Eur J Nutr (2011) 50:587–594
DOI 10.1007/s00394-011-0167-6

ORIGINAL CONTRIBUTION

# Impact of spinach consumption on DNA stability in peripheral lymphocytes and on biochemical blood parameters: results of a human intervention trial

**Beate Moser · Thomas Szekeres · Christian Bieglmayer · Karl-Heinz Wagner · Miroslav Mišík · Michael Kundi · Oliwia Zakerska · Armen Nersesyan · Nina Kager · Johann Zahrl · Christine Hoelzl · Veronika Ehrlich · Siegfried Knasmueller**

Received: 26 July 2010 / Accepted: 10 January 2011 / Published online: 9 March 2011


**Abstract**
*Introduction* A controlled intervention trial was conducted to assess the impact of spinach consumption on DNA stability in lymphocytes and on health-related biochemical parameters.
*Methods* The participants ($n = 8$) consumed homogenised spinach (225 g/day/person) over a period of 16 days. DNA migration was monitored in single cell gel electrophoresis—comet assays under standard conditions, which reflect single- and double-strand breaks, after treatment of nuclei with lesion-specific enzymes (formamidopyrimidine glycosylase, FPG and endonuclease III, ENDO III) and after treatment of intact cells with $H_2O_2$ before, during and after intervention.
*Results* While no reduction in DNA damage was observed under standard conditions after different time intervals of spinach intake, other endpoints, namely ROS sensitivity and DNA migration attributable to the formation of oxidatively damaged DNA bases (i.e. pyrimidines-ENDO III-sensitive sites and purines-FPG sensitive sites) were reduced 6 h after consumption of the first portion and after 11 days of continuous consumption. In the case of ENDO III-sensitive sites, also after 16 days, a decrease in comet formation was observed. At the end of a 40 days washout period, the DNA stability parameters were not significantly different from the background values. Other biochemical parameters which were significantly altered by spinach intake were the folate (+27%) and homocysteine (−16%) concentrations in blood, and it was found in an earlier human study that folate may prevent oxidative damage to DNA bases.
*Conclusions* Taken together, our results show that moderate consumption of spinach causes protection against oxidative DNA damage in humans and that this phenomenon is paralleled by alterations of health-related biochemical parameters.

**Keywords** Intervention trial · Spinach · DNA damage · Antioxidant

B. Moser · M. Mišík · O. Zakerska · A. Nersesyan · N. Kager · C. Hoelzl · V. Ehrlich · S. Knasmueller (✉)
Institute of Cancer Research, Internal Medicine I, Medical University of Vienna, Borschkegasse 8a, 1090 Vienna, Austria
e-mail: siegfried.knasmueller@meduniwien.ac.at

T. Szekeres · C. Bieglmayer
Clinical Institute for Medical and Chemical Laboratory Diagnostics, Medical University of Vienna, 1090 Vienna, Austria

K.-H. Wagner
Department of Nutritional Sciences, University of Vienna, 1090 Vienna, Austria

M. Kundi
Institute of Environmental Hygiene, Medical University of Vienna, 1090 Vienna, Austria

J. Zahrl
Food Effect, Consultancy for Crop Production and Nutrition, 1210 Vienna, Austria

## Introduction

It is assumed that dietary constituents which improve the stability of DNA prevent health effects related to genomic instability such as cancer, ageing, arteriosclerosis cardiovascular disorders, infertility and heritable diseases [1]. Therefore, strong efforts were made during the last decades to identify such components in human foods [1].

Spinach is a rich source of chemoprotective substances such as folic acid, flavonoids, lutein, zeaxanthine, ß-carotene and chlorophylls [2–4], which may contribute to the maintenance of the genetic material. The latter compounds are potent antioxidants, and a recent in vitro study showed that the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic) acid (ATBS) radical scavenging activity and also the oxygen radical absorbance capacity (ORAC) values and metal chelating properties of spinach are higher than those of other commonly consumed vegetables such as carrots, tomatoes, potatoes, green beans and kale [5].

Results of an older single cell gel electrophoresis (SCGE) study by Pool-Zobel and coworkers [6] indicated that the consumption of the vegetable may protect humans against formation of single- and double-strand breaks but not against formation of oxidised purines in lymphocytes, while Porrini et al. [3] found in a more recent small trial substantial reduction in DNA migration induced by reactive oxygen species (ROS) in the same experimental system after a 3-week intervention period. Comparisons with findings of earlier investigations indicate that spinach may have a high protective potency as in many human intervention trials with other types of plant foods no evidence for protective effects was seen (details see [7–9]).

The main aim of the present study was to investigate the potential DNA-protective properties of spinach in more detail. Therefore, we conducted an intervention trial which had, for reasons of comparison, a similar design as the aforementioned Italian study [3]. However, we analysed DNA migration additionally at different time points and included further endpoints of DNA stability. Damage was measured under standard SCGE conditions, migration attributable to reactive oxygen species (ROS) was determined after hydrogen peroxide ($H_2O_2$) treatment of the cells and formation of oxidised DNA bases was monitored by use of lesion-specific enzymes (formamidopyrimidine glycosylase, FPG and endonuclease III, ENDO III).

In addition, also a number of biochemical blood parameters related to the health status was measured, namely homocysteine, folate, iron, triglycerides, glucose, high- and low-density lipoprotein (HDL and LDL) and the vitamins A, E and $B_{12}$. Furthermore, also the effects of spinach intake on the numbers of different types of blood cells and on the haematocrit and haemoglobin concentrations were monitored.

## Materials and methods

### Chemicals

Low (LMA) and normal melting agarose (NMA) were obtained from Gibco (Paisley, UK), anorganic salts, hydrogen peroxide, Trizma® base, Triton X-100, dimethyl sulfoxide (DMSO), RPMI 1640, Histopaque-1077, bovine serum albumin fraction V (BSA), ethidium bromide and trypan blue were purchased from Sigma–Aldrich (Steinheim, Germany). FPG and ENDO III were a gift of M. Dušínska (Slovak Medical University, Bratislava, Slovakia). The BIO-RAD® protein assay came from BIO-RAD® (Munich, Germany).

### Study design

The study was approved by the Ethical Commission of the Medical University of Vienna, and informed consent was obtained from all participants ($n = 8$, four men and four women). All of them were enrolled at the same time and healthy non-smokers and consumed a mixed diet, and none of them consumed pharmaceutical drugs and dietary supplements 8 weeks before and during the study. The demographic characteristics of the subjects are summarised in Table 1. The participants were asked to record the types and amounts of foods they consumed during the intervention and were asked at the end of the trial if they differed from their usual dietary habits. According to the information they provided, the food consumptions patterns and the caloric intake were not or only marginally altered. This latter assumption is also supported by the fact that no significant weight changes were seen after the intervention and at the end of the washout period as compared with the start of the study (the average weight of the participants at the start of the trial was in kilogram 74.5 ± 18.3, at the end of the intervention phase 77.2 ± 17.6 and at the end of the washout phase 76.4 ± 20.1).

Five days before and during spinach consumption and also 5 days before the end of the washout period, the participants were asked to reduce consumption of antioxidant rich fruits, vegetables, beverages and spices (for details see [10]), to consume only moderate amounts of alcohol and to abstain from exhausting physical exercises, which may have an impact on the outcome of the study (for details see [10]).

The design of the study is shown schematically in Fig. 1. After a run-in phase of 5 days, the participants consumed 225 g of spinach/day over a total period of 16 days. Blood samples were collected at the beginning of the study (T0), 6 h after consumption of the first portion of spinach (T1), on day 11 (T2), on day 16 (T3) after

**Table 1** Demographic characteristics of the study group (Mean ± SD)

| Sex (m/f) | Age (years) | Height (m) | Weight (kg) | BMI | Pulse (b/min) |
|---|---|---|---|---|---|
| 4/4 | 53.3 ± 6.6 | 1.7 ± 0.1 | 74.5 ± 18.3 | 24.4 ± 3.8 | 68.2 ± 9.3 |

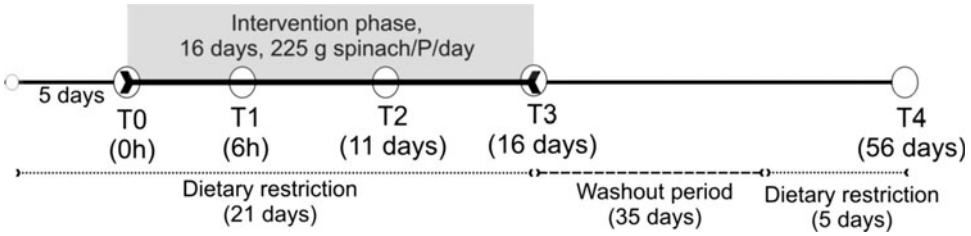


**Fig. 1** Study design

continuous consumption and after a washout period of 40 days (T4). The meals were prepared by the participants from deep frozen unprocessed homogenised spinach (Iglo, Vienna, Austria) and consumed as part of their normal daily diet. To each portion, 8.0 g of corn oil was added to improve the absorption of carotenoids [11, 12]. The participants in the control group were asked to consume an identical amount of corn oil and add it to their regular diet, as it can be not excluded that the additional oil consumption may lead to improved uptake of other fat soluble dietary antioxidants other than those contained in spinach.

## Sampling and isolation of lymphocytes

Blood was collected by venipuncture. For SCGE assays, 5.0 ml was collected in heparinised tubes (10 ml, Becton–Dickinson, Plymouth, UK). Peripheral lymphocytes were isolated with Histopaque 1077 (Sigma–Aldrich, Steinheim, Germany) by centrifugation (800 rpm, 15 min). The cells were suspended in autologous plasma supplemented with DMSO (10%). Subsequently, the suspensions were deep frozen according to the procedure described by Duthie et al. [13], i.e. the cells were frozen slowly in polystyrene boxes stored overnight at −80 °C and finally transferred to liquid nitrogen. We used this procedure in earlier trials and obtained high viability rates of the cells. For the determination of the biochemical parameters and for the cell counts, blood was collected in different tubes and processed as described in detail by Schaenzler and Bieger [14].

## Single cell gel electrophoresis assays

DNA migration was determined under alkaline standard electrophoresis conditions and after treatment of the cells with $H_2O_2$ according to international guidelines [15, 16]. Exposure of isolated nuclei to lesion-specific enzymes was performed as described in detail in the article by Collins et al. [17]. All parameters were measured in one participant at all time points at the same day. In total, 90 slides were prepared per day per participant and analysed in different four electrophoresis procedures.

Briefly, the cells were transferred to agarose-coated slides (1.5% normal melting and 0.5% low-melting agarose) and lysed in buffer (pH 10) for 1 h. After 20-min unwinding and 20-min electrophoresis (300 mA, 25 V at 4 °C, pH > 13), the gels were stained with ethidium bromide (20 μg/ml). In order to measure the sensitivity of the cells towards ROS-induced DNA damage, they were exposed to $H_2O_2$ (25 μM, 5 min on ice); subsequently, the nuclei were isolated, and DNA damage determined under standard conditions. Additionally, nuclei were treated after lyses with the lesion-specific enzymes FPG and ENDO III or with the respective enzyme buffers. To determine the optimal amounts of the enzymes, we conducted experiments with different dilutions of the enzymes prior to the main study (data not shown). After lyses, the slides were washed twice in enzyme reaction buffer (pH 8.0) for 8 min.

The incubation time was 30 min for FPG and 45 min for ENDO III (37 °C). After 40 min unwinding and 30 min electrophoresis (300 mA, 25 V at 4 °C, pH > 13), the gels were stained with ethidium bromide (20 μg/ml). For each endpoint, three slides were made in parallel and 150 cells were analysed from each sample for comet formation by use of an automated image analysis system (Comet Assay IV, Perceptive Instruments, UK). To calculate the endogenous formation of oxidised pyrimidines and purines, the extent of DNA migration measured with the buffers was subtracted from the values obtained with the respective enzymes.

The viability of the cells was assessed in all experiments with the trypan blue exclusion technique [18] since a decline of the viability has been postulated to affect the outcome of SCGE measurements [16, 19]; in the present study, the vitality of the cells was in all samples sufficiently high and all of them were analysed.

## Determination of vitamin concentrations and biochemical parameters in blood

The concentrations of the vitamins A (all-trans retinol), E (α-tocopherol), $B_{12}$ and folic acid were measured in serum samples.

For vitamin C analyses, stabilized Li-heparin plasma was used. For the quantification of plasma-homocysteine, blood samples were collected and transported in chilled EDTA tubes. After centrifugation, all samples were stored at −20 °C until analyses. In HPLC analyses, the signals from the UV detector were integrated and analysed with the Chromeleon software from Dionex (Sunnyvale, CA, USA).

Vitamin A and vitamin E were measured with commercial test kits from Chromsystems (Munich, Germany). Briefly, the method used an isocratic HPLC system with an UV detector set at 325 nm for vitamin A analyses and was switched during the elution to 295 nm for vitamin E measurements. Interassay coefficients of variations were 5% (9%) at 1.8 (4.0) µmol/l vitamin A and 2% (8%) at 20.1 (42.9) µmol/l vitamin E, respectively.

For vitamin C analyses, the plasma samples were stabilized immediately after isolation by addition of a precipitation reagent, which was a component of an HPLC-kit from Immundiagnostik (Bensheim, Germany). Following isocratic elution, vitamin C was measured at 254 nm. Interassay coefficients of variations were 12% (10%) at 21 (91) µmol/l.

Vitamin $B_{12}$, folic acid and homocysteine were measured by immunoassays with an Architect System. The analyser and the kits were from Abbott Laboratories (Abbott Park, IL, USA). Interassay coefficients of variations were 4% (6%) at 351 (857) pmol/l vitamin $B_{12}$, 7% (5%) at 9.6 (28.1) nmol/l folic acid and 5% (4%) at 13.4 (24.2) µmol/L, respectively.

Iron levels were monitored photometrically with the triazin assay [20, 21]. Total serum cholesterol, HDL and LDL were determined with the Olympus enzyme assay [22], and triglycerides were determined as described by Tietz [21]. Uric acid was monitored with the uricase test [23], and glucose was determined with the hexokinase assay [24]. C-reactive protein (CRP) with turbidimetric immunoassays [25]. Photometric measurement was conducted with an Olympus AU5400 automated analyser (Melville, NY, US).

### Cell counts

Blood cells were enumerated with an automated haematology analyser (Sysmex XE 2100, TOA, Medical Electronics, Kobe, Japan), as described by Ruzicka et al. [26].

### Statistics

Sample size was determined based on the results of a previous study [3]. A reduction in DNA migration by 50% translates into an effect size of $eta^2 = 0.3$ (assuming uncorrelated time points). To have a power of 95% to detect an effect of this size at the 1% level of significance (considering multiple endpoints), a sample of 8 subjects evaluated at 5 time points is necessary.

Results of the biochemical analyses were tested for normality by Kolmogorov–Smirnov tests (Dallal-Wilkinson-Lilliefors *p* values). Because of deviations from the assumption of normality, non-parametric statistical tests were performed (Friedman rank test and Dunn's post hoc test).

The medians of DNA migration from fifty cells per slide were arcsine transformed to comply with the sphericity assumption that was tested by Mauchley's sphericity test. Differences between time points were tested by within-subject ANOVA, and comparisons with the baseline (T0) were performed by application of linear contrasts. Triplicate measurements for each participant and time point were tested for outliers by Grubb's test. In case one of these measurements was deviating significantly, the respective slide was excluded from further analysis. In total, 34 slides out of 840 were excluded. For all tests, p values $\leq 0.05$ were considered significant. No adjustment for multiple endpoints was applied.

## Results

The results of the SCGE assays are summarised in Fig. 2a. It can be seen that consumption of the vegetable caused a decrease in DNA migration under standard conditions after 6 h which persisted up to 11 days. However, the reduction in comet formation was not significant in this experiment. After treatment of the cells with $H_2O_2$ and after treatment of the nuclei with the lesion-specific enzymes, a clear increase in DNA migration was observed.

It can be seen in Fig. 2b that a significant decline of ROS ($H_2O_2$)-induced damage (24.2%) was found after spinach consumption. The results obtained with lesion-specific enzymes are shown in Fig. 2c, d. Again, pronounced protective effects were observed shortly after the start of the intervention which decreased gradually. In the case of ENDO III, the reduction in migration was still significant after 16 days of spinach consumption. It is also notable that with all endpoints a similar pattern of protection was observed, i.e. the most pronounced decrease in comet formation was seen immediately after the beginning of the study and the effects decreased as a function of time.

The results of the biochemical measurements, conducted with samples that were collected at the beginning and at the end of the intervention phase (T0 and T3) and after the washout period (T4), are summarised in Table 2.

It can be seen that the folic acid levels increased after spinach consumption by 27.2%; also the concentrations of the vitamins C and $B_{12}$ were higher at the end of intervention but this effect did not reach significance. The majority of the other blood parameters was not altered by spinach intake. The most remarkable change was the decrease in homocysteine (−16.6%), also the CRP level was reduced (−5.4%), but this latter effect was not significant.

In addition, we monitored a number of other clinical parameters such as the counts of erythrocytes, leucocytes, lymphocytes, monocytes and neutrophils as well as

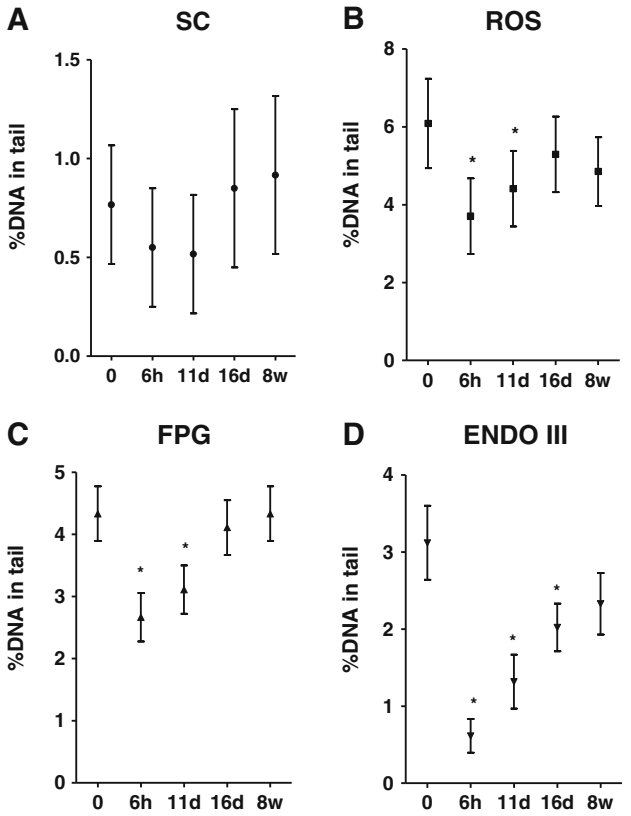


**Fig. 2** Impact of spinach consumption on DNA migration in peripheral lymphocytes. The participants of the study ($n = 8$) consumed the vegetable over a period of 16 days (225 g per person per day). Blood samples were collected at the beginning (T0), 6 h (T1), 11 days (T2) and 16 days after consumption and after a washout period (of 40 days). DNA migration was measured under standard electrophoresis conditions (SC), which reflect single- and double-strand breaks (2A) and after exposure to $H_2O_2$ (25 μM, 5 min) to monitor ROS sensitivity (2B). To measure the endogenous formation of oxidised purines and pyrimidines, the nuclei were incubated with the lesion-specific enzymes FPG (2C) and ENDO III (2D). Points indicate mean values of %DNA in tail, and whiskers indicate 95% confidence intervals. *Stars mark* statistically significant differences from the baseline (T0), $p \leq 0.05$

hematocrit, haemoglobin and clotting; none of them was significantly altered at the end of the consumption period (16 days, data not shown).

## Discussion

Overall, the results of our study indicate that spinach consumption protects humans against oxidative DNA damage and leads to significant alterations of a number of health-related biochemical parameters.

The observation of DNA-protective effects of the vegetable was not unexpected. Pool-Zobel et al. [6] found in a study in which the participants consumed spinach (10 g/P/d) after intake of fruit and vegetable juice over 2 weeks, a decrease in strand breaks under standard conditions, but no significant reduction in ENDO III-sensitive sites. In contrast, the reduction in DNA damage under standard conditions did not reach significance in our study, but, as described earlier, we found a pronounced decline of DNA migration attributable to formation of oxidised purines and pyrimidines (Fig. 2c, d). One of the reasons for these differences may be that the study by Pool-Zobel and coworkers [6] was not well controlled (no washout period was included) and that dried plant material was used. It is also interesting that no clear effect of spinach consumption was found in this trial after the first week. In a study by Porrini et al. [3] ($n = 9$), only two time points were monitored, namely at beginning of the trial and at the end (3 weeks later). The authors detected a significant (ca. 50%) reduction in $H_2O_2$-induced DNA migration after spinach consumption, and no other endpoints of DNA stability were monitored. As described earlier, we observed also a protective effect after $H_2O_2$ treatment in our study. The strongest reduction in comet formation (−39%) was seen shortly (6 h) after consumption of the first portion of spinach. This effect lasted until day 11 (−27%, $p \leq 0.05$) while after 16 days of intake, the effect (reduction by 13%) was not significant (Fig. 2). At the end of the washout period, the extent of DNA migration was higher as that seen after spinach consumption. In the case of oxidatively damaged pyrimidines (ENDO III-sensitive sites), the levels were approximately 30% lower as those found at the start of the trial, which may be taken as an indication that the washout was not complete. However, since the difference is statistically not significant, no firm conclusions can be drawn from this observation.

The observation of a pronounced effect shortly after consumption of the first meal can be taken as an indication that the antioxidant effects caused by the constituents of spinach are at least partly attributable to direct scavenging effects and not to indirect mechanisms such as induction of antioxidant enzymes, which are seen only after extended time periods [27]. Our observation of antioxidant effects of spinach intake is partly supported by the findings by Schirrmacher et al. [28] who found a slight induction of SOD and a modest reduction in the malondialdehyde levels in plasma of humans after a 10-day consumption trial.

Only few intervention trials have been conducted in which the time course of DNA-protective effects was studied in SCGE experiments (for review, see [9]). The results of the present study show that the strongest decrease in DNA migration occurred shortly (6 h) after the start of the consumption. Subsequently, comet formation decreased as a function of the duration of the intervention time, and no significant effects (except with ENDO III-sensitive sites) were seen after 16 days. The results obtained in a previous intervention studies depended strongly on the type

of foods consumed, and no consistent patterns were found. For example, the most pronounced reduction in regard to oxidation of pyrimidines was seen after 8 h in a trial with vitamin C, while no effect was detected after continuous consumption over 4 weeks [29]. With phenolics-enriched fruit juice and also with apples, no time dependency of the reduction in the comets was seen [30–32], while a gradual increase in protection was seen in a trial in which the participants consumed polyphenolics derived from olive oil [33].

The results of the measurements of the biochemical parameters show clearly that consumption of spinach causes a pronounced increase in the folic acid levels in blood (Table 2). It is well known that spinach is a rich source of dietary folate [34, 35]. Our finding is in agreement with the results of Hannon-Fletcher et al. [36], who conducted an intervention trial in which the participants consumed spinach over a period of 6 weeks. In this context, it is notable that several investigations showed that intake of folate is associated with an improvement of the antioxidant status [37–39]. An inverse association between folate levels in serum and prevention of DNA damage has been shown in a number of micronucleus studies with lymphocytes [40]. In SCGE studies, no effects were seen in experiments in which comet formation was monitored under standard conditions [41, 42], but a clear association between folate levels and FPG sensitive sites which are indicative for oxidatively damaged purines was seen in breast cancer patients [43]. In the present study, a significant reduction in the homocysteine concentrations was observed in plasma (Table 2); this effect may be due to reduction in this compound to methionine by folate (42). It has been reported that high homocysteine levels lead to release of ROS as a consequence of oxidation of homocysteine disulfide [44]; however, in vitro experiments demonstrated that homocysteine is not a good ROS precursor and does not induce damage of calf thymus [45]. Therefore, it is likely that other modes of action of folate or other constituents of spinach account on its antioxidant effects. As mentioned earlier, it contains high levels of the carotenoid lutein [46], which is a potent antioxidant in vitro [47]. The compound was tested in a human intervention trial by Zhao et al. [48]; 12 mg was consumed per person per day over a total period of 57 days, and no reduction in comet formation was found after treatment of lymphocytes with $H_2O_2$, while a significant decrease in DNA migration was observed after ≥43 days of continues uptake. Since cooked spinach contains between 6.5 and 7.5 mg of lutein per 100 g [49], the dose of the carotenoid given in the aforementioned trial is contained in the vegetable consumed by the participants in the present study. Spinach contains also relatively high amounts of chlorophyll (1250 μg/g [50]) but it is unlikely that the pigment which is a potent antioxidant when tested in vitro [51] accounts for the effects in the lymphocytes as it is only poorly absorbed in the GI tract [50].

We also expected an increase in vitamin A in serum, since spinach contains substantial amounts of β-carotene [52], but the vitamin status of the participants was quite high before the beginning of the trial, and it is known that only a minor fraction of β-carotene is converted to the vitamin [53].

Pronounced protection against oxidative DNA damage was seen in earlier studies only with certain types of

**Table 2** Impact of spinach consumption ($n = 8$) on different biochemical parameters and on the vitamins status (Mean ± SD)

| Endpoint | Time point (mean ± SD) | | |
|---|---|---|---|
| | T0[a] | T3[b] | T4[c] |
| Homocysteine (μM/L) | 9.97 ± 1.87 | 8.33 ± 1.75* | 10.15 ± 2.42 |
| Uric acid (mg/dL) | 5.24 ± 1.55 | 5.33 ± 1.69 | 5.59 ± 1.68 |
| Triglycerides (mg/dL) | 129.87 ± 142.20 | 130.75 ± 86.02 | 146.13 ± 93.15 |
| Glucose (mg/dL) | 98.75 ± 10.50 | 93.88 ± 11.03 | 95.25 ± 9.79 |
| Cholesterol (mg/dL) | 213.00 ± 33.60 | 214.75 ± 29.96 | 208.88 ± 26.65 |
| HDL (mg/dL) | 60.25 ± 17.96 | 59.88 ± 17.18 | 57.75 ± 17.49 |
| CRP (mg/dL) | 0.23 ± 0.13 | 0.22 ± 0.30 | 0.22 ± 0.22 |
| LDL (mg/dL) | 132.75 ± 24.89 | 128.73 ± 22.93 | 121.90 ± 19.23 |
| Iron (mg/dL) | 101.75 ± 38.56 | 99.63 ± 27.85 | 95.38 ± 32.83 |
| Vitamins (μM/L) | | | |
| All-trans retinol | 2.62 ± 0.77 | 2.37 ± 0.66 | 2.45 ± 0.77 |
| α-tocopherol | 34.51 ± 9.42 | 33.79 ± 5.84 | 33.59 ± 6.56 |
| Vitamin $B_{12}$ | 364.00 ± 211.39 | 396.00 ± 215.10 | 343.38 ± 208.58 |
| Vitamin C | 60.60 ± 18.22 | 70.61 ± 21.74 | 63.69 ± 13.55 |
| Folic acid | 22.44 ± 5.55 | 28.54 ± 7.84* | 16.33 ± 5.41 |

*$p < 0.05$

[a] Beginning of the intervention

[b] End of intervention, day 16

[c] End of washout period, day 40

vegetables, for example with Brussels sprouts and water cress (for review see [7]), but the lack of clear effect in trials in which the participants consumed relatively large amounts of a mixed fruit and vegetable diet (600 g/p/day) [30] or a vegetable/fruit concentrate (500 g/p/day) [54] indicates that only certain plant-derived foods have pronounced DNA-protective capacity. Our findings indicate that spinach is one of them and that it alters and shows several disease-related biochemical parameters. Taken together, the results suggest that its consumption has a beneficial impact on the health status of humans.

**Acknowledgments** The authors are thankful to Dipl. Ing. Barbara Schausberger for discussion and help in the design of the study and IGLO Austria GmbH for financial support of the study.

## References

1. Knasmüller S, Johnson I, DeMarini D, Gerhäuser C (eds) (2009) Chemoprevention of cancer and DNA damage by dietary factors. Wiley, Weinheim
2. Micozzi MS, Beecher GR, Taylor PR, Khachik F (1990) Carotenoid analyses of selected raw and cooked foods associated with a lower risk for cancer. J Natl Cancer Inst 82:282–285
3. Porrini M, Riso P, Oriani G (2002) Spinach and tomato consumption increases lymphocyte DNA resistance to oxidative stress but this is not related to cell carotenoid concentrations. Eur J Nutr 41:95–100
4. Humphries JM, Khachik F (2003) Distribution of lutein, zeaxanthin, and related geometrical isomers in fruit, vegetables, wheat, and pasta products. J Agric Food Chem 51:1322–1327
5. Zhou KQ, Yu LL (2006) Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. LWT Food Sci Technol 39:1155–1162
6. Pool-Zobel BL, Bub A, Muller H, Wollowski I, Rechkemmer G (1997) Consumption of vegetables reduces genetic damage in humans: first results of a human intervention trial with carotenoid-rich foods. Carcinogenesis 18:1847–1850
7. Hoelzl C, Knasmuller S, Misik M, Collins A, Dusinska M, Nersesyan A (2009) Use of single cell gel electrophoresis assays for the detection of DNA-protective effects of dietary factors in humans: recent results and trends. Mutat Res 681:68–79
8. Moller P, Loft S (2004) Interventions with antioxidants and nutrients in relation to oxidative DNA damage and repair. Mutat Res 551:79–89
9. Nersesyan A, Hoelzl C, Ferk F, Misik M, Knasmüller S (2009) Use of single cell gel electrophoresis assays in dietary intervention trials. In: Dhawan A, Anderson D (eds) The comet assay in toxicology. Royal Society of Chemistry, Cambridge, pp 253–282
10. Hoelzl C, Glatt H, Meinl W, Sontag G, Haidinger G, Kundi M, Simic T, Chakraborty A, Bichler J, Ferk F, Angelis K, Nersesyan A, Knasmuller S (2008) Consumption of Brussels sprouts protects peripheral human lymphocytes against 2-amino-1-methyl-6-phenylimidazo 4, 5-b pyridine (PhlP) and oxidative DNA-damage: results of a controlled human intervention trial. Mol Nutr Food Res 52:330–341
11. Brown MJ, Ferruzzi MG, Nguyen ML, Cooper DA, Eldridge AL, Schwartz SJ, White WS (2004) Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. Am J Clin Nutr 80:396–403
12. van Het Hof KH, West CE, Weststrate JA, Hautvast JG (2000) Dietary factors that affect the bioavailability of carotenoids. J Nutr 130:503–506
13. Duthie SJ, Pirie L, Jenkinson AM, Narayanan S (2002) Cryopreserved versus freshly isolated lymphocytes in human biomonitoring: endogenous and induced DNA damage, antioxidant status and repair capability. Mutagenesis 17:211–214
14. Schaenzler N, Bieger WP (2004) Blut- und Laborwerte. Druckerei Auer, Donauwörth
15. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 35:206–221
16. Hartmann A, Agurell E, Beevers C, Brendler-Schwaab S, Burlinson B, Clay P, Collins A, Smith A, Speit G, Thybaud V, Tice RR (2003) Recommendations for conducting the in vivo alkaline Comet assay. 4th international comet assay workshop. Mutagenesis 18:45–51
17. Collins A, Dušinská M (2002) Oxidation of cellular DNA measured with the comet assay. In: Armstrong D (ed) Oxidative stress biomarkers and antioxidant protocols. Humana Press Inc., Totowa, pp 147–160
18. Lindl T, Bauer J (1994) Zell- und Gewebekultur. Stuttgart, Jena, New York
19. Burlinson B, Tice RR, Speit G, Agurell E, Brendler-Schwaab SY, Collins AR, Escobar P, Honma M, Kumaravel TS, Nakajima M, Sasaki YF, Thybaud V, Uno Y, Vasquez M, Hartmann A (2007) Fourth International Workgroup on Genotoxicity testing: results of the in vivo Comet assay workgroup. Mutat Res 627:31–35
20. Goodwin J, Murphy B, Guillemette M (1966) Direct measurement of serum iron and binding capacity. Clin Chem 12:47–57
21. Tietz N (ed) (1995) Clinical guide to laboratory tests. WB Saunders company, Philadelphia
22. Kattermann R, Jaworek D, Moller G, Assmann G, Bjorkhem I, Svensson L, Borner K, Boerma G, Leijnse B, Desager JP et al (1984) Multicentre study of a new enzymatic method of cholesterol determination. J Clin Chem Clin Biochem 22:245–251
23. Thomas L (2005) Uric acid. In: Thomas L (ed) Labor und Diagnose. Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik TH Books Verlöagsgesellschaft, Frankfurt/Main, pp 280–289
24. Thomas L (ed) (2005) Labor und Diagnose—Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik. TH-Books Verlagsgessellschaft, 6. Aufl, Frankfurt/Main
25. Grant G, Silverman L, Christenson R (1987) Amino acids and proteins. In: Tietz N (ed) Fundamentals of clinical chemistry. WB Saunders comp, Philadelphia, p 334
26. Ruzicka K, Veitl M, Thalhammer-Scherrer R, Schwarzinger I (2001) The new hematology analyzer Sysmex XE-2100: performance evaluation of a novel white blood cell differential technology. Arch Pathol Lab Med 125:391–396
27. Huber WW, Grasl-Kraupp B, Schulte-Hermann R (1996) Hepatocarcinogenic potential of di(2-ethylhexyl)phthalate in rodents and its implications on human risk. Crit Rev Toxicol 26:365–481
28. Schirrmacher G, Skurk T, Hauner H, Grassmann J (2010) Effect of *Spinacia oleraceae* L. and *Perilla frutescens* L. on antioxidants and lipid peroxidation in an intervention study in healthy individuals. Plant Foods Hum Nutr 65:71–76
29. Moller P, Viscovich M, Lykkesfeldt J, Loft S, Jensen A, Poulsen HE (2004) Vitamin C supplementation decreases oxidative DNA damage in mononuclear blood cells of smokers. Eur J Nutr 43:267–274
30. Moller P, Vogel U, Pedersen A, Dragsted LO, Sandstrom B, Loft S (2003) No effect of 600 grams fruit and vegetables per day on oxidative DNA damage and repair in healthy nonsmokers. Cancer Epidemiol Biomarkers Prev 12:1016–1022

31. Weisel T, Baum M, Eisenbrand G, Dietrich H, Will F, Stockis JP, Kulling S, Rufer C, Johannes C, Janzowski C (2006) An anthocyanin/polyphenolic-rich fruit juice reduces oxidative DNA damage and increases glutathione level in healthy probands. Biotechnol J 1:388–397
32. Guarnieri S, Riso P, Porrini M (2007) Orange juice vs vitamin C: effect on hydrogen peroxide-induced DNA damage in mononuclear blood cells. Br J Nutr 97:639–643
33. Salvini S, Sera F, Caruso D, Giovannelli L, Visioli F, Saieva C, Masala G, Ceroti M, Giovacchini V, Pitozzi V, Galli C, Romani A, Mulinacci N, Bortolomeazzi R, Dolara P, Palli D (2006) Daily consumption of a high-phenol extra-virgin olive oil reduces oxidative DNA damage in postmenopausal women. Br J Nutr 95:742–751
34. McKillop DJ, Pentieva K, Daly D, McPartlin JM, Hughes J, Strain JJ, Scott JM, McNulty H (2002) The effect of different cooking methods on folate retention in various foods that are amongst the major contributors to folate intake in the UK diet. Br J Nutr 88:681–688
35. Subar AF, Block G, James LD (1989) Folate intake and food sources in the US population. Am J Clin Nutr 50:508–516
36. Hannon-Fletcher MP, Armstrong NC, Scott JM, Pentieva K, Bradbury I, Ward M, Strain JJ, Dunn AA, Molloy AM, Kerr MA, McNulty H (2004) Determining bioavailability of food folates in a controlled intervention study. Am J Clin Nutr 80:911–918
37. Meguid NA, Dardir AA, El-Sayed EM, Ahmed HH, Hashish AF, Ezzat A (2010) Homocysteine and oxidative stress in Egyptian children with Down syndrome. Clin Biochem 43:963–967
38. Xu Y, Wang H, Wang Y, Zheng Y, Sun G (2010) Effects of folate on arsenic toxicity in Chang human hepatocytes: involvement of folate antioxidant properties. Toxicol Lett 195:44–50
39. Moens AL, Vrints CJ, Claeys MJ, Timmermans JP, Champion HC, Kass DA (2008) Mechanisms and potential therapeutic targets for folic acid in cardiovascular disease. Am J Physiol Heart Circul Physiol 294:H1971–H1977
40. Fenech M (2001) The role of folic acid and Vitamin B12 in genomic stability of human cells. Mutat Res 475:57–67
41. Zijno A, Andreoli C, Leopardi P, Marcon F, Rossi S, Caiola S, Verdina A, Galati R, Cafolla A, Crebelli R (2003) Folate status, metabolic genotype, and biomarkers of genotoxicity in healthy subjects. Carcinogenesis 24:1097–1103
42. Milic M, Rozgaj R, Kasuba V, Orescanin V, balija M, Jukic I (2010) Correlation between folate and vitamin B12 and markers of DNA stability in healthy men: preliminary results. Acta Biochim Pol 57:339–345
43. Hussien MM, McNulty H, Armstrong N, Johnston PG, Spence RA, Barnett Y (2005) Investigation of systemic folate status, impact of alcohol intake and levels of DNA damage in mononuclear cells of breast cancer patients. Br J Cancer 92:1524–1530
44. Ramakrishnan S, Sulochana KN, Lakshmi S, Selvi R, Angayarkanni N (2006) Biochemistry of homocysteine in health and diseases. Indian J Biochem Biophys 43:275–283
45. Muniz P, Saez P, Iradi A, Vina J, Oliva MR, Saez GT (2001) Differences between cysteine and homocysteine in the induction of deoxyribose degradation and DNA damage. Free Radic Biol Med 30:354–362
46. Bunea A, Andjelkovic M, Socaciu C, Bobis O, Neacsu M, Verhe R, Van Camp J (2008) Total and individual carotenoids and phenolic acids content in fresh, refrigerated and processed spinach (*Spinacia oleracea* L.). Food Chem 108:649–656
47. Santocono M, Zurria M, Berrettini M, Fedeli D, Falcioni G (2007) Lutein, zeaxanthin and astaxanthin protect against DNA damage in SK-N-SH human neuroblastoma cells induced by reactive nitrogen species. J Photochem Photobiol B 88:1–10
48. Zhao X, Aldini G, Johnson EJ, Rasmussen H, Kraemer K, Woolf H, Musaeus N, Krinsky NI, Russell RM, Yeum KJ (2006) Modification of lymphocyte DNA damage by carotenoid supplementation in postmenopausal women. Am J Clin Nutr 83:163–169
49. Olmedilla B, Granado F, Southon S, Wright AJ, Blanco I, Gil-Martinez E, van den Berg H, Thurnham D, Corridan B, Chopra M, Hininger I (2002) A European multicentre, placebo-controlled supplementation study with alpha-tocopherol, carotene-rich palm oil, lutein or lycopene: analysis of serum responses. Clin Sci (Lond) 102:447–456
50. Ferruzzi MG, Blakeslee J (2007) Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. Nutr Res 27:1–12
51. Waters MD, Stack HF, Jackson MA, Brockman HE, De Flora S (1996) Activity profiles of antimutagens: in vitro and in vivo data. Mutat Res 350:109–129
52. Tang G, Qin J, Dolnikowski GG, Russell RM, Grusak MA (2005) Spinach or carrots can supply significant amounts of vitamin A as assessed by feeding with intrinsically deuterated vegetables. Am J Clin Nutr 82:821–828
53. Wagner KH, Elmadfa I (2003) Biological relevance of terpenoids. Overview focusing on mono-, di- and tetraterpenes. Ann Nutr Metab 47:95–106
54. Van Den Berg R, Haenen G, Van Den Berg H, Bast A (1999) Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. Food Chem 66:511–517