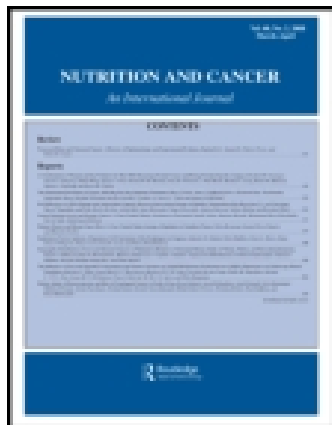


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Nicotinic acid supplementation: Effects on niacin status, cytogenetic damage, and poly(ADP-ribosylation) in lymphocytes of smokers

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Nicotinic Acid Supplementation: Effects on Niacin Status, Cytogenetic Damage, and Poly(ADP-Ribosylation) in Lymphocytes of Smokers

Geja J. Hageman, Rob H. Stierum, Marcel H. M. van Herwijnen,
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Abstract: As a substrate for poly(ADP-ribose) polymerase (PARP; EC, 2.4.2.30), an enzyme that is activated by DNA strand breaks and is thought to facilitate efficient DNA repair, NAD⁺ and its precursor nicotinic acid (niacin) are involved in the cellular defense against DNA damage by genotoxic compounds. In this study, the effect of nicotinic acid supplementation on cytogenetic damage and poly(ADP-ribosylation) was evaluated in a human population that is continuously exposed to genotoxic agents, e.g., smokers. By use of a placebo-controlled intervention design, 21 healthy smokers received supplementary nicotinic acid at 0–100 mg/day for 14 weeks. An increased niacin status, as assessed from blood nicotinamide concentrations and lymphocyte NAD⁺ concentrations, was observed in groups supplemented with 50 and 100 mg/day. This effect was most pronounced in subjects with lower initial NAD⁺ levels. An increased niacin status did not result in decreased hypoxanthine guanine phosphoribosyltransferase variant frequencies and micronuclei induction in peripheral blood lymphocytes (PBLs). Sister chromatid exchanges in PBLs, however, were increased after supplementation with nicotinic acid. This increase was positively associated with the daily dose of nicotinic acid. No effects of nicotinic acid supplementation were found for *ex vivo* (\pm)-7 β ,8 α -dihydroxy-9 α ,10 α -epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-induced poly(ADP-ribosylation), although the small number of samples that could be analyzed (n = 12) does not allow firm conclusions. Because no evidence was found for a decrease in cigarette smoke-induced cytogenetic damage in PBLs of smokers after nicotinic acid supplementation of up to 100 mg/day, it is concluded that supplemental niacin does not contribute to a reduced genetic risk in healthy smokers.

Introduction

Since the identification of the niacin cofactor NAD⁺ as the substrate of the enzyme poly(ADP-ribose) polymerase (PARP; EC 2.4.2.30), which is activated by DNA strand

breaks and appears to play a role in DNA repair, cell division, and cell differentiation (1,2), niacin deficiency has been postulated to increase cancer risk (3,4). PARP uses NAD⁺ as a substrate to form homopolymers composed of ADP-ribose subunits; high levels of DNA strand breaks have been found to induce extensive polymer formation, with a concomitant lowering of cellular NAD⁺ levels, which may ultimately lead to ATP depletion and cell death (5–7). In addition, inhibition of PARP has been reported to decrease DNA repair efficiency, increase the potency of genotoxic compounds, and enhance cell transformation and carcinogenesis (8).

Little is known about the role of poly(ADP-ribosylation) in carcinogenesis in general and, in particular, with respect to the recovery from DNA damage *in vivo* (6). However, as a consequence of niacin deficiency or suboptimal niacin intake, poly(ADP-ribosylation) may be impaired, resulting in an accumulation of DNA damage and increased cancer risk. In rats, niacin deficiency has been found to result in decreased tissue NAD⁺ levels, decreased hepatic poly(ADP-ribose) polymer concentrations, and increased *ex vivo* oxygen radical-induced DNA strand break formation in hepatocytes and lymphocytes (9,10). In addition, Weitberg (4) showed that blood lymphocyte NAD⁺ levels were elevated after supplementation of two healthy human subjects with nicotinic acid at 100 mg/day for eight weeks. The occurrence of *ex vivo* oxygen-radical induced DNA strand breaks was lower in lymphocytes from these subjects than in lymphocytes from nonsupplemented subjects.

Although the estimated daily niacin intake in The Netherlands, 14–17 mg niacin equivalents (as nicotinic acid) for adult men, is sufficient to prevent niacin deficiency-related disorders such as pellagra (11), it is hypothesized that a further increase of the niacin status, especially in individuals who are exposed to genotoxic agents, may contribute to a reduced genetic and cancer risk through an improved action of poly(ADP-ribosylation) and enhanced DNA repair. Therefore, the aim of the present study was to investigate whether cigarette smoke-related cytogenetic damage can be

decreased in peripheral blood lymphocytes (PBLs) of healthy smokers through an increase of the niacin status by supplementation with nicotinic acid. For this purpose, male smokers were supplemented with nicotinic acid for 14 weeks in a placebo-controlled study. Niacin status was monitored by determination of blood nicotinamide (free nicotinamide and nicotinamide converted from NAD⁺ and NADP⁺) and NAD⁺ in PBLs. PARP activation was assessed as poly(ADP-ribose) polymer formation in PBLs after *ex vivo* treatment with (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene [(±)-anti-BPDE]. To evaluate whether tobacco smoke-induced DNA damage can be lowered by increased nicotinic acid intake, sister chromatid exchanges (SCEs), hypoxanthine guanine phosphoribosyl transferase (*hprt*) variant frequencies (VFs), and micronuclei (MN) frequencies were determined in PBLs before and at the end of the nicotinic acid supplementation period.

Materials and Methods

Materials

All chemicals were purchased from Merck (Darmstadt, Germany), unless otherwise specified. Cell culture media and supplements were obtained from GIBCO (Breda, The Netherlands).

Study Population

Twenty-one healthy men who smoked ≥10 cigarettes/day enrolled in this study after approval for the study protocol was obtained from the Medical Ethical Committee of Maastricht University and University Hospital. Initially, the study was to include 24 smokers, divided among 4 dose groups, but because of rigid selection criteria, 21 remained eligible. This number of participants was considered sufficient to detect an increase in niacin status, at least in the highest-dose groups (4). Because it is not known to what extent a change in cytogenetic parameters can be achieved, a total of six subjects per dose group was chosen mainly for practical and technical reasons. Each participant served as his own control; results of different parameters obtained before the start of the supplementation were compared with values obtained during the last two weeks of the supplementation period. Because of the small number of participants who were eligible, the lowest-dose group (25 mg/day) consisted of three,

rather than six, participants. Participants were informed about possible side effects of nicotinic acid intake, and all subjects officially agreed to participate in the study by giving their written informed consent. A standard questionnaire was used to obtain data on age, weight, height, alcohol and coffee consumption, smoking habits, and use of medication. The characteristics of the subjects are shown in Table 1.

Nicotinic Acid Supplementation and Study Protocol

To detect possible dose-dependent effects of nicotinic acid supplementation on parameters of cytogenetic damage and poly(ADP-ribosylation), subjects were divided into several dose groups (Table 1). Subjects were assigned to the dose groups at random, but in such a way that the mean number of cigarettes smoked per day was similar for all groups. A second criterion was that the consumption of alcoholic beverages should not be significantly different between the groups. These variables were previously found to affect cytogenetic parameters (16). To reduce possible side effects (e.g., flushing and itching), individuals in the highest-dose group (100 mg/day) received three doses at 33.3 mg/day. Capsules containing nicotinic acid (C.A.S. 59-67-6) were obtained from the Pharmacy Unit of the University Hospital Maastricht. Identical capsules were used for placebo administration. Supplementation was performed according to a double-blind protocol design, and subjects were advised to eat and drink sufficient water or soft drinks during intake of the capsules.

The nicotinic acid supplementation period lasted 14 weeks, since this was expected to be long enough to detect measurable changes in cigarette smoke-associated DNA damage in PBLs *in vivo*. Nicotinic acid supplementation of two subjects for eight weeks has been reported to result in a maximum elevation of lymphocyte NAD⁺ levels (4).

Before the start of the nicotinic acid supplementation period, (±)-anti-BPDE-induced poly(ADP-ribosylation), SCE frequencies, *hprt* VFs, and MN frequencies were determined in PBLs twice for each individual within a two-week period. Also, on two occasions during the presupplementation period, the levels of the main nicotinic acid metabolites, nicotinamide and NAD⁺, were determined in blood and isolated lymphocytes, respectively, to assess niacin status before nicotinic acid administration. At the end of nicotinic acid supplementation, blood nicotinamide levels, lymphocyte NAD⁺ levels, SCE and MN frequencies, VFs, and (±)-

Table 1. Characteristics of the Study Population^a

	Nicotinic Acid Intake, mg/day			
	0 (n = 6)	25 (n = 3)	50 (n = 6)	100 (n = 6)
Age, yr	31.8 ± 13.1	42.0 ± 9.5	36.2 ± 9.2	28.3 ± 9.1
Smoking behavior, cigarettes/day	18.8 ± 8.9	21.6 ± 2.8	19.7 ± 3.3	18.5 ± 5.8
Alcohol consumption, glasses/wk	10.8 ± 5.9	14.3 ± 14.0	11.2 ± 11.9	14.2 ± 8.0
Coffee consumption, cups/day	3.8 ± 2.5	8.0 ± 2.0	5.7 ± 2.9	5.5 ± 1.9

^a: Values are means ± SD.

anti-BPDE-induced poly(ADP-ribosylation) were determined twice in a two-week period.

Determination of NAD⁺ Levels in Isolated PBLs

Within 2 hours after collection of the blood sample, 10 ml of blood were diluted in an equal volume of phosphate-buffered saline (PBS) and layered onto three-quarters volume of Lymphoprep (Nycomed, Oslo, Norway) in 50-ml culture tubes before centrifugation for 20 minutes at 800 *g*. Interphases containing PBLs were collected, pooled in 50-ml culture tubes, and washed twice with PBS. Cells were counted using a hemocytometer and dissolved in RPMI 1640 medium to a final density of 1×10^8 cells/ml. Then 60 μ l of cell suspension were resuspended in 540 μ l of ice-cold RPMI 1640 medium in 10-ml tubes in duplicate, and 100 μ l of this solution were stored at -20°C for protein determination. Subsequently, pyridine nucleotides were extracted with 2 ml of ice-cold 0.1 M NaOH and 1 mM nicotinamide (Sigma Chemical, Axel, The Netherlands), then washed with 1 ml of the solution. The time between withdrawal of blood samples and the extraction procedure was held as constant as possible. Then samples were neutralized to pH 7.3 with 0.37 M H₃PO₄ and stored at -20°C . For determination of intracellular NAD⁺ levels, an enzymatic cycling assay was applied (12). Frozen extracts were thawed, and reduced pyridine nucleotides in the extract were oxidized by addition of 200 μ l of 2.0 mM phenazine ethosulfate (Sigma Chemical). An aliquot of 200 μ l of the extract [or standard NAD⁺ (Sigma Chemical) in bidest] was added in duplicate to a preincubated reaction mixture containing final concentrations of 1.67 μ mol/ml phenazine ethosulfate, 0.42 μ mol/ml 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2H-tetrazolium bromide, 60 μ mol/ml bicin, pH 7.8, 0.167 mg/ml alcohol dehydrogenase (all from Sigma Chemical), and 0.5 μ mol/ml ethanol and incubated for exactly 30 minutes at 30°C . Absorption of the reaction mixture was measured at 570 nm immediately after incubation at room temperature with a spectrophotometer (model DU 648, Beckman).

Protein Determination

Frozen samples were thawed, and to dissolve the cellular material, an equal volume of 0.2% sodium dodecyl sulfate was added and samples were mixed vigorously for ≥ 30 minutes. Then 20 μ l of the dissolved material were added to 780 μ l of bidest, and protein was determined using the Bio-Rad (Veenendaal, The Netherlands) protein assay kit. Standard bovine serum albumin solutions were prepared in $2.5 \times 10^{-3}\%$ sodium dodecyl sulfate, which did not interfere with the linearity of the calibration curve.

Determination of Nicotinamide in Venous Heparinized Blood

Nicotinamide levels in venous heparinized blood were determined according to the procedure developed by Shibata

and co-workers (13). This method measures free nicotinamide as well as nicotinamide derived from NAD⁺ and NADP⁺. Blood samples were collected and stored at -20°C until analysis. Nicotinamide and the internal standard 1-methylnicotinamide (Sigma Chemical, C.A.S. 114-33-0) were separated on a Supelcosil LC-18S column (250×4.6 mm ID; Supelco, Bellefonte, PA). The injection volume was 100 μ l. The column was eluted isocratically at a flow rate of 1.00 ml/min at room temperature with 97% 10 mM KH₂PO₄, 2.5% acetonitrile, and 0.5% tetrahydrofuran (vol/vol/vol), pH 4.5, delivered by a Kratos Spectroflow 400 solvent delivery system (Interscience, Breda, The Netherlands). Nicotinamide (and 1-methylnicotinamide) was monitored at 260 nm using a programmable photodiode array detector (model 994, Waters, Eitten-Leur, The Netherlands). The detection limit of nicotinamide was 0.05 μ g absolute, corresponding to approximately 3.3 μ g nicotinamide/ml blood. Overall recoveries were 80–90%.

Determination of (\pm)-*Anti*-BPDE-Induced Poly(ADP-Ribosylation)

In a previous study, we showed that (\pm)-*anti*-BPDE treatment of phytohemagglutinin-stimulated lymphocytes for 15 minutes resulted in elevated levels of ethenoribosyladenosine (eRado), the unique nucleoside derived from poly(ADP-ribose) (14), which increased further on prolonged treatment (15). Venous heparinized blood (100 ml) was diluted with an equal volume of PBS, and lymphocytes were isolated as described above for determination of NAD⁺ levels. Next, cells were incubated at 37°C at a density of 1×10^7 cells/ml RPMI 1640 medium with 40 μ M (\pm)-*anti*-BPDE (Midwest Research Institute). The final dimethyl sulfoxide concentration was 0.18%. After 15 minutes, one-half of the cell suspension was added to an equal volume of ice-cold 40% trichloroacetic acid (TCA) and incubated on ice for ≥ 30 minutes. One hundred microliters of cell suspension were used for protein determination before TCA precipitation. The other half of the cell suspension was incubated for another 20 minutes, then precipitated with TCA. TCA-insoluble material from both time points (15 and 35 min) was pelleted by centrifugation at 3,600 rpm for 15 minutes at 4°C . Pellets were stored at -20°C before determination of eRado, according to the procedure of Jacobson and co-workers (14) with minor modifications as described elsewhere (15), except [*adenine*-¹⁴C]poly(ADP-ribose) was obtained from permeabilized lymphocytes.

Measurement of Cytogenetic Parameters

SCE frequencies in human PBLs were determined as described previously (16). Before microscopic evaluation, the slides were encoded. Slides were evaluated by a well-trained observer, and SCE scores were checked by a second independent observer. From one subject, for each time point,

≥20 metaphases containing ≥40 chromosomes were analyzed for SCEs.

Hprt VFs were analyzed by assessment of 6-thioguanine resistance according to a short-time procedure with bromodeoxyuridine labeling and immunocytochemical staining, as described previously (17). Slides were encoded before microscopic analysis and evaluated by a well-trained observer.

MN frequencies were determined using the procedure of Fenech and Morley (18) with a slight modification. Aliquots of 0.4 ml of venous heparinized blood were cultured in 5 ml of RPMI 1640 complete medium and 0.2 ml of phytohemagglutinin (±52 µg/ml final concn) to stimulate T cell proliferation. To block cytokinesis, 44 hours after culturing, cytochalasin B (Sigma Chemical) was added to a final concentration of 6 µg/ml. After an additional culture period of 28 hours, cultures were harvested as described above and slides were prepared and stained with 3% Giemsa for 20 minutes. Before microscopic analysis, slides were encoded. Per subject, for each time point, 1,000 binucleated cells were analyzed for the presence of MN by a well-trained observer and checked by a second observer.

Statistical Analysis

Possible differences between parameters of cytogenetic damage and niacin status before and at the end of the nicotinic acid supplementation period were statistically analyzed by means of the nonparametric Wilcoxon signed rank test. Differences between supplementation groups were analyzed using analysis of variance with Dunnett's *t*-test for multiple comparisons. Possible correlations between nicotinic acid intake and changes in parameters of niacin status and DNA damage and repair were statistically analyzed using non-parametric Spearman's rank correlation test. Because one subject became ill during the nicotinic acid supplementation period, his results were excluded from the data set.

Results

The effect of daily supplementation with various doses of nicotinic acid for 14 weeks on niacin status of the subjects, as determined by blood nicotinamide and lymphocyte NAD⁺ concentrations, is shown in Figures 1 and 2. Blood nicotinamide concentrations were increased at the end of the supplementation period in all dose groups; this increase was statistically significant for the group that received 100 mg/day ($p < 0.05$, Wilcoxon signed rank test). At the individual level, an increase in blood nicotinamide was observed at the end of the supplementation period in 9 of 11 subjects receiving nicotinic acid at 50–100 mg/day. In addition, the increase in blood nicotinamide concentrations was dose related: a significant positive association was found between the dose administered and the increase in blood nicotinamide ($r = 0.75$, $p = 0.001$, Spearman rank correlation test). Lymphocyte NAD⁺ concentrations were also increased after nico-

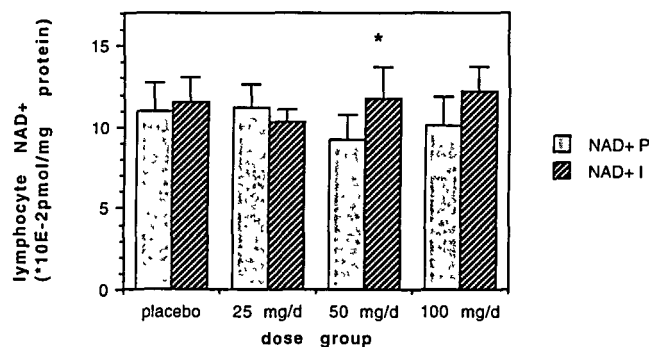


Figure 1. NAD⁺ concentrations in peripheral blood lymphocytes before (NAD⁺ P) and after (NAD⁺ I) nicotinic acid supplementation. Values are means ± SD. *, Statistically significantly different from initial NAD⁺ concentration ($p < 0.05$, Wilcoxon signed rank test).

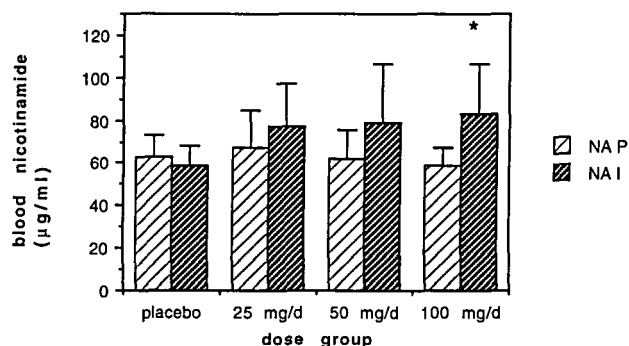


Figure 2. Blood nicotinamide concentration before (NA P) and after (NA I) nicotinic acid supplementation. Values are means ± SD. *, Statistically significantly different from initial blood nicotinamide concentration ($p < 0.05$, Wilcoxon signed rank test).

tinic acid supplementation at 50 and 100 mg/day. This increase was statistically significant only for the group that received nicotinic acid at 50 mg/day ($p < 0.05$, Wilcoxon signed rank test). A weak dose-effect association was found between the dose of nicotinic acid administered and the increase in lymphocyte NAD⁺ concentrations ($r = 0.42$, $p = 0.07$, Spearman rank correlation test).

In Figure 3, results for *ex vivo* (±)-anti-BPDE-induced poly(ADP-ribosylation) in lymphocytes are shown, as determined by eRado levels at 15 and 35 minutes after start of the treatment. Presupplementation measurements are compared with measurements at the end of the supplementation period. Because of loss of polymers during sample preparation and cleanup, sufficient material was available for eRado analysis for only 12 subjects. Although mean eRado levels at 15 minutes tended to be decreased at the end of the supplementation period in the placebo group, for the other groups no differences were observed between initial eRado levels and levels at the end of the supplementation period.

Table 2 presents results of replicate measurements of SCE frequencies in PBLs at baseline and at the end of the supplementation period for each dose group. SCE frequencies were significantly increased after nicotinic acid supple-

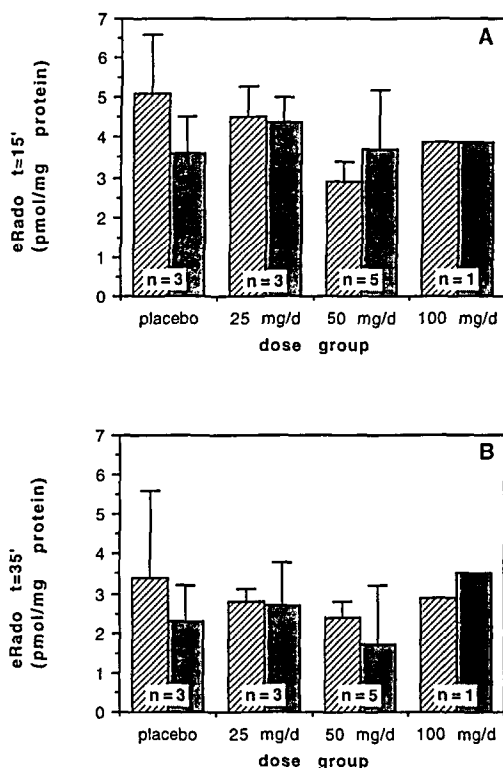


Figure 3. Poly(ADP-ribosylation) in isolated peripheral blood lymphocyte 15 min (A) and 35 min (B) after *ex vivo* treatment with (\pm)-7 β ,8 α -dihydroxy-9 α ,10 α -epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene before (hatched bars) and after (stippled bars) nicotinic acid supplementation. Values are means \pm SD. eRado, ethenoribosyladenosine.

mentation at 100 mg/day ($p < 0.05$, Wilcoxon signed rank test), whereas in the nonsupplemented group, mean SCE frequencies tended to decrease. In addition, an increase in SCE frequencies was found for all nicotinic acid-supplemented subjects compared with the nonsupplemented group ($p < 0.05$, Wilcoxon signed rank test). Moreover, a significant association was found between daily nicotinic acid intake and changes in SCE frequencies ($r = 0.51$, $p < 0.05$, Spearman rank correlation test). The observed increase for the two highest-dose groups, however, was still within the range of SCE frequencies for the nonsupplemented subjects.

No effect of nicotinic acid supplementation was found on MN frequencies in PBLs (Table 3). A significant decrease

in *hprt* VFs at the end of the supplementation period was observed only for the group receiving nicotinic acid at 100 mg/day ($p < 0.05$, Wilcoxon signed rank test; Table 4). No significant effect of nicotinic acid supplementation on VFs was found for the lower-dose groups or when all supplemented subjects were analyzed as one group. No correlation (Spearman rank correlation test) was found between individual changes in cytogenetic parameters and individual changes in niacin status (intracellular NAD⁺ and plasma nicotinic acid).

Discussion

In experimental studies with rats, niacin deficiency resulted in an increased sensitivity to *ex vivo* oxygen radical-induced DNA strand breaks in lymphocytes and hepatocytes and an increased rate of *N*-nitrosodimethylamine-induced hepatic tumor incidence (10,19). In addition, inhibition of PARP resulted in increased SCE frequencies in cell cultures (20,21), which led to the hypothesis that a sufficient niacin status or niacin supplementation may be a preventive factor in carcinogenesis (3,4). Niacin is the main precursor for NAD⁺, which is a substrate for the DNA repair-related enzyme PARP. Consequently, sufficient niacin nutrition may contribute to an increased resistance of cells to DNA damage.

Nicotinic acid and nicotinamide have been reported to be precursors for NAD⁺ synthesis in resting lymphocytes (22). In our study, the blood niacin status of the groups that received nicotinic acid at ≥ 50 mg/day was increased, although not all subjects in these supplementation groups showed an increase in blood nicotinamide or lymphocyte NAD⁺ concentrations. The daily doses supplied in our study were approximately equivalent to two (25 mg) to eight times (100 mg) the average daily intake of nicotinic acid for men in The Netherlands (11). However, when we compared subjects with a relatively low initial niacin status (lymphocyte NAD⁺ $< 9.9 \times 10^{-2}$ pmol/mg protein) with subjects with a high initial niacin status (NAD⁺ $> 11.2 \times 10^{-2}$ pmol/mg protein) in our study, the effect of supplemental nicotinic acid was apparent in the first group only. All supplemented subjects with a relatively low niacin status ($n = 5$) showed an increase in lymphocyte NAD⁺ averaging 2.8×10^{-2} pmol/mg protein, whereas supplemental

Table 2. SCE Frequencies in PBLs Before and After Intervention^{a,b}

Nicotinic Acid Intake, mg/day	n	SCEs/Cell					
		Before intervention			After intervention		
		First	Second	Mean	First	Second	Mean
0	6	6.1 \pm 1.1	6.7 \pm 1.3	6.3 \pm 1.1	6.3 \pm 1.1	5.5 \pm 1.0	5.8 \pm 0.9
25	3	6.8 \pm 1.1	7.0 \pm 0.8	7.0 \pm 1.0	7.6 \pm 0.1	7.7 \pm 0.8	7.6 \pm 0.4
50	6	6.6 \pm 0.5	5.8 \pm 0.9	6.2 \pm 0.8	7.5 \pm 0.8	6.4 \pm 1.3	6.8 \pm 1.3
100	5	5.1 \pm 1.0	5.2 \pm 0.6	5.2 \pm 0.6	6.4 \pm 0.6	6.2 \pm 0.8	6.2 \pm 0.7*
All supplemented subjs	14	6.2 \pm 1.1	5.6 \pm 0.8	6.0 \pm 0.9	7.2 \pm 0.8	6.6 \pm 1.1	6.8 \pm 1.1†

a: Values (means \pm SD) are replicate measurements (2-wk interval) and means of both measurements before and after end of intervention. SCE, sister chromatid exchange; PBLs, peripheral blood lymphocytes.

b: Statistical significance is as follows: significantly different from before intervention: *, $p < 0.05$; †, $p < 0.02$ (Wilcoxon signed rank test).

Table 3. MN Frequencies in PBLs Before and After Intervention^a

Nicotinic Acid Intake, mg/day	n	MN/1,000 Binucleated Cells					
		Before intervention			After intervention		
		First	Second	Mean	First	Second	Mean
0	6	5.7 ± 3.4	4.2 ± 1.8	4.8 ± 1.7	5.8 ± 4.8	6.5 ± 3.3	6.2 ± 2.6
25	3	6.0 ± 3.6	6.3 ± 3.2	6.0 ± 1.0	5.7 ± 2.3	6.7 ± 2.1	6.2 ± 1.8
50	6	8.0 ± 2.0	5.2 ± 3.1	6.6 ± 2.2	6.3 ± 2.7	5.8 ± 3.4	6.1 ± 2.9
100	5	4.0 ± 2.2	4.0 ± 1.4	4.0 ± 1.6	3.0 ± 3.3	6.4 ± 4.0	4.7 ± 1.6
All supplemented subjs	14	6.1 ± 2.9	5.0 ± 2.6	5.5 ± 2.1	5.0 ± 3.1	6.2 ± 3.2	5.6 ± 2.2

a: Values (means ± SD) are replicate measurements (2-wk interval) and means of both measurements before and after end of intervention. MN, micronuclei.

Table 4. VFs in PBLs Before and After Intervention^{a,b}

Nicotinic Acid Intake, mg/day	n	VF, ×10 ⁻⁶					
		Before intervention			After intervention		
		First	Second	Mean	First	Second	Mean
0	6	7 ± 6	13 ± 12	9 ± 7	5 ± 5	3 ± 3	3 ± 2
25	3	18 ± 24	3 ± 2	11 ± 11	3 ± 2	18 ± 12	11 ± 7
50	5 ^c	7 ± 10	8 ± 14	8 ± 12	25 ± 6	15 ± 16	19 ± 12
100	5	7 ± 4	21 ± 15	11 ± 6	2 ± 1	7 ± 6	5 ± 3*
All supplemented subjs	13	9 ± 13	10 ± 13	10 ± 9	10 ± 12	12 ± 12	11 ± 10

a: Values (means ± SD) are replicate measurements (2-wk interval) and means of both measurements before and after intervention. VFs, variant frequencies.

b: Statistical significance is as follows: *, significantly different from before intervention ($p < 0.05$, Wilcoxon signed rank test).

c: Cell cultures of 1 subject showed labeling indexes <5%, indicating poor reliability of estimation of VFs (17); these data were excluded from analyses of VFs.

nicotinic acid in the highest status group ($n = 4$) was without effect on niacin status, since lymphocyte NAD⁺ was slightly decreased (-0.65×10^{-2} pmol/mg protein). It is concluded that supplementation with nicotinic acid appears to be effective only in individuals with a relatively low niacin status. Weitberg (4) reported a more pronounced increase in lymphocyte NAD⁺ after supplementation of two healthy men with nicotinic acid at 100 mg/day (from 4 to 23 pmol/10⁶ cells). In our study, much higher initial levels of NAD⁺ were measured (31–108 pmol/10⁶ cells), which are comparable to concentrations found by others in lymphocytes (22). The more pronounced increase in lymphocyte NAD⁺ reported by Weitberg may therefore be ascribed to the relatively low initial niacin status of their subjects. Our data also suggest that the intracellular NAD⁺ concentration most likely is homeostatically controlled once a critical concentration is reached (our data indicate that this concentration is approx 11–12 × 10⁻² pmol/mg protein).

The efficacy of supplemental nicotinic acid to increase cellular NAD⁺ concentrations appeared to be dependent on the initial niacin status and did not lead to an improved status in all supplemented subjects. Therefore, we performed additional analyses to evaluate whether changes in intracellular NAD⁺ were related to effects on cytogenetic damage and DNA repair in PBLs. We did not observe significant associations between changes in intracellular NAD⁺ and effects on parameters of cytogenetic damage or poly(ADP-ribosylation) in PBLs. This is in contrast to results of Weitberg (4), who reported that PBLs of two subjects were less sen-

sitive to *ex vivo* reactive oxygen-induced DNA strand breaks after eight weeks of supplementation with nicotinic acid at 100 mg/day. This was attributed to enhanced poly(ADP-ribosylation) and repair of oxidative lesions in DNA and appears to be in agreement with the role of PARP in base excision repair of oxidative DNA lesions (6).

Previously, we observed that PARP is activated on treatment of PBLs with (±)-*anti*-BPDE (15). Because cigarette smoke contains numerous genotoxic substances, including reactive oxygen species-producing compounds and polycyclic aromatic hydrocarbons (23,24), a reduction in cigarette smoke-associated cytogenetic damage was expected after improvement of the niacin status of the volunteers in our study. No clear effects, however, were observed for cigarette smoke-induced MN and VFs in PBLs. In addition, an increase in niacin status in supplemented subjects was not accompanied by changes in MN or VFs, since in those subjects who responded to supplemental niacin, defined as an increase in lymphocyte NAD⁺ >2.0 × 10⁻² pmol/mg protein ($n = 6$), no significant changes were found in MN or VFs. Nor was (±)-*anti*-BPDE-induced poly(ADP-ribosylation) enhanced in relation to an increased niacin status.

SCE frequencies showed a tendency to be increased in all supplemented groups. In addition, increases in SCE frequencies were positively associated with the daily dose of nicotinic acid supplementation and with individual increases in blood nicotinamide concentrations ($r = 0.51$, $p < 0.05$; $r = 0.33$, $p = \text{NS}$, respectively, Spearman rank correlation test). This enhancing effect of supplementation on SCE frequencies may

be explained by the inhibiting effect of nicotinamide on PARP. Inhibition of PARP by nicotinamide, which is a noncompetitive inhibitor (5), has been reported to result in increased SCE frequencies *in vitro* (20,21). We were able to obtain data on (\pm)-*anti*-BPDE-induced poly(ADP-ribosylation) for 12 subjects only, which is not sufficient to draw conclusions with respect to the effect of nicotinic acid supplementation on poly(ADP-ribosylation). Data obtained for these 12 subjects do not indicate that poly(ADP-ribosylation) is greatly altered after supplementation with nicotinic acid.

VFs in PBLs were decreased after supplementation with nicotinic acid at 100 mg/day, but none of the other supplemented groups showed decreased VFs. VFs showed large variation between replicate measurements (Table 4). Moreover, the reduction in VFs found for the group receiving 100 mg/day was similar to the reduction observed for the placebo group and small compared with the variation observed for replicate measurements. Therefore, the results obtained in this study for VFs are considered less reliable. Although MN frequencies were less prone to intraindividual variation, no effect of supplementation with nicotinic acid was found for MN induction. It appears that, in addition to exposure to cigarette smoke-related genotoxic agents, other unknown endogenous or exogenous factors determine the occurrence of MN frequencies and VFs. These factors appear to exert stronger effects on MN frequencies and VFs in PBLs than changes in niacin status of the subjects in our study.

In our study, no consistent results were found for the effect of supplementation of healthy human subjects with nicotinic acid on poly(ADP-ribosylation), cytogenetic damage, and consequently genetic risk. Recent experimental studies with rats also yielded equivocal results with respect to the effect of niacin deficiency on poly(ADP-ribosylation) and DNA damage. Zhang and co-workers (10) reported an increased PARP activity in niacin-deficient rats with low liver NAD⁺ concentrations, which was accompanied by an increased sensitivity to *ex vivo*-induced oxygen free radical-induced DNA strand breaks. On the other hand, Rawling and colleagues (9,25) reported lowered tissue NAD⁺ and ADP-ribose polymer concentrations in the liver of niacin-deficient rats, but treatment with diethylnitrosamine enhanced hepatic accumulation of ADP-ribose polymers, finally resulting in similar frequencies of altered hepatic foci, a preneoplastic lesion, compared with controls. The results of these animal studies as well as data obtained from our intervention study with human subjects suggest that niacin deficiency or supplemental niacin during prolonged periods may have minor effects on metabolism of poly(ADP-ribose) after carcinogen exposure. Results obtained until now indicate that an improved niacin status does not appear to contribute significantly to a reduced genetic and cancer risk.

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