Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 19 (2008) 746 – 753
Journal of Nutritional Biochemistry

Niacin deficiency causes oxidative stress in rat bone marrow cells but not through decreased NADPH or glutathione status☆

Kitty Tang, Heidi Sham, Evon Hui, James B. Kirkland*
Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1
Received 27 April 2007; received in revised form 4 September 2007; accepted 5 October 2007

Abstract

Niacin (vitamin B_3), in the form of NADPH, is required for the regeneration of glutathione (GSH), which is the substrate of GSH peroxidase. In this study, we examined the effect of dietary niacin deficiency on protein and DNA oxidation in bone marrow cells of Long-Evans rats. Western blotting was used to measure 2,4-dinitrophenylhydrazine-reactive protein carbonyl products, and the Biotrin OxyDNA method was used to measure 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG). The levels of both protein carbonyls and 8-oxodG were increased by 50% in niacin-deficient bone marrow cells. To examine whether this oxidant damage involves altered metabolism of pyridine nucleotides and glutathione, both oxidized and reduced forms of pyridine nucleotides (NAD^+, NADH, $NADP^+$, NADPH) and glutathione (GSSG and GSH) were quantified in total and nucleated bone marrow cells. NAD and $NADP^+$ levels were decreased 80% and 22%, respectively, by niacin deficiency. NADPH and GSH were not depleted by niacin deficiency, showing that oxidant injury was not due directly to impairment of this pathway. Oxidative stress, of uncertain etiology, may play a role in the observed genomic instability and sensitivity to leukemogenesis in bone marrow cells during niacin deficiency.

Keywords: Niacin; DNA; Protein; Oxidative stress; NADPH; Glutathione; Rat; Bone marrow

1. Introduction

Oxidative stress refers to a shift in the balance of reactive oxygen species (ROS) versus the endogenous defense systems which handle these metabolites. Many factors can impair defenses, including a poor supply of reducing cofactors or low expression or activity of protective enzymes. Enhanced oxidation may result from stresses such as irradiation, inflammation or xenobiotic metabolism. ROS oxidize cellular macromolecules such as DNA, proteins and lipids to induce cell damage, which may encourage many degenerative conditions, including neurological disorders, cancer, cardiovascular disease, rheumatoid arthritis, lupus and the acceleration of the aging process. Nutritional status has a large impact on oxidant defense; deficiencies of vitamin E [1], vitamin C [2], selenium [3] and sulfur amino acids [4] are known to enhance oxidant stress-related pathologies.

Reduced and oxidized nicotinamide adenine dinucleotide (NADH, NAD^+) and nicotinamide adenine dinucleotide phosphate (NADPH, $NADP^+$) are essential coenzymes throughout metabolism, and they participate with a number of enzymes in oxidant defense and xenobiotic detoxification pathways. In addition, it has been shown that NADH and NADPH are able to directly scavenge oxidants like hypohalous acids, produced during inflammation [5]. However, the most significant role for NADPH in oxidant defense is through the regeneration of reduced glutathione (GSH), which is required for the metabolism of hydrogen peroxide and fatty acid hydroperoxides by GSH peroxidases. NAD(P)H quinone-oxidoreductase may exert a protective effect against redox cycling of xenobiotics and oxidative stress [6–8], and its activity is also NADPH-dependent. Conversely, other enzymes use NADH or NADPH to generate ROS to be used in bacterial killing, oxidative reactions or cell signaling events [9].

☆ This work was supported by the National Cancer Institute of Canada and the Cancer Research Society.
* Corresponding author. Tel.: +1 519 824 4120x56693; fax: +1 519 763 4381.
E-mail address: jkirklan@uoguelph.ca (J.B. Kirkland).

0955-2863/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2007.10.003

Human cancer patients are frequently niacin-deficient and are faced with extreme exposures to genotoxic drugs which damage bone marrow cells, leading to myelosuppression and the long-term development of secondary cancers [10]. In our animal model, niacin deficiency causes a large decrease in bone marrow NAD^+ and poly(adenosine diphosphate ribose) (poly(ADP-ribose)) [11,12], dramatic genomic instability [12,13] and increased myelosuppresion [14] and leukemogenesis [15]. From a mechanistic perspective, we have shown that niacin deficiency causes delayed repair of single-strand breaks [13], accumulation of double-strand breaks [13], increased recombination [12], increased chromatid and chromosome breaks [13] and deregulation of p53 expression, apoptosis and cell cycle arrest [16].

These findings have been logical with respect to the roles of poly(ADP-ribose) formation in DNA repair, chromosome sorting and apoptosis [17]. At the same time, reviewers have suggested that niacin deficiency may impair oxidant defense, leading to DNA damage that could play a role in the sensitivity to bone marrow suppression and leukemogenesis observed during niacin deficiency. In the current studies, we have investigated whether niacin deficient animals demonstrate signs of increased oxidative stress in cellular DNA and proteins. We measured the levels of protein carbonyl groups and 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) in the bone marrow cells of Long-Evans rats. On finding evidence of niacin deficiency-induced oxidant stress in these end points, we have characterized the status of all pyridine nucleotide pools, and reduced and oxidized glutathione levels, and found that the accumulation of oxidant damage cannot be explained by impaired NADPH or GSH pools.

2. Materials and methods

2.1. Chemicals

Reagents for gel electrophoresis were purchased from BioRad Laboratories (Mississauga, Canada). The Pierce BCA Protein Assay Kit was obtained from MJS Biolynx (Brockville, Ontario), while oxidized GSH (GSSG) and 2-vinylpyridine were purchased from Fisher Scientific (Ottawa, Ontario). All other chemicals were purchased from Sigma-Aldrich Canada (Oakville, Ontario).

2.2. Animal model

The use of animals in this experiment was approved by the University of Guelph Animal Care Committee, and animal treatment was in accordance with the guidelines of the Canadian Council on Animal Care. Weanling male Long-Evans rats (Charles River Canada, St. Constant, QC, Canada), weighing between 35 and 50 g, were matched by weight and assigned to either niacin-deficient (ND) or pair-fed (PF) control groups. The ND and niacin-replete PF diets were identical except that PF diet contained 30 mg of added niacin/kg of diet (United States Biochemical, Cleveland, OH, USA). The dietary protein was composed of a mixture of 7% casein and 6% gelatin. The proportion of casein to gelatin was required to limit the content of tryptophan, which is the substrate for an alternate pathway for NAD^+ synthesis [17]. The ND rats were fed ad libitum, while the PF rats received the same quantity of diet that their ND counterpart consumed in the previous day. The animals were maintained on experimental diets for 3 weeks, housed individually in suspended wire-bottomed cages and given free access to water with a 12-h light cycle. Their body weight was monitored twice weekly. After 3 weeks, rats were anaesthetized with isoflurane (Baxter, Mississauga, Ontario, Canada) and decapitated. Bone marrow was collected from both femurs and tibias using phosphate-buffered saline (PBS), and total and nucleated cells were counted using a Z2 model Coulter cell counter (Beckman Coulter, Miami, FL, USA).

2.3. Immunoblotting of 2,4-dinitrophenylhydrazine-reactive carbonyl groups

Bone marrow cells were analyzed for levels of protein carbonyls [18], a biomarker of oxidative protein damage [19]. A 2-ml aliquot of bone marrow suspension (total cells, 25×10^6 cells/ml) was pelleted and resuspended in lysing buffer containing leuprotinin (10 μg/L) and aprotinin (10 μg/L). An equal volume of sodium dodecyl sulfate (SDS) (12%) was added and then two volumes of 10 mM 2,4-dinitrophenylhydrazine (DNPH) dissolved in 2M HCl, followed by a 30-min incubation at room temperature, was neutralized with 1 N NaOH and 1% SDS in 100 mM Tris (pH 7.4) to constitute a final volume of 300 μl. Protein content was determined using the Pierce BCA Protein Assay Kit.

Equal quantities of protein (5 μg per lane) were separated on 8% gradient SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gels and electrophoresed overnight at 25 V. Proteins were then transferred to nitrocellulose membrane filters (Hybond-C pure, Amersham, Baie d'Urse, QC, Canada) for 2 h at 200 mA. Membranes were blocked for 1 h in PBS with 3% skim milk powder with 0.05% Tween (PBSMT) and then incubated with primary antibody (anti-DNPH, Sigma-Aldrich) at 1:8000 dilution in PBSMT for another h. Membranes were washed with PBS with 0.05% Tween and incubated with secondary antibody (goat antirabbit-horse radish peroxidase [HRP], IgG, Sigma-Aldrich) at a 1:20000 dilution in PBSMT for 1 h. Membranes were washed again and treated with ECL+ (Mandel Scientific, Guelph, Canada) and exposed to film for 3 min. All washes and incubations were conducted at room temperature. Protein carbonyl smears were quantified using thresholding functions of Northern Exposure densitometry software, version 6.0. Secondary antibody alone did not cause a significant signal on these blots. Treatment of freshly isolated bone marrow cells with 50-μM cumene hydroperoxide for 30 min caused a doubling of the densitometry signal using the above method.

2.4. 8-oxodG analysis using Biotrin OxyDNA kit and flow cytometry

An aliquot of bone marrow suspension was used to determine oxidative DNA damage in the form of 8-oxodG. This biomarker was quantified using a commercial kit (OxyDNA, Biotrin, BD Biosciences, Mississauga, Ontario, Canada), based on the high affinity binding of avidin to 8-oxodG lesions in DNA [20,21]. This binding appears to be due to a fortuitous similarity between the structures of oxidatively modified guanine and biotin (the physiological ligand) [20]. Avidin-fluorescein isothicyanate (FITC) has been shown to specifically illuminate the nuclei of cultured cells following hydrogen peroxide exposure, and this was effectively competed by biotin and 8-oxodG, but not guanine [21].

The kit protocol was modified to improve baseline accuracy. All bone marrow cell suspensions were divided into two 2-ml samples containing 50×10^6 cells, only one of which was treated with the active probe. Samples were washed extensively with PBS, fixed by addition of 2% paraformaldehyde for 15 min, washed twice in PBS, then fixed and permeabilized with ice-cold 70% ethanol and stored at −20°C until ready to be analyzed. Thawed samples were centrifuged and washed with PBS and wash solution provided in the Biotrin OxyDNA kit. Subsequently, blocking solution was prepared, and samples were incubated for 1 h at 37°C. After two additional washings of the cell suspension, one of the samples was incubated with avidin-FITC solution for 1 h in the dark. Samples were then centrifuged twice with wash solution, followed by washing with PBS. Propidium iodide dye (10 μg/ml) was added to differentiate cell cycle phases (G1, G2). Tubes without avidin-FITC served as blanks, and the difference in fluorescence was interpreted as 8-oxodG content. We have found problems in the past with autofluorescence in bone marrow cells, and the nonprobe blank is an important addition to this method. Treatment of freshly isolated bone marrow cells for 10 min with 400 μM cumene hydroperoxide caused a 30% increase in fluorescence.

2.5. Determination of pyridine nucleotide levels

Bone marrow cell suspensions were divided into aliquots for the following: (*a*) acid extraction, (*b*) alkaline extraction, (*c*) GSSG analysis, (*d*) determination of total GSH and (*e*) red blood cell lysis (to which five volumes of RBC lysis buffer (150 mM NH4Cl, 10 mM KHCO3, 0.1 mM Na2-EDTA) were added and the lysis suspension was divided as above). This generated a total of eight fractions from each rat, used to determine 12 end points. Since mature erythrocytes lack mitochondria and are more susceptible to oxidant stress (due to the presence of hemoglobin and high concentrations of oxygen) [22,23], we wanted to see if the cellular differences would affect the metabolism of pyridine nucleotides and GSH. Thus, we performed the same assays on total bone marrow cells and nucleated bone marrow cells. Pyridine nucleotide levels were measured by the enzymatic recycling methods of Shah et al. [24] and Jacobson and Jacobson [25] with slight modifications. PCA (1 M) or sodium hydroxide (0.05 M containing 0.5 mM cysteine) was added to the bone marrow cell pellet to extract the oxidized and the reduced pyridine nucleotides, respectively, and stored at −80°C. Alkaline extracts were incubated at 60°C for 5 min to destroy NAD^+ and $NADP^+$. The acidic extracts were neutralized to around pH 6.8 with potassium phosphate buffer (2 M KOH and 0.33 M KH_2PO_4), and the alkaline extracts were neutralized to around pH 7.2 with 0.02 M H_3PO_4. β-NAD^+, and β-$NADP^+$ was used to generate a standard curve for the analysis of NAD^+/H and $NADP^+$/H, respectively. Standards and samples (50 μl per well) were aliquoted into a 96-well plate, followed by assay cocktail [100 μl per well; NAD^+/H assay: 0.1 M bicine, 0.5 M ethanol, 4.17 M EDTA, 0.83 mg/ml bovine serum albumin (BSA), 0.42 mM 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 1.66 mM phenazine ethosulfate (PES); $NADP^+$/H assay: 0.1 M bicine, 10 mM $MgCl_2$, 10 mM DL-isocitrate, 0.83 mg/ml BSA, 0.42 mM MTT and 1.66 mM PES] and enzyme (20 μl per well; NAD^+/H assay: 0.1 M alcohol dehydrogenase; $NADP^+$/H assay: 10 mM isocitrate dehydrogenase). Absorbances of the colorimetric end products were measured at 540 nm for NAD^+ and NADH or 570 nm for $NADP^+$ and NADPH with a spectrophotometer. The concentration of various pyridine nucleotides was calculated based on the standard curve with a correlation coefficient of $R^2>0.99$.

2.6. Glutathione assay

Total GSH and GSSG levels were assayed by a modification of the methods of Anderson [26] and Griffith [27], using 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) as a substrate. Addition of 2-vinylpyridine removes GSH, thereby allowing the total GSH and GSSG levels to be determined using the same enzymatic recycling method, after which GSH is derived by subtraction. Aliquots of the original bone marrow cell suspension and lysis suspension were centrifuged, and the cell pellets were obtained. Proteins were precipitated by the addition of 5-sulfosalicylic acid (SSA), and the SSA supernatant was stored at −80°C. SSA supernatants and GSSG standards were partially neutralized to around pH 6.8 with triethanolamine. 2-vinylpyridine (1 M) was added at 1:100 for derivatization of GSH and determination of GSSG, while ethanol was added to samples for total GSH analysis and incubated for 1 h at room temperature. GSSG standards and samples (20 μl per well) were assayed in a 96-well plate. The DTNB-GSH reductase cycling was initiated by the addition of the assay cocktail (185 μl per well) made up of 0.248 mg/ml NADPH, 6 mM DTNB and 120 U/ml GSH reductase. The reaction was monitored at 405 nm spectrophotometrically. The total GSH and GSSG content in the samples were

calculated from the standard curve, which had a correlation coefficient of R^2>0.99.

2.7. Statistical analysis

All data were expressed as mean±S.E.M. Significant differences were determined by Student *t* test; *P*<.05 was considered significant.

3. Results

3.1. Niacin deficiency increases the levels of protein carbonyls

Fig. 1B shows an example of a Western blot of bone marrow cell proteins treated with DNPH and illuminated with anti-DNPH antibodies. Equal quantities of protein were loaded in each lane, and Fast Green staining of membranes for total protein appeared identical between PF and ND samples. In preliminary experiments, we found that treatment of freshly isolated bone marrow cells with 50 μM cumene hydroperoxide for 30 min caused a doubling of the anti-DNPH signal density. In the analysis of bone marrow from the experimental diet groups, PF lanes were consistently lighter than ND lanes. There are two stronger bands in the lower molecular weights range in the ND that seem reproducible, but the increased intensity of immunoreactivity occurs across the range of molecular weights. Optical densitometry was used to quantify the intensity of the lanes. Compiled densitometry data showed that immunoreactive protein carbonyl levels were 50% higher in animals on ND diets compared to animals on PF diets (Fig. 1A).

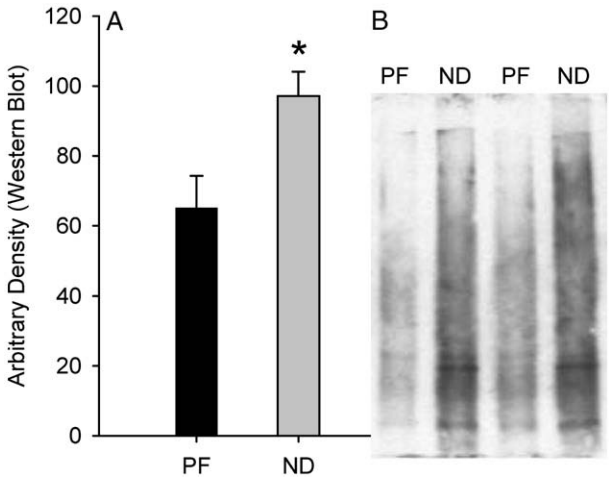

Fig. 1. Effect of niacin deficiency on total protein carbonyls in rat bone marrow cells. (A) Densitometric analysis. Bone marrow protein carbonyls were derivatized with DNPH, separated via SDS-PAGE and quantified by Western immunoblotting using antibodies against DNPH. Oxidized protein bands were visualized with ECL+, then quantified using thresholding functions of Northern Exposure densitometry software (version 6.0). *Significant differences from PF values (*P*<.05, *t* test, one-tailed). Values are means±S.E.M. (*n*=13). (B) An example of a Western blot.

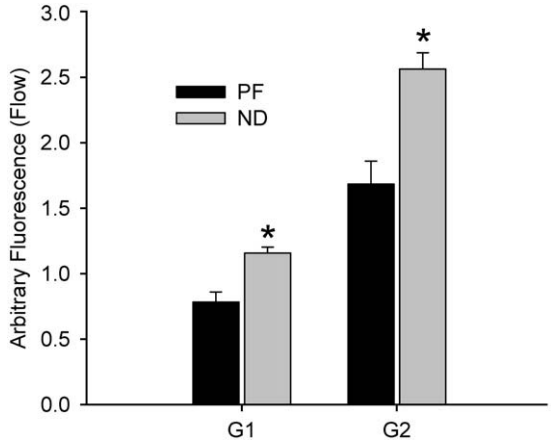

Fig. 2. Effect of niacin deficiency on 8-oxydeoxyguanosine (8-oxodG) in rat bone marrow cells. DNA damage was quantified using an avidin-FITC conjugate, quantified by flow cytometry. Propidium iodide staining was used to differentiate cell cycle phases. Parallel samples without avidin-FITC were used to correct for autofluorescence. As expected, FITC fluorescence, or 8-oxodG levels, were twice as high in G2 versus G1 cells. *Significant differences from PF values (*P*<.05, *t* test, 1-tailed). Values are means±S.E.M. (*n*=13).

3.2. Niacin deficiency increases the levels of 8-oxodG

Levels of 8-oxodG were measured as an indicator of oxygen radical-induced DNA oxidative damage using flow cytometry (Fig. 2). PI staining was used to separate G1 and G2 cell populations. Each bone marrow sample was divided equally, and half was treated with avidin-FITC reagent. Avidin binds with high affinity to 8-oxodG residues in DNA [20,21]. In preliminary experiments, we found that treatment of freshly isolated bone marrow cells for 10 min with 400 μM cumene hydroperoxide caused a 30% increase in fluorescence. Following subtraction for nonspecific autofluorescence in each sample, ND treatment increased FITC fluorescence in G1 cells by 48% and in G2 cells by 50%. The extent of increase was similar in both G1 and G2 cells, but the arbitrary measure of 8-oxodG was twice as high in G2 vs. G1 cells, reflecting the 2-fold increase in DNA content.

3.3. Effect of niacin status on pyridine nucleotide metabolism

Niacin deficiency decreased NAD^+ levels by about 80% in both the total (Fig. 3A) and nucleated bone marrow cell populations (Fig. 3B). The data were consistent with our previous findings [8] and confirmed the niacin-deficient status of the animals. Despite the large decrease in NAD^+, NADH levels were not significantly decreased by niacin deficiency in total or nucleated cell populations (Fig. 3). NADP pools are, as expected, predominantly in the reduced form (NADPH) (Fig. 4). The only significant change in oxidized and reduced pools in either cell fraction is a 22% decrease in $NADP^+$ in total cells (Fig. 4A).

3.4. Effect of niacin status on GSH metabolism

There was no significant effect of niacin deficiency on total GSH levels in total or nucleated bone marrow

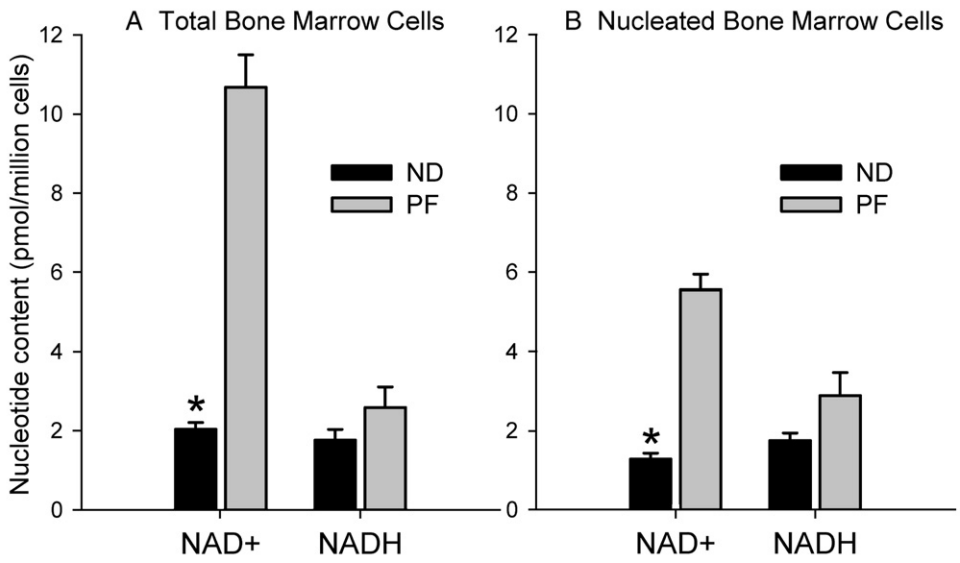

Fig. 3. Effect of niacin deficiency on bone marrow NAD^+ and NADH levels. Pyridine nucleotide contents were measured via enzymatic cycling. Levels of NAD^+ and NADH in total bone marrow cells (A) and nucleated bone marrow cells (B). *Significant differences from PF values ($P<.05$, t test, 2-tailed). Values are means±S.E.M. ($n=12$).

populations (Fig. 5, sum of GSH and GSSG bars). GSH levels were unaffected by diet in either cell population. GSSG in the total cell population decreased by 26% in the ND group, while GSSG in the nucleated cell population was unaffected.

4. Discussion

This study has shown that niacin deficiency causes the accumulation of oxidative damage to protein and DNA components of bone marrow cells. It is hard to know if this is due to enhanced formation or impaired clearance of ROS, or decreased repair of damaged proteins and DNA. Similar findings were documented by Arun et al. [28], who found that nicotinic acid supplementation of stored whole blood reduced the levels of lipid peroxidation products and protected vitamin E pools. The studies described in this paper showed that dietary niacin deficiency also causes oxidant stress in vivo in a tissue that has been shown to be sensitive to niacin status [11,12]. Other investigators working in vivo have shown that niacin supplementation can decrease the levels of oxidant stress associated with alcoholic pellagra in humans [29] and oxidant stress induced by paraquat in rats [30].

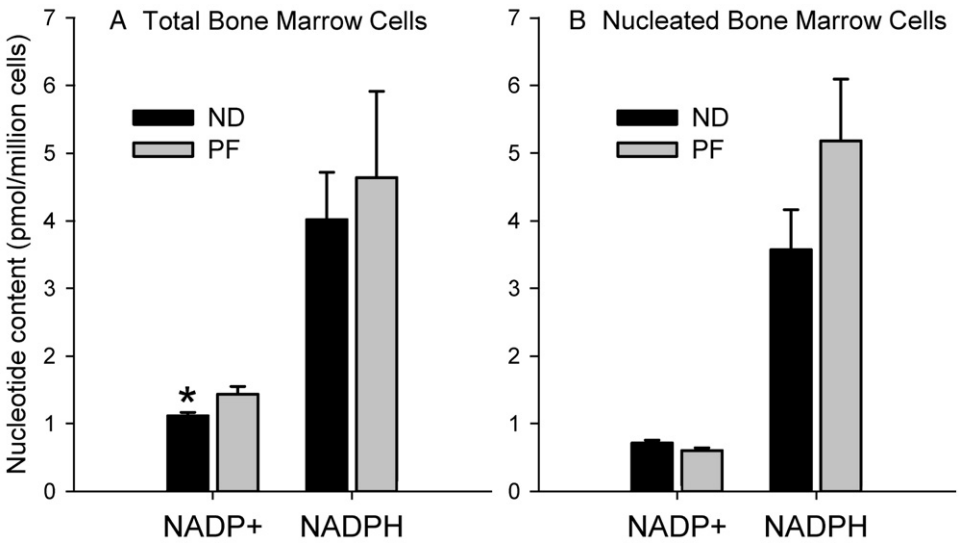

Fig. 4. Effect of niacin deficiency on bone marrow $NADP^+$ and NADPH levels. Pyridine nucleotide contents were measured via enzymatic cycling. Levels of $NADP^+$ and NADPH in total bone marrow cells (A) and nucleated bone marrow cells (B). *Significant differences from PF values ($P<.05$, t test, 2-tailed). Values are means±S.E.M. ($n=12$).

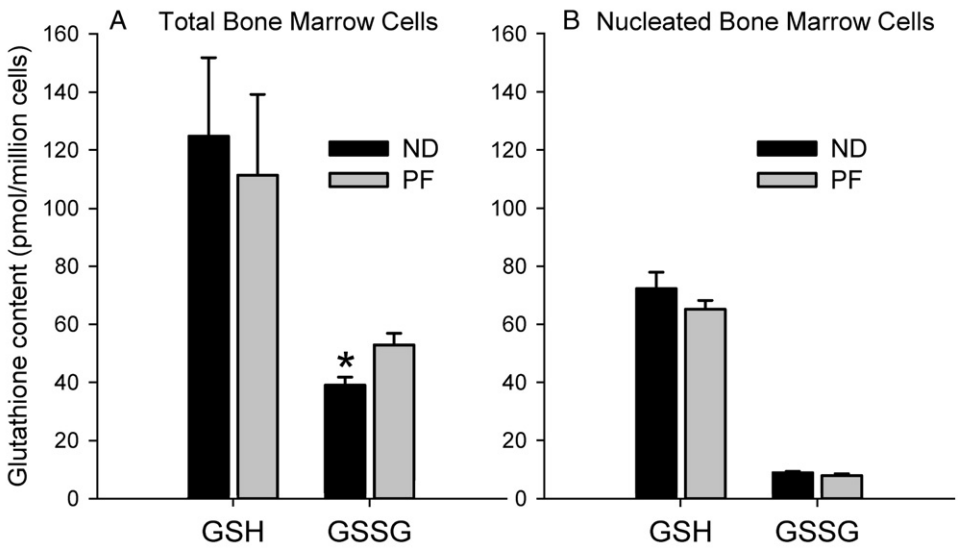

Fig. 5. Effect of niacin deficiency on total GSH and GSSG levels in rat bone marrow cells. Total GSH and GSSG were quantified by enzymatic cycling. Levels of total GSH and GSSG in total bone marrow cells (A) (n=11) and nucleated bone marrow cells (n=12). *Significant differences from PF values (P<.05, t test, 2-tailed). Values are means±S.E.M.

In past discussions of niacin status and oxidant stress, it has been suggested or assumed that oxidant stress is impacted during niacin deficiency by the ability to regenerate GSH through the use of NADPH in the glutathione reductase reaction. Thus, in the second part of the study, we examined the levels of $NADP^+$/NADPH and GSSG/GSH. Despite the dramatic depletion in NAD^+, levels of NADH and NADPH were not affected by niacin deficiency. Deficient bone marrow cells were able to adjust for the limitation in NAD^+ and maintain essential redox functions. Given that NADH is the direct source of most of the reducing equivalents for the electron transport chain, the ability to maintain NADH suggests that aerobic cellular energy production would not be compromised during niacin deficiency. Similarly, it has been shown that cultured cells continue to divide at a normal rate with NAD^+ depletions of up to 90%, as long as they are not exposed to stresses like DNA damage [31,32], which further deplete NAD^+, and require cellular responses regulated by ADP-ribosylation reactions. It has been shown that poly(ADP-ribose) formation is significantly impaired in our in vivo bone marrow model [11] and in cell culture models on niacin deficiency [32].

Despite an 80% decrease in NAD^+, NADPH was adequately maintained by the pentose phosphate pathway, providing sufficient substrate for GSH regeneration in the GSH reductase reaction. This is supported by the lack of any decrease in GSH during niacin deficiency. The measurements of GSSG appear to be somewhat elevated, especially in the total cell population. It is recognized that the oxidative environment of red blood cells makes these extracts prone to conversion of GSH to GSSG during processing, and this could have been minimized through some changes in methodology [33], but the period of anoxia during the recovery of bone marrow cells may be hard to fully overcome. However, the values in the nucleated cell population are more relevant to leukemogenesis, and the loss of GSH in these samples appears to be minimal. None of the NADPH, GSH or GSSG measurements in either population indicate that niacin deficiency could be limiting GSH regeneration. Taken together, these results showed that the reductive roles in oxidant defense were not compromised and the increased level of oxidant stress in niacin-deficient bone marrow cells resulted from other events.

The NAD and NADP pools represent redox couples, and the electronegativity of these can be estimated using the Nernst equation, as described by Schafer and Buettner [34]. The half-cell reduction potentials of the NAD and NADP couples are shown in Table 1. It should be noted that the whole cell data used for these Nernst calculations does not reflect bound to free fractions and subcellular localization, which may lead to significant differences in the true functionality of these couples. As expected, the NAD couples are less electronegative than the NADP couples.

Table 1
Half-cell reduction potentials (E_{hc}) of NAD and NADP couples

Cell population/diet	Half-cell reduction potential of NADH/NAD^+	Half-cell reduction potential of NADPH/$NADP^+$
Total cells		
PF	−298	−330
ND	−314	−331
Nucleated cells		
PF	−308	−343
ND	−320	−336

E_{hc} values are derived from the Nernst Equation, $E_{hc} = E°' - (59.1/2)$ log ([reduced nucleotide]/[oxidized nucleotide]) assuming $E°' = -316$ mV for NAD and −315 mV for NADP couples as described by Schafer and Buettner (2001).

Of interest, the major impact of niacin deficiency is to cause a substantial increase in the electronegativity of the NAD couple due to the depletion of NAD^+. As noted in the results, and seen in E_{hc} values in Table 1, there was no corresponding shift in the NADP couples. It is possible that some of the effects of niacin deficiency are not due to limitations in NAD^+ per se but rather due to the shift in electronegativity of this couple, which could affect the regulation of many cellular processes. In addition to controlling the activity of redox sensitive enzymes, the NAD^+/NADH ratio can regulate the activity of various transcription factors, leading to changes in gene expression. This includes transcription factors that regulate circadian rhythms [35] and repressor proteins involved in the control of cell growth and differentiation [36]. Redox state may directly control the expression of genes involved in DNA repair [37], raising the possibility that, during niacin deficiency, oxidant stress could be constant but lesion removal impaired. We have recently examined the impact of niacin deficiency on DNA excision repair in response to alkylation damage [13]. In these experiments, we found that niacin deficiency did not affect lesion removal or incision steps of the repair process but delayed repair during either the patch synthesis or ligation steps. This fits well with the knowledge that catalytically inactive poly(ADP-ribose) polymerase 1 and 2 tend to bind to strand breaks and block the repair process, and this appears to take place in our bone marrow cells during niacin deficiency, due to the lack of available NAD^+ [13]. Oxidative DNA lesions are removed by a similar process of excision repair, and our data on alkylation injury does not support the idea that niacin deficiency leads to an accumulation of damaged bases by inhibiting DNA glycosylase function.

The GSH/GSSG couple is very influential in controlling the configuration of cellular proteins through the reduction state of protein thiols (cysteine side chains). This includes the activity of several important transcription factors that regulate a variety of stress responses [34]. However, there were no diet-induced changes in GSH or GSSG concentrations in the nucleated cell population that would indicate that shifts in thiol-dependent transcriptional activity are a factor in niacin deficiency.

There are many other possible mechanisms by which niacin deficiency could cause accumulation of oxidant damage in cells. The tumor suppressor protein p53 directs cell cycle arrest, DNA repair and apoptosis. We have shown that its expression is significantly altered during niacin deficiency and that cell cycle arrest and apoptosis are impaired [16]. This could allow cells with an accumulation of oxidant injury to survive when they would normally be removed by apoptosis. The sirtuins are NAD-dependent deacetylases that regulate p53 function and the compaction of chromatin structure [38]. Under low NAD conditions, histones may become hyperacetylated, and DNA may be more open to oxidant damage. In addition to the possible effects of the NAD redox state on gene expression, there are a myriad of potential cell signaling events controlled by cyclic ADP-ribose formation and mono ADP-ribosylation reactions that could lead to changes in gene expression and the eventual expression of a genomically unstable, nonapoptotic phenotype [17,39]. One intriguing connection that could bring these together is enhanced expression of NADPH oxidases (Nox), which produce reactive oxygen in cells, controlling many aspects of cell function. Of interest, enhanced Nox activity has been shown to enhance cell division and inhibit apoptosis [40], while inducing oxidant damage to cellular constituents, producing an oncogenic pattern similar to what we observe in niacin deficient bone marrow cells.

In this study, we have demonstrated that increased oxidant damage to DNA and protein occurs in niacin deficient bone marrow but is not a result of decreased NADPH or GSH levels. The source of imbalance between oxidant stress and defense in niacin deficient bone marrow is of uncertain etiology, and defining this may help to explain the genomic instability and sensitivity of these cells to leukemogenesis.

References

[1] Sen CK, Khanna S, Roy S. Tocotrienols: vitamin E beyond tocopherols. Life Sci 2006;78:2088–98.
[2] Burk RF, Christensen JM, Maguire MJ, Austin LM, Whetsell Jr WO, May JM, et al. A combined deficiency of vitamins E and C causes severe central nervous system damage in guinea pigs. J Nutr 2006;136:1576–81.
[3] Thomson CD. Assessment of requirements for selenium and adequacy of selenium status: a review. Eur J Clin Nutr 2004;58:391–402.
[4] Mathers J, Fraser JA, McMahon M, Saunders RD, Hayes JD, McLellan LI. Antioxidant and cytoprotective responses to redox stress. Biochem Soc Symp 2004:157–76.
[5] Prutz WA, Kissner R, Koppenol WH, Ruegger H. On the irreversible destruction of reduced nicotinamide nucleotides by hypohalous acids. Arch Biochem Biophys 2000;380:181–91.
[6] Tampo Y, Yonaha M. Enzymatic and molecular aspects of the antioxidant effect of menadione in hepatic microsomes. Arch Biochem Biophys 1996;334:163–74.
[7] Kappus H, Sies H. Toxic drug effects associated with oxygen metabolism: redox cycling and lipid peroxidation. Experientia 1981; 37:1233–41.
[8] Zhang L, Smith MT, Bandy B, Tamaki SJ, Davison AJ. Role of quinones, active oxygen species and metals in the genotoxicity of 1,2,4-benzenetriol, a metabolite of benzene. In: Nohl H, Esterbauer H, Rice-Evans C, editors. Free Radicals in the Environment, Medicine and Toxicology, vol. 8. London: Richelieu Press; 1994. p. 521–62.
[9] Ammendola R, Ruocchio MR, Chirico G, Russo L, De Felice C, Esposito F, et al. Inhibition of NADH/NADPH oxidase affects signal transduction by growth factor receptors in normal fibroblasts. Arch Biochem Biophys 2002;397:253–7.
[10] Felix CA. Secondary leukemias induced by topoisomerase-targeted drugs. Biochim Biophys Acta 1998;1400:233–55.
[11] Boyonoski AC, Spronck JC, Gallacher LM, Jacobs RM, Shah GM, Poirier GG, et al. Niacin deficiency decreases bone marrow poly(ADP-ribose) and the latency of ethylnitrosourea-induced carcinogenesis in rats. J Nutr 2002;132:108–14.
[12] Spronck JC, Kirkland JB. Niacin deficiency increases spontaneous and etoposide-induced chromosomal instability in rat bone marrow cells in vivo. Mutat Res 2002;508:83–97.
[13] Kostecki LM, Thomas M, Linford G, Lizottee M, Toxopeus L, Bartleman AP, et al. Niacin deficiency delays DNA excision repair and

increases spontaneous and nitrosourea-induced chromosomal instability in rat bone marrow. Mutat Res 2007;625:50–61.
[14] Boyonoski AC, Gallacher LM, ApSimon MM, Jacobs RM, Shah GM, Poirier GG, et al. Niacin deficiency in rats increases the severity of ethylnitrosourea-induced anemia and leukopenia. J Nutr 2000;130: 1102–7.
[15] Bartleman AP, Jacob RA, Kirkland JB. Niacin supplementation decreases the incidence of alkylation-induced non-lymphocytic leukemia in Long-Evans rats. Nutr Cancer 2008 [in press].
[16] Spronck JC, Nickerson JL, Kirkland JB. Niacin deficiency alters p53 expression and impairs etoposide-induced cell cycle arrest and apoptosis in rat bone marrow cells. Nutr Cancer 2007;57: 88–99.
[17] Kirkland JB. Niacin. In: Rucker R, Zempleni J, Suttie JW, et al, editors. Handbook of Vitamins, Fourth Edition. 4th ed. New York, NY: Taylor and Francis; 2007. p. 191–232.
[18] Shacter E, Williams JA, Lim M, Levine RL. Differential susceptibility of plasma proteins to oxidative modification: examination by western blot immunoassay. Free Radic Biol Med 1994;17:429–37.
[19] Requena JR, Levine RL, Stadtman ER. Recent advances in the analysis of oxidized proteins. Amino Acids 2003;25:221–6.
[20] Conners R, Hooley E, Clarke AR, Thomas S, Brady RL. Recognition of oxidatively modified bases within the biotin-binding site of avidin. J Mol Biol 2006;357:263–74.
[21] Struthers L, Patel R, Clark J, Thomas S. Direct detection of 8-oxodeoxyguanosine and 8-oxoguanine by avidin and its analogues. Anal Biochem 1998;255:20–31.
[22] Atamna H, Ginsburg H. Heme degradation in the presence of glutathione. A proposed mechanism to account for the high levels of non-heme iron found in the membranes of hemoglobinopathic red blood cells. J Biol Chem 1995;270:24876–83.
[23] Baskurt OK, Meiselman HJ. Blood rheology and hemodynamics. Semin Thromb Hemost 2003;29:435–50.
[24] Shah GM, Poirier D, Duchaine C, Brochu G, Desnoyers S, Lagueux J, et al. Methods for biochemical study of poly(ADP-ribose) metabolism in vitro and in vivo. Anal Biochem 1995;227:1–13.
[25] Jacobson EL, Jacobson MK. Pyridine nucleotide levels as a function of growth in normal and transformed 3T3 cells. Arch Biochem Biophys 1976;175:627–34.
[26] Anderson ME. Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol 1985;113:548–55.
[27] Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem 1980;106:207–12.
[28] Arun P, Padmakumaran Nair KG, Manojkumar V, Deepadevi KV, Lakshmi LR, Kurup PA. Decreased hemolysis and lipid peroxidation in blood during storage in the presence of nicotinic acid. Vox Sang 1999;76:220–5.
[29] Varella MJ-F, Ernesto TL, Garcia CP, do Rosario Del Lama Unamuno M, Afonso JA, Vannucchi H. Intestinal permeability and oxidative stress in patients with alcoholic pellagra. Clin Nutr 2006;25:977–83.
[30] Ghazi-Khansari M, Nasiri G, Honarjoo M. Decreasing the oxidant stress from paraquat in isolated perfused rat lung using captopril and niacin. Arch Toxicol 2005;79:341–5.
[31] Durkacz BW, Omidiji O, Gray DA, Shall S. (ADP-ribose)n participates in DNA excision repair. Nature 1980;283:593–6.
[32] Jacobson EL, Nunbhakdi-Craig V, Smith DG, Chen HY, Wasson BL, Jacobson MK. ADP-ribose polymer metabolism: implications for human nutrition. In: Poirier GG, Moreau P, editors. ADP-Ribosylation Reactions. New York: Springer-Verlag; 1992. p. 153–62.
[33] Giustarini D, Dalle-Donne I, Colombo R, Milzani A, Rossi R. An improved HPLC measurement for GSH and GSSG in human blood. Free Radic Biol Med 2003;35:1365–72.
[34] Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med 2001;30:1191–212.
[35] Rutter J, Reick M, Wu LC, McKnight SL. Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. Science 2001;293:510–4.
[36] Zhang Q, Piston DW, Goodman RH. Regulation of corepressor function by nuclear NADH. Science 2002;295:1895–7.
[37] Grillo C, D'Ambrosio C, Scaloni A, Maceroni M, Merluzzi S, Turano C, et al. Cooperative activity of Ref-1/APE and ERp57 in reductive activation of transcription factors. Free Radic Biol Med 2006;41: 1113–23.
[38] Kirkland JB, Zempleni J, Buckles LK, Christman JK. Vitamin-Dependent Modifications of Chromatin: Epigenetic Events and Genomic Stability. In: Rucker RB, Zempleni J, Suttie JW, et al, editors. Handbook of vitamins. 4th ed. New York, NY: Taylor and Francis; 2007. p. 521–44.
[39] Kirkland JB. Niacin and carcinogenesis. Nutr Cancer 2003;46:110–8.
[40] Lambeth JD. Nox enzymes, ROS, and chronic disease: an example of antagonistic pleiotropy. Free Radic Biol Med 2007;43:332–47.