

## Prevention of Oxidative DNA Damage in Rats by Brussels Sprouts

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The alleged cancer preventive effects of cruciferous vegetables could be related to protection from mutagenic oxidative DNA damage. We have studied the effects of Brussels sprouts, some non-cruciferous vegetables and isolated glucosinolates on spontaneous and induced oxidative DNA damage in terms of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in groups of 6–8 male Wistar rats. Excess oxidative DNA damage was induced by 2-nitropropane (2-NP 100 mg/kg).

Four days oral administration of 3 g of cooked Brussels sprouts homogenate reduced the spontaneous urinary 8-oxodG excretion by 31% ( $p < 0.05$ ) whereas raw sprouts, beans and endive (1:1), isolated indolyl glucosinolates and breakdown products had no significant effect. An aqueous extract of cooked Brussels sprouts (corresponding to 6.7 g vegetable per day for 4 days) decreased the spontaneous 8-oxodG excretion from  $92 \pm 12$  to  $52 \pm 15$  pmol/24 h ( $p < 0.05$ ). After 2-NP administration the 8-oxodG excretion was increased to  $132 \pm 26$  pmol/24 h ( $p < 0.05$ ) whereas pretreatment with the sprouts extract reduced this to  $102 \pm 30$  pmol/24 h ( $p < 0.05$ ).

The spontaneous level of 8-oxodG in nuclear DNA from liver and bone marrow was not significantly affected by the sprouts extract whereas the level decreased by 27% in the kidney ( $p < 0.05$ ). In the liver 2-NP increased the 8-oxodG levels in nuclear DNA 8.7

and 3.8 times ( $p < 0.05$ ) 6 and 24 h after dose, respectively. The sprouts extract reduced this increase by 57% ( $p < 0.05$ ) at 6 h whereas there was no significant effect at 24 h. In the kidneys 2-NP increased the 8-oxodG levels 2.2 and 1.2 times ( $p < 0.05$ ) 6 and 24 h after dose, respectively. Pretreatment with the sprouts extract abolished these increases ( $p < 0.05$ ). Similarly, in the bone marrow the extract protected completely ( $p < 0.05$ ) against a 4.9-fold 2-NP induced increase ( $p < 0.05$ ) in the 8-oxodG level.

These findings demonstrate that cooked Brussels sprouts contain bioactive substance(s) with a potential for reducing the physiological as well as oxidative stress induced oxidative DNA damage in rats. This could explain the suggested cancer preventive effect of cruciferous vegetables. The correspondence between the urinary excretion and 8-oxodG levels in 2-NP target organs supports its being the main repair product that reflects the rate of guanine oxidation in DNA.

**Keywords:** Brussels sprouts, oxidative DNA damage, 2-nitropropane, 8-oxodG, chemoprevention

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## INTRODUCTION

In epidemiological studies, a diet rich in green and yellow vegetables is consistently associated with a low risk of epithelial cancers in the airways and upper gastric intestinal tract.<sup>[1,2]</sup>

Cruciferous vegetables, e.g. broccoli, cauliflower, kale and Brussels sprouts, have particular cancer preventive effects in observational epidemiology and animal carcinogenesis studies.<sup>[3-9]</sup> However, the cancer preventive mechanism of cruciferous vegetables and the active principles are not fully understood. These vegetables are rich in glucosinolates which are hydrolysed by myrosinase contained in the plants and liberated by processing and/or storage. Many of the resulting isothiocyanate breakdown products have potent pharmacological and toxicological effects. The indolyl derivatives are unstable and their further breakdown and condensation products are potent inducer of cytochrome P450 (CYP) enzymes, particularly the CYP 1A subfamily, and some phase II enzymes.<sup>[10-12]</sup> Other resulting isothiocyanates with aryl, alkyl and/or sulphinyl side chains, such as sulphoraphane isolated from broccoli, are potent inducers of particular phase II enzymes and may inhibit chemical carcinogenesis.<sup>[9,13,14]</sup>

A specific cancer preventive mechanism of cruciferous vegetables could be related to reduction of oxidative DNA damage, particular guanine oxidation, which is the most abundant and mutagenic oxidative modification known.<sup>[15]</sup> Similarly, green tea and its extracts can reduce oxidative DNA damage, measured by 8-oxodG formation, induced by the hepatocarcinogen 2-nitropropane (2-NP) in rats.<sup>[16]</sup> Indeed, in an intervention study in humans 300 g of Brussels sprouts as compared with non-cruciferous vegetables reduced the urine excretion of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), a repair product of guanine oxidation and biomarker of the rate of oxidative DNA damage.<sup>[15,17]</sup> The effects of cruciferous vegetables on the rate of DNA damage induced by oxidative stress and the levels of 8-oxodG in the

relevant organs or surrogate tissue have not been investigated.

The aim of the present study was to establish an animal model for prevention of oxidative DNA damage and to investigate if Brussels sprouts components reduce spontaneous as well as induced oxidative DNA damage. To that end the effect of raw and cooked Brussels sprouts, isolated indolyl glucosinolates and non-cruciferous vegetables on urinary 8-oxodG excretion was investigated. In addition the effect of an extract of Brussels sprouts on spontaneous and 2-NP induced 8-oxodG excretion and levels in nuclear DNA in target organs were studied.

## MATERIALS AND METHODS

### Chemicals

2-Nitropropane was purchased from Aldrich Chemical Co. (Milwaukee, WI), 2'-deoxyguanosine (dG), nuclease P1, corn oil was from Sigma (St. Louis, MO), and alkaline phosphatase was from Boehringer Mannheim (Germany). 8-OxodG standard was synthesized as previously described<sup>[18,19]</sup> and calibrated against pure compound from Sigma (St. Louis, MO) and WAKO (Germany).

*Isolated Glucosinolates* Glucosinolates were isolated from freeze dried broccoli (*Brassica oleracea* L. cv. *botrytis* (L.) Alef. var. *cymosa* Duch., cultivar "Skiff") as previously described.<sup>[20]</sup> Indolyl glucosinolates, mainly glucobrassicin and neoglucobrassicin, represented 70% of the total glucosinolates. A fraction of the glucosinolates was incubated for 3 days with myrosinase isolated from rape seed, thus converting the glucosinolates, into indole-3-yl methanol, indole-3-yl aldehyde and indole-3-yl acetonitrile and other products as determined by HPLC.<sup>[20]</sup>

*Vegetable Homogenates* Brussels sprouts, endive and green beans were bought at the local vegetable market. Homogenates were prepared of raw Brussels sprouts, Brussels sprouts microwave cooked for 15 min, and a

1:1 mixture of endive and beans microwave cooked for 20 min. The homogenates were diluted 25% with distilled water.

**Brussels Sprouts Extract** One kilogram fresh Brussels sprouts obtained from a local supermarket was washed successively with cold tap water and distilled water, then ground in a home-mixer (Braun Multipress) with 40 ml distilled water, and the juice and residues cooked together in a microwave oven for 3 min. The cooked materials were squeezed through gauze and the residues were repeatedly washed with distilled water until the colour changed (from green to white). The combined solution (cooked juice and water extract from the residues) was frozen at  $-80^{\circ}\text{C}$ , lyophilised and resuspended in distilled water to a final volume of 600 ml.

### Animals and Treatment

Male Wistar rats, 6 weeks old and of 200–220 g body weight, were housed 3 to a cage with aspen wood bedding (Finntapwei, Finland) in an environmentally controlled animal facility operating on a 12 h dark/light cycle at  $22\text{--}24^{\circ}\text{C}$  and 55% humidity. The rats were given free access to tap water and standard laboratory diet (Altromin 1314, Lage, Germany). For 24-h urine collection the rats were placed in individual metabolic cages.

**Glucosinates and Myrosinase Study** Three groups of 6 rats were treated with either (1) the intact or (2) the myrosinase treated glucosinolate powder (0.22 g/kg body weight), corresponding to 35% (w/w) freeze dried broccoli in the diet, or (3) the reaction buffer (control) by gastric gavage twice daily for 3 days. After a last dose on the fourth day urine was collected for 24 h for 8-oxodG determination.

**Vegetables Study** Three groups of 8 rats were treated with 4 ml of either homogenate, corresponding to 3 g of vegetable, by gastric gavage for 4 days. Before the vegetable homogenate treatment and after the last dose on the fourth day urine was collected for 24 h for 8-oxodG determination.

**Brussels Sprouts Extract Study** Four groups of 12–16 rats were used. Two groups were pretreated with 4 ml of the Brussels sprouts extract, corresponding to 6.7 g vegetables, per day by gastric gavage for 4 days whereas the other two groups were pretreated with an identical volume of water. After the last dose of extract or water one group for each of the two pretreatments received 2-NP (100 mg/kg body weight) i.p. in corn oil (5 ml/kg BW) whereas the other two groups received only corn oil in a similar dose. Six hours after 2-NP or corn oil, half of the rats from all groups were killed, and 24 h after 2-NP or corn oil the remaining half were killed. The liver, kidney and bone marrow (from the two femurs of each rat and only at 24 h) were collected, frozen and stored at  $-80^{\circ}\text{C}$ . Before pretreatment 24 h urine was collected from each rat by means of metabolic cages. After the 2-NP dose and until the animals were killed, i.e. for 6 h or 24 h, urine was collected again by means of metabolic cages.

### Analysis of 8-oxodG and dG

Tissues and cell suspensions were analysed by a modification of previously described procedures.<sup>[18,19]</sup> Samples of liver and kidney were, homogenised with HEPES 5 mM buffer (N-hydroxyethyl]piperazine-N=[2-ethanesulfonic acid]), sucrose 70 mM, manitol 250 mM, pH 7.4 on ice. The pellet after centrifugation at 1000 g containing the nuclei was resuspended in 0.15 M NaCl, 1  $\mu\text{l}/\text{mg}$  tissue. The bone marrow cells were flushed from the femurs, washed and suspended in phosphate buffered saline (pH 7.4). Two hundred  $\mu\text{l}$  of either suspension was transferred to 1.8 ml ice-cold TE buffer (150 mM NaCl, 10 mM Tris, 10 mM  $\text{Na}_2\text{EDTA}$ , pH 8.0), and 200  $\mu\text{l}$  1% dodecylsulphate sodium salt (SDS) was added. After vortexing for 30 s and incubation in a water bath at  $37^{\circ}\text{C}$  for 10 min, 200  $\mu\text{l}$  3 M sodium acetate (pH 5.2), 550  $\mu\text{l}$  5 M sodium perchlorate and chloroform/isoamyl alcohol (24:1) 2 ml were added. After rotation in an extraction bench for 10 min the samples

were centrifuged at 3500 rpm for 10 min and 2 volumes of ice-cold 96% ethanol were slowly added to the separated supernatant (non-organic phase). The DNA was allowed to precipitate at  $-20^{\circ}\text{C}$  overnight followed by centrifugation at 3000 rpm for 5 min. The DNA precipitate was washed with 70% ethanol, dried with a gentle stream of nitrogen gas, dissolved in  $200\ \mu\text{l}$  20 mM sodium acetate (pH 4.5), and digested to nucleoside level at  $37^{\circ}\text{C}$  with  $20\ \mu\text{l}$  (5 U/sample) of Nuclease P1 (Sigma, ST. Louis, MO) for 30 min and  $20\ \mu\text{l}$  (1 U/sample) of alkaline phosphatase (Boehringer Mannheim, Germany) for 60 min. The amounts of 8-oxodG and dG were measured using a HPLC system with electrochemical and UV detector as previously described.<sup>[21]</sup> The urine concentration of 8-oxodG was measured by HPLC with electrochemical detection as previously described.<sup>[22]</sup>

### Statistics

Different treatment groups were compared with multifactorial analysis of variance and the

method of least significant difference as post hoc test. Probability values less than 0.05 were considered statistically significant.

### RESULTS

Administration of the homogenate of cooked Brussels sprouts significantly decreased the urinary excretion of 8-oxodG by 31% ( $p=0.005$ ) whereas the raw Brussels sprouts homogenate had no significant effect ( $p=0.45$ ; Figure 1). In the rat group treated with a homogenate of cooked beans and endive there was a non-significant decrease of 19% ( $p=0.11$ ). In the rats treated with isolated indolyl glucosinolates with or without myrosinase activation or with the incubation buffer there were no significant differences in the urinary excretion of 8-oxodG (Figure 1).

After pretreatment with the aqueous extract of Brussels sprouts and administration of corn oil the 24 h urine 8-oxodG excretion was decreased by 43% (95% confidence interval: 27–60%)

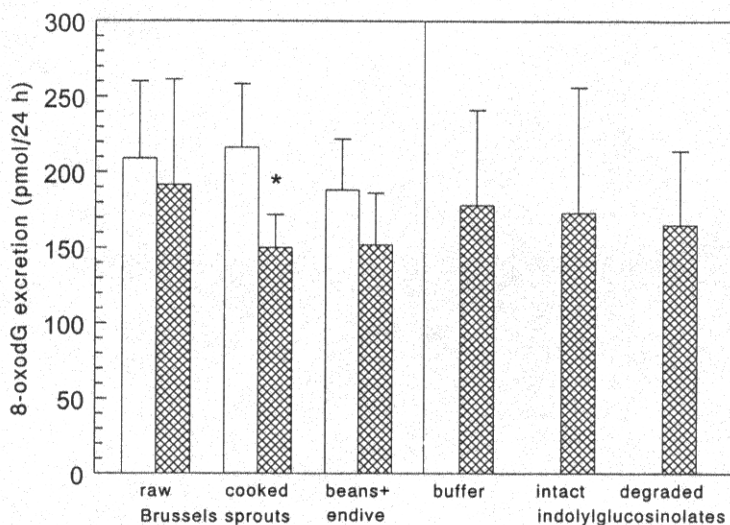


FIGURE 1 Twenty-four hour urinary excretion of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), a repair product of guanine oxidation and biomarker of the rate of oxidative DNA damage, in rats before (open bars) and after (hatched bars) treatment with a homogenate of raw Brussels sprouts, microwave cooked Brussels sprouts or microwave cooked endive and beans, corresponding to 3 g of vegetable per day (left panel) or with isolated indolyl glucosinolates with or without myrosinase degradation or with the incubation buffer (right panel) by gastric gavage for 4 days. Values are mean with SD of 8 rats per group. \* denotes  $p < 0.05$  versus value before treatment.

comparing with the group administered water and corn oil, whereas the excretion was decreased by 58% (48–68%,  $p < 0.05$ ) if comparing with the value obtained in the group before pretreatment (Figure 2). The spontaneous 6 h urinary 8-oxodG excretion was decreased by 15% in the group pretreated with the extract as compared with the water pretreated group. The spontaneous levels of 8-oxodG in the target organs were not significantly decreased by pretreatment with the Brussels sprouts extract except in the kidneys with a decrease of 30% (19–41%,  $p < 0.05$ ) 24 h after the last dose (Figure 3). If the 6 and 24 h after vehicle groups were pooled the decrease in spontaneous 8-oxodG level in kidney amounted to 27% (14–41%,  $p < 0.05$ ).

In the groups pretreated with water, 2-NP increased the 8-oxodG excretion by 33% (9–57%,

$p < 0.05$ ) and 43% (27–60%,  $p < 0.05$ ) in the urine collected 6 and 24 h after administration, respectively (Figure 2). Pretreatment with Brussels sprouts extract completely abolished the 2-NP induced increase in 8-oxodG excretion, i.e. there were relative decreases of 29% and 28% in the urine collected 6 and 24 h after dose, respectively ( $p < 0.05$ ).

In the liver 2-NP had increased the 8-oxodG levels in nuclear DNA to 8.7 and 3.8 times the control value ( $p < 0.05$ ) at 6 and 24 h after dose, respectively (Figure 3). Pretreatment with the Brussels sprouts extract reduced the increase by 57% ( $p < 0.05$ ) at 6 h whereas there was no significant effect 24 h after 2-NP administration. In the kidneys 2-NP increased the 8-oxodG levels in nuclear DNA to 2.2 times ( $p < 0.05$ ) and 1.2 times ( $p < 0.05$ ) the control values at 6 h and 24 h

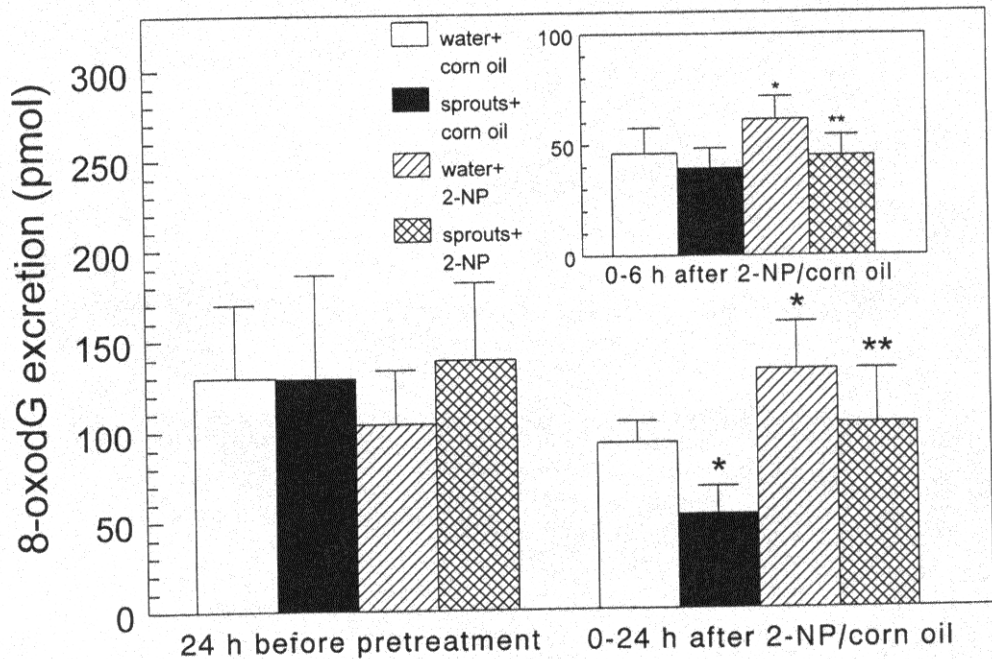


FIGURE 2 The effects of pretreatment with an extract of cooked Brussels sprouts followed by administration of 2-nitropropane (2-NP) on the urinary excretion of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), a repair product of guanine oxidation and biomarker of the rate of oxidative DNA damage in rats. For each of the 4 treatment combinations 15–16 rats were allocated. Urine was collected twice from each rat, for 24 h before pretreatment ( $n = 15-16$ /group) and for 6 h ( $n = 6-8$ /group) or 24 h ( $n = 6-8$ /group) after administration of 2-NP 100 mg/kg or corn oil preceded by pretreatment with water or the extract for 4 days. Values are mean with SD. \* denotes  $p < 0.05$  versus water + vehicle group; \*\* denotes  $p < 0.05$  versus water + 2-NP group.

TABLE I Estimated excess levels of 8-oxodG in nuclear DNA from target tissues and 24 h urinary excretion of 8-oxodG after administration of 2-nitropropane in rats. The excess was estimated as the difference between values at 6 h and 24 h in liver and kidneys and between the 24 h and control value in bone marrow, assuming  $2 \times 10^9$  dG per cell

Target	Assumed no of cells	Excess 8-oxodG	Contribution to body burden
Liver (6 g)	$6 \times 10^8$	3 per $10^5$ dG	$3.6 \times 10^{13}$ molecules = 60 pmol
Kidneys (2 g)	$2 \times 10^8$	0.4 per $10^5$ dG	$0.2 \times 10^{13}$ molecules = 3 pmol
Bone marrow (0.5 g)	$0.5 \times 10^8$	2 per $10^5$ dG	$0.2 \times 10^{13}$ molecules = 3 pmol
Excess urinary excretion			40 pmol

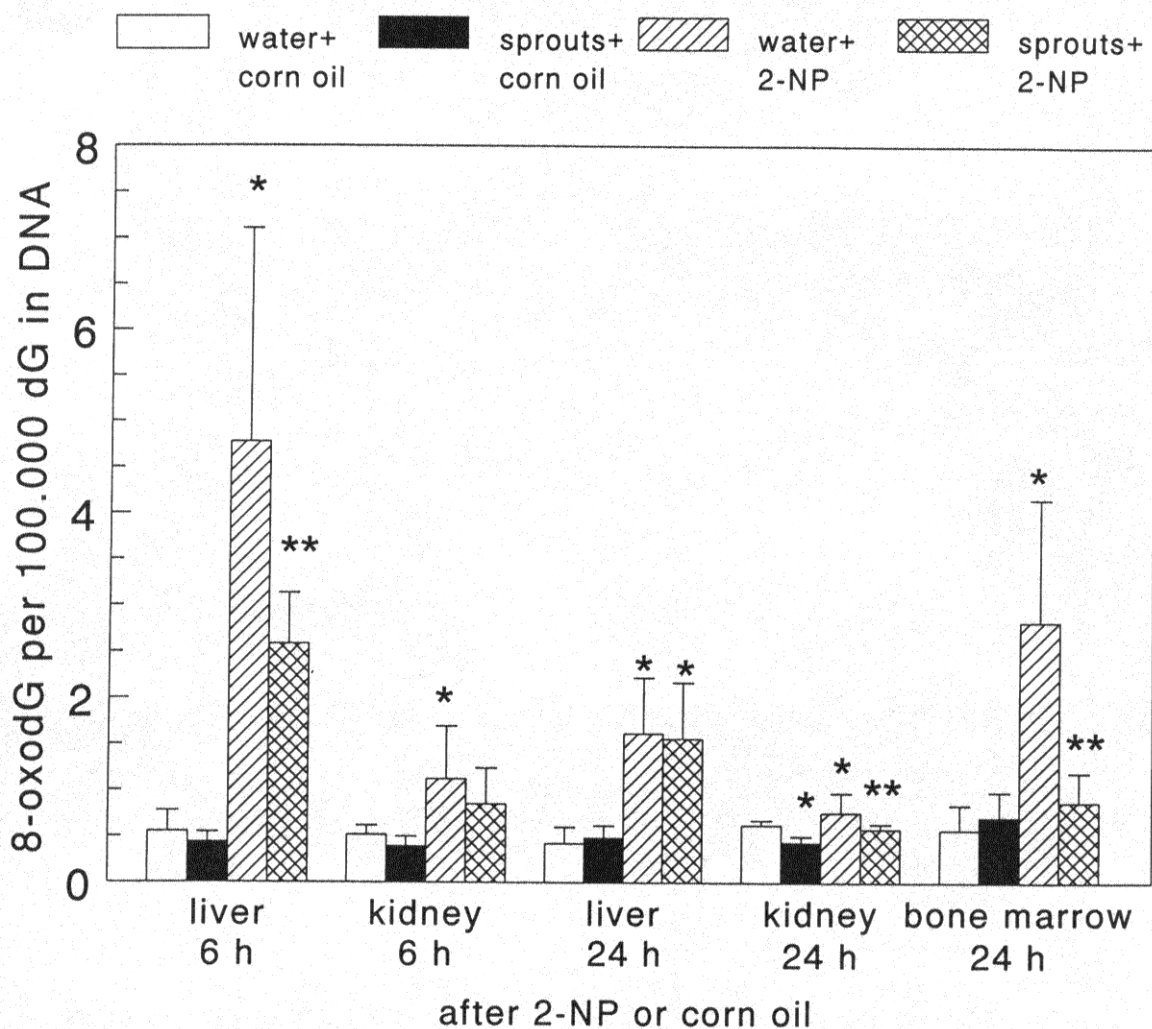


FIGURE 3 Level of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in DNA from liver, kidney and bone marrow 6 and 24 h after administration of 2-nitropropane (2-NP, 100 mg/kg or vehicle) preceded by treatment with water or an extract of cooked Brussels sprouts for 4 days. Values are mean with SD of 6-8 rats per group. \* denotes  $p < 0.05$  versus water + vehicle group; \*\* denotes  $p < 0.05$  versus water + 2-NP group.

after dose. Pretreatment with the sprouts extract abolished these increases ( $p < 0.05$ ; Figure 3). Similarly, in the bone marrow the extract protected completely against a 4.9-fold increase ( $p < 0.05$ ) in the 8-oxodG in nuclear DNA 24 h after 2-NP treatment ( $p < 0.05$ , Figure 3).

## DISCUSSION

Cooked Brussels sprouts and in particular an extract from these vegetables decreased the spontaneous rate of oxidative DNA damage as shown by reduced 8-oxodG excretion. Moreover, the extract prevented 2-NP induced 8-oxodG formation as shown by the reduced urinary excretion of 8-oxodG and the lowering of the induced nuclear levels of 8-oxodG in liver, kidney and bone marrow. In contrast, raw Brussels sprouts, non-cruciferous vegetable or isolated intact or myrosinase degraded indolyl glucosinolates, had no effects. This indicates that cooked Brussels sprouts contain bioactive substance(s) with a potential for reducing the physiological as well as oxidative stress induced oxidative DNA modification. Remarkably, this applies to the overall DNA modification measured by urinary 8-oxodG excretion and the organ specific changes measured by the nuclear DNA level of oxidised guanine.

In a previous study in humans cooked Brussels sprouts decreased 8-oxodG excretion whereas non-cruciferous vegetables had no effect.<sup>[17]</sup> The present data confirm and extend this observation in rats indicating that the protective effect may be specific for cruciferous vegetables which are characterised by their content of glucosinolates. This large family of compounds and their transformation products have various potent pharmacological and toxicological effects among which some could be responsible for the beneficial effect on oxidative DNA damage. Moreover, cooking was essential for the protective effect. Previously, antioxidants, including vitamin E, ellagic acid and tea extracts have been

shown to protect the liver from 8-oxodG induction by 2-NP although other signs of hepatotoxicity were unaffected.<sup>[16,23]</sup>

The indolyl isothiocyanates liberated from myrosinase catalysed breakdown of e.g. glucobrassicin and neoglucobrassicin, are highly unstable and ultimately condense to products such as indole-3-carbazole, which are potent inducers of cytochrome P450 enzymes, particularly CYP1A1 and CYP1A2 in the liver and intestine.<sup>[12,24]</sup> However, in cell cultures responsive to CYP1A1 inducers, indole-3-carbazole increased 8-oxoGua formation along with the enzyme activity.<sup>[24]</sup> In the present study the breakdown products or intact indolyl glucosinolates, including substantial amounts of glucobrassicin, had no effect on the 8-oxodG excretion although this dose caused considerable induction of cytochrome P450 activity as assessed by the oxidative metabolism of antipyrine and metronidazole.<sup>[20]</sup> Moreover, CYP1A1 and CYP1A2 induction by 3-methylcholanthrene enhanced the ability of 2-NP to induce DNA strand breaks in the liver.<sup>[25]</sup>

This is in keeping with the notion that reactive oxygen species generated by metabolism of 2-NP-nitronate catalysed by CYP's are responsible for the specific hepatocarcinogenicity of 2-NP,<sup>[26-29]</sup> whereas the present effects in the bone marrow could be due to metabolism by myeloperoxidase. Thus, a similar effect of CYP1A induction in the present study could have partly outbalanced a protective effect of the Brussels sprouts extract on the 2-NP induced 8-oxodG formation in the liver, in which the protection appeared less than e.g. in the bone marrow without relevant CYP1A activity. Accordingly, other glucosinolates than the indolyl derivatives may be responsible for the reduction in oxidative DNA damage. The protective effect of the sprouts extract was apparent in the kidney and from the urinary excretion of 8-oxodG without 2-NP induced oxidative stress. This indicates that the effect of the sprouts extract is more likely to be an antioxidative effect than related to inhibition of 2-NP metabolism with ROS generation.

Brussels sprouts induce a host of phase II enzymes and possibly other defence mechanism.<sup>[9,12,30,31]</sup> Isothiocyanates with alkyl, aryl and/or sulphanyl side chains are abundant as their glucosinolate precursors in cruciferous vegetables and induce glutathione S-transferases (GTS's), glutathione peroxidase, UDP-glucuronosyltransferases, NAD(P)H:quinone reductase, levels of reduced glutathione and heme oxygenase, via the antioxidant responsive element.<sup>[9,13,14,32]</sup> Antioxidant functions could at least be related to glutathione levels, GST's functioning as glutathione peroxidase and heme oxygenase catalysed conversion of the prooxidant heme to the antioxidant bilirubin.<sup>[33]</sup> These isothiocyanates are monofunctional inducers of the phase II enzymes with a possible inhibitory effect on CYP enzymes<sup>[34,35]</sup> in contrast to the indolyl glucosinolate products which act via the Ah receptor inducing both CYP's and phase II enzymes.<sup>[24,36]</sup> Moreover, several isothiocyanates have been shown to suppress chemical carcinogenesis from many different compounds as recently reviewed by Verhoeven *et al.*<sup>[9]</sup> Sulphoraphane is a particularly potent inducer of the antioxidant responsive element effects of phase II detoxication enzymes and other protectants,<sup>[32,37]</sup> as well as a potent inhibitor of CYP2E1 which may contribute to its anti-genotoxic effects.<sup>[38]</sup> This compound inhibited neoplastic transformation *in vitro* as well as the formation of mammary tumours in rats treated with 9,10-dimethyl-1,2-benzanthracene.<sup>[37]</sup> Accordingly, the protective effects of cooked Brussels sprouts with respect to oxidative DNA damage may reside within some of these isothiocyanate compounds. The homogenisation used for the present sprouts products would be sufficient to activate myrosinase and thus liberate the compounds. Nevertheless, it is still intriguing that the effect was confined to the cooked sprouts although this could have prevented further enzymatic degradation. Actually, the glucosinolates in Brussels sprouts are rather resistant to cooking.<sup>[39]</sup> As the sprouts

extract was prepared from both juices and residues after micro wave cooking the minced vegetables it is unlikely that large amounts of compound have escaped as possible in steam or water blanching.<sup>[39]</sup>

Cruciferous vegetables are rich in ascorbic acid, carotenoids and many other potential antioxidants, which could contribute to the effects. However, this could not explain their specific protective effect in comparison with other vegetables. *In vitro*, extracts from cooked Brussels sprouts even enhanced lipid peroxidation in liver microsomes whereas some other assays and purified glucosinolates showed weak antioxidant effects.<sup>[40]</sup> Accordingly, direct antioxidant effects are unlikely to explain the protective effects of Brussels sprouts on oxidative DNA damage.

Presence of other electrochemically active DNA and RNA adducts, including 8-aminodeoxyguanosine, 8-oxoguanosine, and 8-aminoguanosine, has been reported in the liver of 2-NP treated rats.<sup>[41]</sup> However, our assays do not include RNA adducts, 8-aminoguanosine elutes far earlier than 8-oxodG in our HPLC system (data not shown) and there were no unknown peaks in our chromatograms from tissues of 2-NP treated rats and thus no possibility to evaluate whether the sprouts extract had any effects on amino related DNA adducts.

Urinary excretion and nuclear DNA levels of 8-oxodG are used as biomarkers of oxidative DNA damage.<sup>[15]</sup> The major part of 8-oxodG in DNA probably arises from oxidation of the base within the DNA whereas incorporation of oxidized nucleotides from the cellular pool is probably of minor quantitative importance although highly mutagenic and thus of large qualitative importance.<sup>[42]</sup> The repair of 8-oxodG in DNA results in 8-oxodG or 8-oxoGua by nucleotide excision and base excision, respectively.<sup>[43]</sup> Recently, the human 8-oxoGua glycosylase was cloned by several groups<sup>[44,45]</sup> whereas nucleotide excision repair was shown to contribute to the repair of 8-oxodG in DNA.<sup>[46]</sup>



It is intriguing that the spontaneous urinary excretion of 8-oxodG was reduced by half without a corresponding decrease in the levels in the nuclear DNA in liver and bone marrow. It is unlikely that artefactual formation of 8-oxodG which may occur during sample preparation<sup>[15,47]</sup> was responsible as comparison were made between groups and our values of 8-oxodG in liver DNA are in the lower end of the published range. After 4 days treatment with the extract steady state should have been reached and the urinary excretion and nuclear levels of 8-oxodG should thus reflect the damage rate and the balance between damage and repair rates, respectively.<sup>[15]</sup> Accordingly, the repair rate may be down regulated if the damage rate is decreased leaving a constant 8-oxodG level in the DNA. Part of the decrease in urinary 8-oxodG excretion could also be due to a reduced rate of oxidation of dGTP in the nucleotide pool which is sanitised by specific enzymes with 8-oxodG as end product.<sup>[15,48]</sup> The third major potential source of urinary 8-oxodG relates to cell and mitochondria turn over.<sup>[15]</sup> However, it is unlikely that the Brussels sprouts extract could change these turnover rates to an extent that could explain the reduced urinary 8-oxodG excretion. Moreover, the 40% increase in 8-oxodG excretion induced by 2-NP could correspond to most of the possible output from repair of the nuclear DNA in the target organs with possible metabolic activation. These consisted mainly of the liver which represents about 3.5% of the body mass and had an 8-fold increased 8-oxodG level in the nuclear DNA, i.e. a 28% increase in body burden by simple arithmetic. The kidneys had only a 2-fold increase, the bone marrow represents a very small part of the cells of the body and other organs, except perhaps the lungs and intestine, are less likely to generate reactive oxygen species from 2-NP. Indeed, 2-NP administration had no effects on the 8-oxodG levels in the nuclear DNA from rat testes (unpublished data). In terms of number of molecules the increase in urinary excretion could account for

60% of the estimated excess 8-oxodG induced in the target organs (Table I).

In conclusion the present study demonstrates that cooked Brussels sprouts contain bioactive substance(s) with a potential for reducing the physiological as well as oxidative stress induced oxidative DNA modification in rats. Accordingly, this could partly explain the suggested cancer preventive effect of cruciferous vegetables. The correspondence between urinary excretion and 8-oxodG levels in 2-NP target organs supports that it is a main repair product and reflects the rate of guanine oxidation in DNA.

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