those with levels ≥50nmol/l. Therefore, no association between vitamin D and oxidation-induced DNA damage was observed in this study. However, some points are worth noting. DNA damage has many causes, and levels reflect a balance between induction and repair of damage, and repair mechanisms are normally upregulated when DNA damage increases [32]. The first step in base excision repair of oxidation-induced damage is by the action of the enzyme 8-oxoguanine DNA glycosylase (hOGG1), the human analogue of FPG, which is of microbial origin [32,33]. Interestingly, expression of hOGG1 was reportedly higher in vitamin D deficient subjects with elevated DNA damage [29]. It is possible that no difference in DNA damage was seen in relation to vitamin D status in this current study due to more efficient repair in vitamin D deficient subjects. This

requires further study using the DNA repair model of the comet assay (32). In addition, although plasma 25(OH)D concentrations of our 121 young healthy subjects covered a wide range (<20 to >80 nmol/l), only two had plasma 25(OH)D ≥75 nmol/l. The effect of vitamin D on DNA damage may be a threshold one, and the study of more subjects in the 75-100 nmol/l range is needed to help complete the investigation of vitamin D and DNA damage. We highlight here that people with plasma 25(OH)D in this range are rare amongst free living, healthy, non-supplemented subjects, while vitamin D deficiency is very common. Therefore, a controlled supplementation trial to monitor effects of correction of vitamin D deficiency on oxidation-induced DNA damage and repair, along with response of plasma 25(OH) D concentrations, is needed to further investigate the possible role of vitamin D in modulation of DNA damage and in order to help fill the knowledge gaps on the role vitamin D in healthy aging [9,31,36].

To conclude, results of this study showed no association between vitamin D status (as plasma 25(OH)D) and oxidation-induced DNA damage, as measured by the FPG-assisted comet assay, in young healthy adults. Further study is needed into the effect of correction of vitamin D deficiency, which is very common, on DNA damage and repair.

References

1. Ferguson LR, Chen H, Collins AR, Connell M, Damia G, Dasgupta S, Malhotra M et al. (2015). Genomic instability in human cancer: molecular insights and opportunities for therapeutic attack and prevention through diet and nutrition. Sem Cancer Biol 35:S5-S24.
2. Rani V, Deep G, Singh RK, Palle K, Yadav UCS (2016). Oxidative stress and metabolic disorders: pathogenesis and therapeutic strategies. Life Sci doi: 10.1016/j.lfs.2016.02.002.
3. Lenart P, Krejci L (2016). DNA, the central molecule of aging. Mutat Res 786:1-7.
4. Singh AK, Sharma N, Ghosh M, Park YH, Jeong DK (2016). Emerging importance of dietary phytochemicals in fight against cancer: role in targeting cancer stem cells. Crit Rev Food Sci Nutr PMID: 26853447.
5. Ho CK, Choi SW, Siu PM, Benzie IFF (2014). Effects of single dose and regular intake of green tea (*Camellia sinensis*) on DNA damage, DNA repair and heme oxygenase -1 expression in a randomised controlled human supplementation study. Mol Nutr Food Nutr Res 58: 1379-83.
6. Han KC, Wong WC, Benzie IFF (2011). Genoprotective effects of green tea (*Camellia sinensis*) in humans: results of a controlled supplementation trial, Br J Nutr 105:171-179
7. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G (2016). Vitamin D: metabolism, molecular effects of action, and pleiotropic effects. Physiol Rev 96:365-408.
8. Schottker B, Haug U, Schomburg L, Kohrle J, Perna L, Muller H, Hollesczek B, Brenner H (2013). Strong association of hydroxyvitamin D concentrations with all-cause, cardiovascular, cancer and respiratory disease mortality in a large cohort study. Am J Clin Nutr 97:782-793.

9. Spedding S, Vanlint S, Morris H, Scragg R (2013) Does vitamin D sufficiency equate to a single serum 25-hydroxyvitamin D level or are different levels required for non-skeletal diseases? Nutrients 5:5127-5139.

10. Mitri J, Muraru MD, Pittas AG (2011). Vitamin D and type 2 diabetes: a systematic review. Eur J Clin Nutr 65:1005-1015.

11. Norman PE, Powell JT (2015). Vitamin D and cardiovascular disease. Circ Res 114:379-393.

12. Skaaby T (2015). The relationship of vitamin D status to risk of cardiovascular disease and mortality. Dan Med J 62(2): pii B5008.

13. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, Bjelokovic M, Gluud C (2014). Vitamin D supplementation for prevention of mortality in adults. Cochrane Database Syst Rev doi: 10.1002/14651858.

14. Balvers MGJ, Brouwer-Brolsma EM, Enderberg S, de Groot LCPG, Kok F, Gunnewiek JK (2015). Recommended intakes of vitamin D to optimise health, associated 25-hydroxyvitamin D concentrations, and dosing regimes to treat deficiency: workshop report and overview of current literature. J Nutr Sci doi: 10.1017/jns.2015.10.

15. Institute of Medicine (IOM) (2011). Dietary Reference Intakes for Calcium and Vitamin D. Washington DC, National Academies Press.

16. Heaney RP, Holick MF (2011). Why the IOM recommendations for vitamin D are deficient. J Bone Mineral Res 26:455-457.

17. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the Tolerable Upper Intake Level of vitamin D. EFSA Journal 2012;10(7):2813. doi:10.2903/j.efsa.2012.2813. Available online: www.efsa.europa.eu/efsajournal

18. Ashwell M, Stone EM, Stolte H, Cashman KD, Macdonald H, Lanham-New S, Hiom S, Webb A, Fraser D (2010). UK Food Standards Agency Workshop Report: an investigation of the relative contributions of diet and sunlight to vitamin D status. Br J Nutr 104:603-611.

19. Kimlin MG, Lucas RM, Harrison SL, van der Mei J, Armstrong BK, Whiteman DC, Kricker A et al (2014). The contributions of solar ultraviolet radiation exposure and other determinants to serum 25-hydroxyvitamin D concentrations in Australian adults: the AusD study. Am J Epidemiol 179:864-874.

20. Hilger J, Friedel A, Herr R, Rausch T, Roos F, Wahl DA, Pierroz DD, Weber P, Hoffmann K (2014). A systematic review of vitamin D status in populations worldwide. Br J Nutr 111:23-45.

21. Peng X, Vaishnav A, Murillo G, Alimirah F, Torres KO, Mehta RG (2010). Protection against cellular stress by 25-hydroxy D3 in breast epithelial cells. J Cell Biochem 110:1324-1333.

22. Gradinaru D, Borsa C, Ionescu C, Margina D, Prada GI, Jansen E (2012). Vitamin D status and oxidative stress markers in elderly with impaired fasting glucose and type 2 diabetes mellitus. Aging Clin Exp Res 24: 595-602.

23. Pourghassem GB, Pourteymour FTF, Sadien B, Asghari JM, Farzadi L (2015). Vitamin D status is related to oxidative stress but not high-sensitive C-reactive protein in women with pre-eclampsia. Gynecol Obstet Invest PMID; 26587898.

24. Codoner-Franch P, Tavarez-Alonso S, Simo-Jorda R, Laport-Martin P, Carratala-Calvo A, Alonso-Iglesias E (2012). Vitamin D status is linked to biomarkers of oxidative stress, inflammation, and endothelial activation in obese children. J Pediatr PMID: 22677566.

25. Sepehrmanesh Z, Kolahdooz F, Abedi F, Mazroii N, Assarian A, Asemi Z, Esmaillzadeh A (2015). Vitamin D supplementation affects the Beck depression inventory, insulin resistance, and biomarkers of oxidative stress in patients with major depressive disorder: a randomized, controlled clinical trial. J Nutr 146:243-248.

26. Meerza D, Naseem I, Ahmed J (2012). Effect of 25(OH)2 vitamin D3 on glucose homeostasis an DNA damage in type 2 diabetic mice. J Diab Complic 26:363-368.

27. Richards JB, Valdes AM, Gardner JP, Paximada D, Kimura M, Nessa A, Lu X et al (2007). Higher serum vitamin D concentrations are associated with longer leukocyte telomere length in women. Am J Clin Nutr 86:1420-1425.

28. Fedirko V, Bostick RM, Long Q, Flanders WD, McCulloch ML, Sidelnikov E, Daniel CR (2010). Effects of supplemental vitamin D and calcium on oxidative DNA damage marker in normal colorectal mucosa: a randomized clinical trial. Cancer Epidemiol Biomarkers Prev 19:280-291.

29. Ian N, Luo G, Yang X, Cheng Y, Zhang Y, Wang X, Wang X, et al (2014). 25-hyrdoxyvitamin D3 deficiency enhances oxidative stress and corticosteroid resistance in severe asthma exacerbation. PLOS ONE 9:e111599.

30. Nair-Shalliker V, Fenech M, Forder PM, Clements MS, Armstrong BK (2012). Sunlight and vitamin D affect DNA damage, cell division and cell death in human lymphocytes: a cross-sectional study in South Australia. Mutagenesis 27:609-614.

31. Nair-Salliker V, Armstrong BK, Fenech M (2012) Does vitamin D protect against DNA damage. Mutation Res 733:50-57.

32. Slyskova J, Lorenzo Y, Karlsen MH, Novosadova V, Blomhoff R, Vodicka P et al (2014). Both genetic and dietary factors underlie individual differences in DNA damage levels and DNA repair capacity. DNA Repair 16:66-73.

33. Collins A, Koppen G, Valdiglesias V, Dusinska M, Kruszewski M, Møller P, Rojas E et al for the ComNet project (2014). The comet assay as a tool for human biomonitoring studies – The ComNet project. Mut Res Rev 759:27-39.

34. Wong WC, Szeto YT, Collins AR, Benzie IFF (2005. The comet assay: a biomonitoring tool for nutraceutical research. Curr Topics Nutraceut Res 3:1-14.

35. Hojskov CS, Heickendorff L, Moller HJ (2010) High throughput liquid-liquid extraction and LCMSMS assay for determination of circulating 25(OH) vitamin D3 and D2 in the routine clinical laboratory. Clin Chim Acta 411:114-116.

36. Cashman KD, Kiely M (2011). Towards prevention of vitamin D deficiency and beyond: knowledge gaps and research needs in vitamin D nutrition and public health. Br J Nutr 106;1617-1627.

Table 1

Demographics of the Study Group; results are Mean(SD)

	Total (n=121)	Male (n=44)	Female (n=77)
Age in years	20.69(1.50)	20.70(1.66)	20.69(1.42)
BMI in kg/m^2	21.02(2.31)	20.96(2.12)	21.06(2.43)

Table 2

Plasma 25(OH)D (mean(SD) nmol/l) and the FPG-Assisted Comet Assay Results (%DNA in comet tail) in 121 Healthy Adults Aged 18-26 Years:

Mean(SD) results of 100 nucleoids scored per sample in both A) Buffer-treated and B) FPG- treated gels, and the difference between these two gel treatments are shown

Plasma 25(OH)D	A Buffer-treated gels (pre-existing single stand breaks)	B FPG-treated gels ('total' damage, i.e. A plus breaks created at FPG-sensitive lesions)	B-A (oxidation induced DNA damage revealed by action of FPG)
44.68 (13.03)	1.89 (0.79)	20.47 (3.37)	18.58 (3.39)

Table 3

DNA Damage in those with Plasma 25(OH)D <50 nmol/l and ≥50 nmol/l; results are Mean(SD)

	Plasma 25(OH)D <50nmol/l (n=82)	Plasma 25(OH)D ≥50 nmol/l (n=39)
Plasma 25(OHD) nmol/l	37.3(7.0)	*60.1(8.4)
Buffer-treated gels (A)	1.85(0.80)	1.96(0.77)
FPG-treated gels (B)	20.58(3.66)	20.23(2.70)
Oxidation-induced damage to DNA (B-A)	18.73(3.63)	18.27(2.84)

*p<0.05 compared to vitamin D deficient group (<50nmol/l)

Table 4

DNA Damage in those with Plasma 25(OH)D <50 nmol/l and ≥50 nmol/l; results are Mean(SD)

	Plasma 25(OH)D <50nmol/l (n=82)	Plasma 25(OH)D ≥50 nmol/l (n=39)
Plasma 25(OHD) nmol/l	37.3(7.0)	*60.1(8.4)
Buffer-treated gels (A)	1.85(0.80)	1.96(0.77)
FPG-treated gels (B)	20.58(3.66)	20.23(2.70)
Oxidation-induced damage to DNA (B-A)	18.73(3.63)	18.27(2.84)

*p<0.05 compared to vitamin D deficient group (<50nmol/l)