Antioxidant Metabolism Induced by Quinic Acid. Increased Urinary Excretion of Tryptophan and Nicotinamide

Ronald W. Pero*, Harald Lund and Tomas Leanderson

Institute of Clinical Medical Science, Section of Immunology, Lund University, BMC I:13, SE-221 84 Lund, Sweden

For over 50 years, hippuric/quinic acids were believed to have no biological efficacy. Here data are presented to support the hypothesis that quinic acid is not responsible for any efficacy, but rather that quinic acid nutritionally supports the synthesis of tryptophan and nicotinamide in the gastrointestinal (GI) tract, and that this in turn leads to DNA repair enhancement and NF-kB inhibition via increased nicotinamide and tryptophan production.

Moreover, it is shown that quinic acid is a normal constituent of our diet, capable of conversion to tryptophan and nicotinamide via the GI tract microflora, thus providing an *in situ* physiological source of these essential metabolic ingredients to humans. The concentrations of quinic and hippuric acids in the diet were dependent on each other when analysed in urine, as was evidenced by a significant linear regression analysis that included unsupplemented control subjects ($n = 45$, $p < 0.001$). Thus, these ingredients were identified as major dietary components, and not simply originating from environmental pollution as previously had been thought. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: antioxidant metabolism; DNA repair; hippuric acid; quinic acid; tryptophan; nicotinamide.

INTRODUCTION

The structure of quinic acid was elucidated in 1932 (Fisher and Dandstedt, 1932). The synthesis of quinic acid via the shikimic acid pathway, and its natural occurrence in plants was first reviewed in 1965 (Bohm, 1965). It was also shown in 1940 that benzoic acid was a precursor to the synthesis of hippuric acid in liver slices of man and animals (Borsook and Dubnoff, 1940). Moreover, it was shown that quinic acid could be metabolized in the gastrointestinal (GI) tract by intestinal microflora to hippuric acid (Adamson *et al.*, 1970; Gonthier *et al.*, 2003; Indahl and Scheline, 1973), which was also clear evidence of metabolic aromatization assigned to the shikimate pathway of plants and bacteria (Herrmann, 1995; Herrmann and Weaver, 1993). During this long period of scientific development (i.e. >70 years) no clear biological efficacious role has been described for either quinic or hippuric acids despite their well understood metabolism (Fig. 1).

However, beginning in 1998 water extracts of cat's claw (*Uncaria tomentosa*) were shown to possess DNA repair and immune modulating properties (Sandoval-Chacon *et al.*, 1998; Sheng *et al.*, 1998, 2000a, 2000b; Lamm *et al.*, 2001). Later on this extract was delineated as having the bioactive components; first carboxy alkyl esters (CAEs) (Sheng *et al.*, 2000a, 2000b, 2001; Åkesson
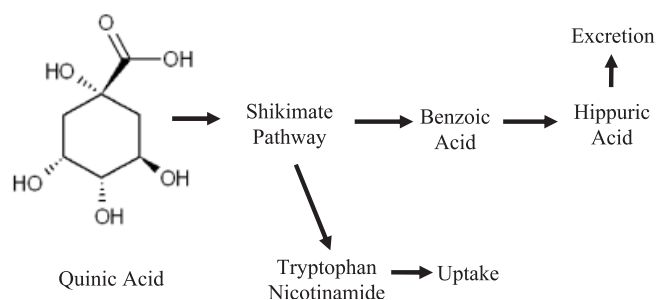

Figure 1. Already known metabolic fate of quinic acid in plants and microorganisms.

et al., 2003a, 2003b), which were then further identified as quinic acid esters (Sheng *et al.*, 2005; Mammone *et al.*, 2006) and finally shown to be quinic acid (Sheng *et al.*, 2005; Åkesson *et al.*, 2005). Because quinic acid was identified as a component in many well-known healthy foodstuffs (Engelhardt and Maier, 1985; Van Gorsel *et al.*, 1992; Beveridge *et al.*, 1999; Jensen *et al.*, 2002), our laboratory attempted to verify quinic acid as a bioactive component in humans, and further to characterize its mode of action as an efficacious component of foods. This study reports on a clinical trial that confirms the efficacy of quinic acid as an antioxidant, and extends its mode of action to include a basic nutritional benefit due to the enhanced metabolism of both tryptophan and nicotinamide, that is simultaneously induced by oral exposure to quinic acid. Hence, quinic acid has been characterized as a pro-metabolite that leads to the induction of efficacious levels of nicotinamide and tryptophan as antioxidants.

* Correspondence to: Professor Ronald W. Pero, Institute of Clinical Medical Science, Section of Immunology, Lund University, BMC I:13, SE-221 84 Lund, Sweden.
E-mail: rwpero@attglobal.net

MATERIALS AND METHODS

Sample collection and preparation. Blood samples were collected by venal puncture using vacutainers (red top, 10 mL) usually in the a.m. after 12 h of fasting. Serum was separated from the blood clot after setting on the bench top for 2 h before centrifugation for 10 min at $1500 \times$ **g**. Sera prepared in this manner were stored at $+4\,^{\circ}\text{C}$ until biochemical analysis usually within 1–30 days of collection.

Urine samples were collected as a random 50 mL sample in the a.m. during the treatment and follow-up (after treatment for 8 months) periods following oral treatment with quinic acid ammonium chelate, but did not include any before treatment baseline sampling as was used to evaluate serum thiol status (see Trial Design below). Urine was spun at $5000 \times$ **g** for 15 min and the supernatant was stored at $+4\,^{\circ}\text{C}$ until analysed within 1 month of collection. After the first analysis all samples were further stored at $-20\,^{\circ}\text{C}$ and used for repeat determinations over time.

Trial design. The trial design for the clinical evaluation of Aqua Bimini (quinic acid ammonium chelate) was carried out at Lund University beginning 28 April 2006. Two male subjects, identity codes HL and RP, received Aqua Bimini manufactured in 500 mL bottles by Aqua Bimini Worldwide (Cederberg Mineral Water Pty Ltd, Vogelfonten Farm, Cederberg, Cape, South Africa, www.aquabimini.com) at 1500 mg/day and 3000 mg/day, respectively for 4–5 consecutive weeks (36 days) in the a.m. on a daily basis. Aqua Bimini has the composition of: 1000 mg Quinmax™ (quinic acid ammonium chelate), 4.7 mg Ca, 6.7 mg Mg, 25 mg Na, 9 mg K, 4.9 mg Cl, 28 mg SO_4 and <0.1 mg F per 1000 mL in sterile water

or bottled in two 500 mL bottles of Aqua Bimini. The volunteers were instructed not to change their diet during the study. In addition, they did not take micronutrient supplements, were disease free and had healthy lifestyle habits (i.e. non-smokers that consumed the equivalent of <2 glasses of wine per day). Repeat serum samples were collected throughout the trial period and then pooled into groups for analysis as 6 weeks before (baseline), 4 weeks of intervention (Aqua Bimini treatment, 36 days) and 8 months dry-out (no treatment). All individual serum sampling points are indicated by the sample dates in Figs 2–4 (*x*-axis).

In addition, a reference group for urinary analytical evaluation of quinic acid metabolites was established for normal no Aqua Bimini-treated individuals who also had healthy lifestyle patterns (e.g. non-smokers, non-vegetarians, disease free, drank <2 glasses of wine per day). This group was used for direct comparison with the Aqua Bimini supplemented subjects. The non-supplemented group consisted of nine individuals who had never taken quinic acid as a supplement of which there were six males and three females aged 12–86 years of age, six of the nine had been taking a general vitamin supplement for at least 3 months at the time of sampling. All individual urine sampling points are indicated by the sample dates recorded in the Table 1 legend. The unexposed (controls) and nutrient supplemented levels in urine of quinic acid, hippuric acid, tryptophan or nicotinamide are also summarized in Table 1. The mean urinary values for each group including both supplemented and unexposed controls were compared by *t*-test statistics to assess the efficacy and metabolism of Aqua Bimini.

Moreover, baseline-controlled levels of protein thiols (antioxidant test parameter) determined in serum samples before supplementation were also compared by *t*-test with the during supplementation levels (4–5 weeks). This
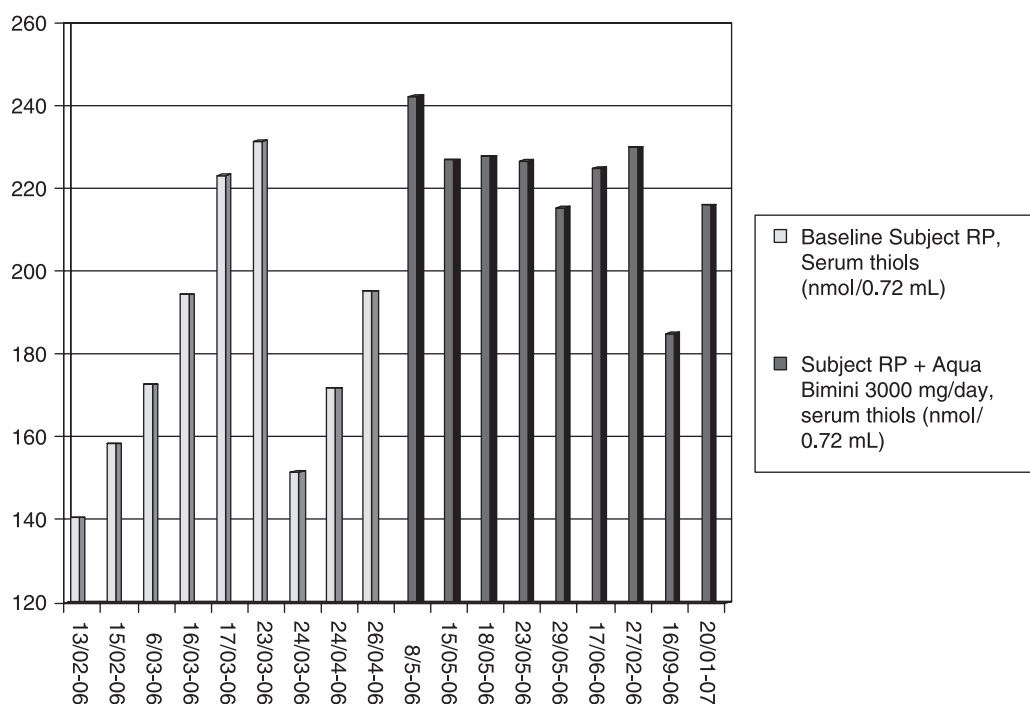

Figure 2. Individual baseline serum protein thiol estimaions determined on Subject RP over 83 days before oral administration of Aqua Bimini at 3000 mg/day for 36 days, and then followed-up with no treatment for 8 months. Indivdual serum sampling points are indicated by the sample dates (*x*-axis).
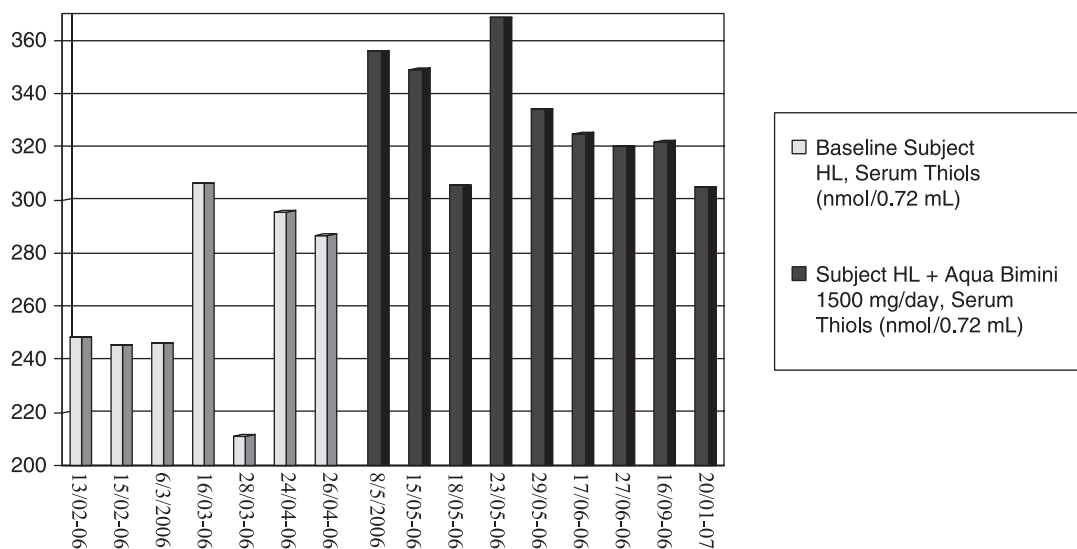
Figure 3. Individual baseline serum protein thiol estimaions deterimned on Subject HL over 83 days before oral administration of Aqua Bimini at 1500 mg/day for 36 days, and then followed-up after no treatment for 8 months. Indivdual serum sampling points are indicated by the sample dates (*x*-axis).
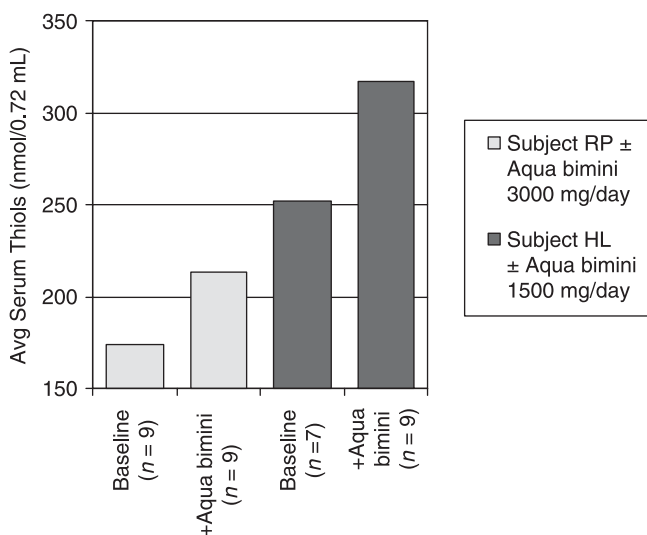

Figure 4. Orally administered Aqua Bimini for 37 days followed by a dry-out period of an additional 32 days enhanced the *in vivo* antioxidant status of trial participants when assessed by the antioxidant parameter of serum protein thiols. Number of samples before ($n = 9$, $n = 7$) and after ($n = 9$, $n = 9$) supplementation with Aqua Bimini are recorded as repeat individual values according to the sample dates presented in Figs 2–3. Both doses of Aqua Bimini (i.e. quinic acid ammonium chelate) were statistically different from their corresponding baseline values analysed by Student's *t*-test ($p < 0.001$).

included all the after supplementation values even the dry-out values (immediately after with no treatment for 8 months) for the same individual. The total individual serum protein thiol values can be viewed in Figs 2–3. In this manner, interindividual variation in serum thiols were randomized out of the protocol, because individual variation in the antioxidant test parameter had been taken out of the design by controlling differences in individual baseline values before the start of treatment. Aging is a multifactorial disorder varying as much as to an individual's genetic makeup. Hence, aging factor variables also support the design of using before and after analyses as an appropriate statistical approach on small samples sizes.

Sampling dates for evaluation of serum thiols or urinary metabolites of quinic acid are recorded as day/month/year throughout. This non-clinical pharmacokinetic research trial evaluating both serum and urine parameters were conducted according to the guidelines of the Declaration of Helsinki for humans. Moreover, informed consent was obtained from all participants that included individual permission to obtain blood and urine samples only for use in this study, and with institutional review approval.

Estimation of serum protein thiols as an *in vivo* antioxidant indicator. The following detailed description is designed to standardize the estimation of the *in vivo* level of serum protein thiols that have been used previously to estimate DNA repair capacity (Pero *et al.*, 2000, 2002, 2005; Banne *et al.*, 2004; Campbell *et al.*, 2005). When a blood sample is collected and the serum isolated, the level of serum protein thiols is an estimate of the concentration between thiols and disulfides existing in protein structures that are in turn equilibrated to the existing oxidant environment *in vivo*. Thus, serum protein thiol analyses are an *in vivo* estimate of the antioxidant status of an individual that is in turn regulated by DNA repair.

Establishing a standard thiol-sensitive curve for quantitative measurement of protein thiols. A stock solution of the colorimetric agent 5,5-dithio-bis-(2-nitrobenzoic acid (DTNB) was prepared using 9.5 mg/mL solid DTNB, 0.1 M K_2HPO_4 and 17.5 mM EDTA. The pH was adjusted to 7.5 and then diluted to the desired volume. A working DTNB solution was prepared by diluting the DTNB stock solution 1:50 with saline. Solutions of L-cysteine were prepared for the standard curve. The standard curve should be in the 0–100 μM range. However, the solutions were made 5 times more concentrated to begin with (they were diluted 5 fold later). A stock solution was made of 50 mM L-cysteine in saline. It was diluted 100 fold to give 500 μM. Then 5–10 solutions of varying concentration were made from this solution. A transparent 96-well flat-bottom microtiter plate was used for the

Table 1. Level of quinic acid metabolites in urine during and estimated immediately after oral administration of either 3000 mg/day or 1500 mg/day Aqua Bimini

Clinical treatment	n	Tryptophan (μM)	Nicotinamide (μM)	Hippuric acid (mM)	Quinic acid (mM)
Controls (No supplement)	9	10 ± 4	23 ± 23	1.1 ± 2	60 ± 42
RP +3000 mg/day QA (36 days)	5	13 ± 5	21 ± 16	2.6 ± 4	231 ± 181
RP 1st 4 mo. after no treatment	11	22 ± 7[a]	71 ± 44[a]	3.6 ± 3[b]	160 ± 57[a]
RP 2nd 4 mo. after no treatment	3	19 ± 6	71 ± 63	2.5 ± 0.6	194 ± 70[b]
Total RP sample mean	19	19 ± 7[a]	58 ± 46[a]	3.1 ± 3[b]	184 ± 103[a]
HL +1500 mg/day QA (36 days)	5	18 ± 5[a]	163 ± 91[a]	1.4 ± 0.8	86 ± 52
HL 1st 4 mo. after no treatment	6	30 ± 8[a]	453 ± 139[a]	1.5 ± 2	84 ± 52
HL 2nd 4 mo. after no treatment	6	17 ± 7	24 ± 7	0.5 ± 0.5	30 ± 17
Total HL sample mean	17	22 ± 9[a]	216 ± 210[a]	1.2 ± 1	65 ± 49

[a] Two-way *t*-test = $p < 0.05$ versus controls; [b] one-way *t*-test = $p < 0.05$ versus controls.
The *n* values shown are the number of repeat samples analysed during the 30 day daily supplement period, the first 4 months after supplement with no treatment period, and followed by the second 4 months after supplement with no treatment period. During treatment period (36 days) for both the Subjects RP and HL: *n* = 5 samples were collected on 8/5/2006, 15/5/2006, 18/5/2006, 23/5/2006 and 29/5/2006; The first 4 months after no treatment period for Subject RP: *n* = 11, samples were collected on 1/6/2006, 4/6/2006, 17/6/2006, 18/6/2006, 19/6/2006, 20/6/2006, 26/6/2006, 27/2006, 29/6/2006, 2/7/2006, 10/7/2006 and for Subject HL: *n* = 6, samples were collected on 1/6/2006, 4/6/2006, 15/6/2006, 27/6/2006, 28/6/2006, 2/7/2006. The second 4 months after no treatment period for Subject RP: *n* = 3, samples were collected on 16/9/2006, 20/12/2006, 20/1/2007, and for Subject HL: *n* = 6, samples were collected on 12/9/2006, 169/2006, 29/9/2006, 13/11/2006, 7/12/2006, 20/1/2007. Mean ± SD values are shown for each group.

absorbance spectrophotometer evaluation. 50 μL L-cysteine solution was mixed with 200 μL working DTNB solution per well. Two to three replicates per concentration were sufficient. A working DTNB blank included 50 μL saline + 200 μL working DTNB. The plate was put on a plate rotator for 15 min. The absorbance was read at 412 nm with a microtiter plate scanner spectrophotometer. The average of every concentration was calculated. The DTNB blank was subtracted from every value, so that 0 μM cysteine should have an absorbance of 0.

The concentration (*x*-axis) vs absorbance (*y*-axis) was plotted and the straightest line was drawn through the points. The *x*/*y*-axis intercept was locked to 0 so that the linear regression analyses gave $y = m(x)$ and not $y = m(x) + b$. In such a way, *y* is the direct absorbance value of standard cysteine solutions, and then the corresponding cysteine solutions that the absorbance values represent were calculated from the factor of $m(x)$, where the slope is *m* (usually equal to 0.01) and *x* is the cysteine concentration or $x = y/m$.

Measuring serum protein thiols. A 100% saturated ammonium sulphate solution was prepared. For a 100 mL solution approximately 50 g ammonium sulphate was used. Sufficient ammonium sulphate was added to the solution so that it did not dissolve any more. 200 μL serum was used for every sample. The serum was precipitated with 800 μL saturated ammonium sulphate (i.e. 80% of saturation), 1.5 mL vials were preferable. They were vortexed to insure mixing and then centrifuged at $12\,000 \times$ **g** for 15 min at room temperature, to give a solid pellet in the bottom of the vial. The supernatant (about 800 μL) was discarded without disturbing the pellet. The pellet was resuspended in 600 μL saline. A transparent 96-well flat-bottom microtiter plate was used. One replicate was prepared as follows: (i) a 50 μL aliquot of serum with 200 μL working DTNB in one well, and (ii) a serum background consisting of a 50 μL aliquot with 200 μL saline in another well. This was repeated three times for each

sample to provide triplicate replications. A DTNB blank (50 μL working DTNB + 200 μL saline) and a saline blank (250 μL saline) were prepared. Again, three replicates were sufficient. The absorbance was read in a microtiter plate scanner set at 412 nm.

Estimating serum protein thiols from the cysteine standard curve. The average was calculated for the DTNB blank and the saline blank. For each replicate, the DTNB blank was subtracted from the sample value (corrected sample). Also subtract the saline blank from every serum value (corrected serum). An example is outlined below to illustrate the calculations. Then the corrected serum value was subtracted from the corrected sample value. This value is the final serum thiol value expressed as the absorbance at 410 nm. The average of this value was calculated for each of the replicates as outlined below.

This value was entered to the standard curve and the equivalent cysteine molar concentration read. This value is the molar concentration of serum thiols expressed as cysteine molar equivalents in each well. It was multiplied by the dilution factor calculated below: After the serum was precipitated and spun in the centrifuge, the pellet was resuspended in 600 μL saline. With the volume increase of the pellet, the final volume was 800 μL. 200 μL pure serum was separated in the beginning, so a 4-fold dilution occurred. The sampled serum was then diluted 5 times when put in the well (50 μL in 250 μL). Altogether this was a 20-fold dilution ($4 \times 5 = 20$). Thus, the thiol value read off the standard curve should be multiplied by a factor of 20. In addition, in order to make the data comparable to the documented and archived data it is necessary to multiply by a factor of 0.722. This is due to previously used experimental factors that are now necessary for comparison with our earlier published studies. Thiols are hereby measured in nmol/0.72 mL.

General conditions of high pressure liquid chromatography (HPLC). HPLC analyses of hippuric acid, quinic

acid, nicotinamide and tryptophan were carried out using a Perkin Elmer 200 LC pump equipped with a UV detector 785 A. The identification of each compound being evaluated by HPLC was also confirmed independently by thin layer chromatography (TLC) analyses. The columns were either C18 150×4.6 mm or C18 80×4.6 mm Perkin Elmer-Brownlee (Pecosphere part no. 0258-0196 or 0258-0166). There was also in tandem but before either the 80 or 150 mm C18 columns, a Perkin Elmer C18 30×4.6 mm Brownlee pre-column (P/N N930-3395). The mobile phase was pumped through the column at 1 mL/min with 1500–5000 psi. The UV detector was set at a wavelength of 200–230 nm depending on the compound being detected. An injection loop of 20 μL was used in all experiments. The data were stored and reprocessed using PE Nelson Turbochrom 4 (S270-0052). C18 columns were regenerated following 30 min washes at 1 mL/min with the following sequence of solvents: acetonitrile: methanol (30:70, v/v/), 100% methanol, methanol: water (50:50), methanol: 0.2% TFA (trifluoroacetic acid) and 100% 0.2% TFA. In all cases quantitative estimates were based on peak height calculations using analytical grade purity of commercially available standard compounds. These general conditions were applied to all HPLC analyses performed in this study.

HPLC sample and standard curve conditions for quantifying quinic acid in serum. Serum samples of 0.2 mL and 30 mL were collected from blood, and precipitated with either ethanol or trichloroacetic acid (TCA). After clean-up the supernatants were dried (ethanol precipitated) or used directly. A standard curve was prepared using quinic acid (Sigma) dissolved in distilled water that obeyed the equation $y = 6013x - 24\,698$ following 20 μL injections of solutions of 0–25 mg/mL.

Single high dose quinic acid time study. A 67 year old apparently healthy male volunteer drank 6 g of quinic acid ammonium chelate (Quinmax™) dissolved in 300 mL water over a 15 min period, and then about 40 mL of peripheral blood (4 red-topped vacutainers) were repeatedly drawn and allowed to clot at room temperature to prepare serum samples by centrifugation. The serum sampling points were 0.7 h, 1.7 h, 2.7 h, 3.7 h, 10.5 h 12.5 h, 22 h, 28 h and 44 h. 30 mL serum samples were precipitated with 50% ethanol, taken to dryness under a stream of air, and re-dissolved in 1 mL of methanol for simultaneous HPLC analysis of quinic acid and hippuric acid. The data were reported as quinic acid present in 30 mL of serum. The column used for this experiment was a C18 150×4.6 mm. The mobile phase was 0.1% TFA. The UV detector was set at 220 nm and quinic acid eluted with a retention of 2.26 min and hippuric acid at 8.3 min. Sensitivity of the method was about $1/30 = 0.033$ mg quinic acid/mL serum.

Repeat doses of 1500 mg and 3000 mg per day quinic acid (i.e. as Quinmax™ or Aqua Bimini) analysed in serum over a 6 week period. Two human volunteers subject HL (20 years) and subject RP (67 years) ingested daily doses of 1500 mg per day or 3000 mg quinic acid per day and serum samples were collected on May 8, May 15, May 18, May 23, May 29 and June 1. These samples were each analysed for quinic acid content in 200 μL serum after precipitation with 2 M TCA. The column used for these experiments was a C18 80×4.6 mm. The

mobile phase was 0.2% TFA: methanol 85:15. The UV detector was set at 215 nm and quinic acid eluted with a retention time of 1.4–1.5 min. The sensitivity for detection of this method was about 1 mg quinic acid/mL serum or a detectable dose of 70 mg/kg in humans, not 21 to 42 mg/kg as used in this study. Thus this protocol could only assess whether the quinic acid levels in serum would accumulate after repeated daily administration of 4–5 weeks to above 70 mg/kg.

HPLC analysis of hippuric acid in serum. Serum samples were stored at $+4\,°C$ and analysed within 1 month of collection. Serum samples (200 μL) were prepared for analysis by precipitating with 2 M TCA (25 μL of 25 M TCA). 20 μL injections of the TCA supernatant into the HPLC were made on serum samples collected during 36 days of Aqua Bimini supplementation and for 30 days of follow-up (no treatment). Serum were analysed by HPLC using a C18 80×4.6 mm and a mobile phase of 0.2% TFA: methanol 75:25. The UV detector was set at 228 nm and hippuric acid eluted with a retention time of 4.0–4.25 min. A standard curve was prepared using hippuric acid (Sigma) dissolved in distilled water of varying concentrations expressed by the equation $y = 511,000x + 6616$ following 20 μL injections of solutions of 0–0.15 mg/mL and then converted to μM before plotting. This standard curve was adequate for detecting and quantifying the levels of hippuric acid in serum, principally because of its increased sensitivity which was about 0.01 mg/mL compared with 1 mg/mL for quinic acid by HPLC.

Preliminary clean-up of urine samples for HPLC analyses. Urine samples were mixed with 1:2:4 v/v/v (urine: 95% ethanol: ethyl acetate) for 10 min with vigorous shaking. The ethyl acetate layer was allowed to separate with gravity at room temperature, and then it was removed with a pipette and evaporated with an air stream to dryness in a vacuum hood. In this manner 1–2 mL of urine were reconstituted in 0.2 mL water or ethanol which represented a 5–10 fold increased concentration over the level in urine. Recovery using this method of extraction of metabolites analysed by spiking with authentic standards was: quinic acid = 53%, hippuric acid = 53%, nicotinamide = 54% and tryptophan = 66%.

Quinic acid and nicotinamide simultaneous detection by HPLC in urine. The urine samples were diluted 1:2:4 v/v/v with ethanol and ethyl acetate, and then the ethyl acetate fraction was dried, solubilized in 0.2 mL water and used for HPLC analyses with 20 μL injections directly onto a C18 80×4.6 mm. The mobile phase was 0.2% trifluoroacetic (TFA): methanol: acetonitrile (70:30): water in a ratio 8:8:84 (v/v/v). The UV detector was set at 215 nm, and quinic acid eluted with a retention of 0.97–1.05 min and nicotinamide at 2.6–2.9 min. The detection limit in urine was about 1.5 mg/mL for quinic acid and for nicotinamide it was 0.015 mg/mL. Between 0–17.5 mg/mL quinic acid, the dose response was expressed as $y = 53.520x + 33.55$, and between 0–0.2 mg/mL nicotinamide the dose response gave the linear regression line of $y = 4\,748\,000x - 0.1174$.

Tryptophan and hippuric acid simultaneous detection by HPLC in urine. The urine samples were diluted 1:2:4 v/v/v with ethanol and ethyl acetate, and then the ethyl

acetate fraction was dried, solubilized in 0.2 mL water and used for HPLC analyses with 20 μL injections directly onto a C18 80 × 4.6 mm. The mobile phase was 70:30 v/v (0.2% TFA: 30% methanol). The UV detector was set at 225 nm. Tryptophan eluted after 5.2–5.4 min and hippuric acid after 3.1–3.4 min. Detection limits for tryptophan and hippuric acid in urine were 0.01 mg/mL and 0.02 mg/mL, respectively. Tryptophan within the dose range 0–0.06 mg/mL yielded a linear regression of $y = 3\,557\,000x + 3.345$ and the dose range 0–0.15 mg/mL gave a similar linear relationship of $y = 4\,592\,000x - 72.46$ for hippuric acid.

RESULTS

Serum protein thiol analyses as *in vivo* estimates of individual antioxidant status

It was possible that since earlier reports of quinic acid efficacy were carried out in rodents, it was indeed not efficacious in humans. Therefore, here serum protein thiol data were determined and summarily presented in Figs 2–3. All the individual data points distinguishing the before (baseline) serum thiol values from the after intervention values including both the short term dryout period from June 2 to August 31, and the longer term dryout period (September 2006 to January 20, 2007) are included in Figs 2–3. It was quite obvious that Aqua Bimini supplementation was associated with a significant increase in reduced thiols (-SH) being present in the proteins. This elevation remained throughout the treatment and follow-up periods. This was considered direct evidence that the redox balance was shifted away from the oxidant state. Because serum proteins contain signal transducing proteins, this was taken as strong evidence of a major shift toward the antioxidant state occurring *in vivo* during supplementation with Aqua Bimini, and remarkably so for at least a 9 month evaluation period. It was concluded that although Aqua Bimini

was a powerful antioxidant, it must be causing these effects as a pro-metabolite by being converted into other compounds that could mediate antioxidant effects.

Figure 4 further clarifies that baseline serum thiol varies from one individual to another, presumably because of genetic and environmental factors. Here serum thiols increased from 175 to 250 nmol cysteine/0.72 mL serum for Subject RP, and 250 to 320 for Subject HL or +31% and +32%, respectively after Aqua Bimini supplementation (Fig. 4). The 30% increase in serum thiols relating to Aqua Bimini ingestion was about equal to the population variability of 30% noted in earlier normal studies (Banne *et al.*, 2004), thus suggesting a reasonable biological effect from Aqua Bimini supplementation. As a result, it was concluded that the antioxidant efficacious effects of Aqua Bimini were clearly present, albeit varying from individual to individual presumably due to genetic factors.

Pharmacokinetic analysis of quinic acid

Characterization of quinic acid metabolism was as follows. (i) A single high dose of 6000 mg was administered orally, and then 30 mL serum samples were prepared from whole blood samples taken from 0.7 to 44 h after exposure. Because the serum samples were precipitated with ethanol and re-dissolved in 1 mL water, this allowed for a 30-fold increase in concentration of any quinic acid present in each blood sample, rendering the method extraordinarily sensitive to even small amounts of quinic acid. The data are presented in Fig. 5.

First of all, quinic acid had maximum uptake into serum after 10.5 h and this was paralleled by the maximum conversion of quinic acid to hippuric acid at about the same time after about 7 h in the systemic circulation (serum). However, it was also shown that only 4400 μg/mL (4.4 mg/mL) quinic acid divided by 30 fold concentration = 147 μg/mL in serum of a volunteer orally administered with 6000 mg quinic acid had the opportunity to accumulate in blood. The theoretical
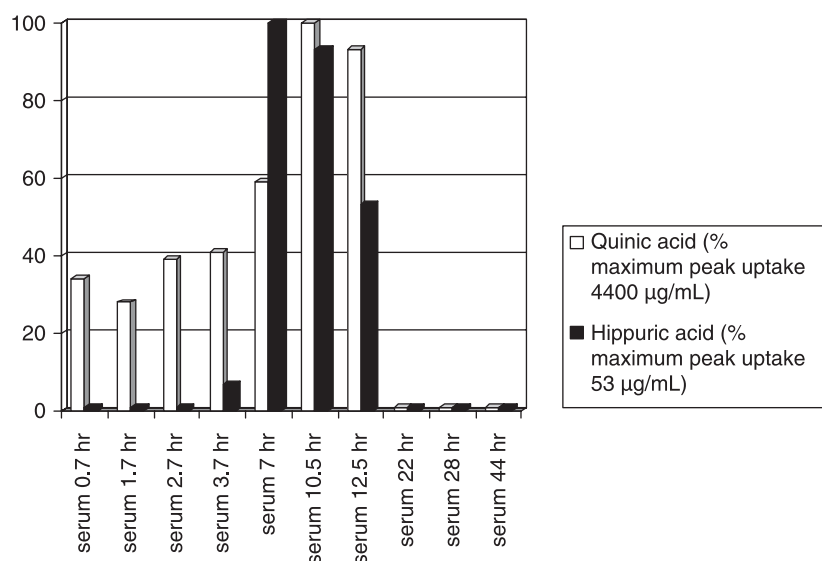

Figure 5. The pharmacokinetics of a single high dose of quinic acid at 6000 mg administered orally in distilled water, and then within 15 min 30 mL serum samples collected at the indicated times. Serum detection of quinic acid, and in relation to its main metabolite hippuric acid, was determined by HPLC as described in Material and Methods. To visualize the maximum time of uptake, the data were presented as a % of maximum uptake: quinic acid = 100% at 12.5 h (4400 μg/mL); hippuric acid = 100% at 7 h (55 μg/mL).

calculation from human whole blood volume of 5 L would then be 6000 mg/5000 mL = 1.2 mg/mL = 1200 μg/mL. This in turn means that only 147 μg/mL of the total dose of 1200 μg/mL was taken up into serum (i.e. 147/1200 = 12.2%), or in other words about 87.8% of the quinic acid was either excreted or metabolized to something else within 44 h.

(ii) The data in Fig. 5 were confirmed and extended by analysing serum after 6 weeks of daily administration and follow-up of either 1500 mg/day or 3000 mg/day of quinic acid chelate (Aqua Bimini). Blood samples were taken and serum prepared six times from day 10 to day 37 after Aqua Bimini repeated oral administration, and then subsequently analysed for quinic acid. None was found in any of the blood serum samples regardless of how long they had been treated daily with quinic acid ammonium chelate. It was concluded that quinic acid did not accumulate in the blood stream after repeat daily oral administrations when analysed by the established sensitivity of detection even after 27 repeated doses (i.e. day 37 to day 10 = 27 doses) of Aqua Bimini, and total doses of quinic acid were equal to 40 500 mg for 1500 mg/day or 81 000 mg for 3000 mg/day, respectively). If quinic acid had accumulated they would have been easily detectable by this HPLC method.

(iii) Finally, because 87.8% of the quinic acid was not found in serum, it was questioned whether it was either being excreted in urine or possibly metabolized to other compounds. Urine samples collected for 4–5 consecutive weeks during oral daily treatment (36 days and during 8 months of follow-up (i.e. after no treatment) of Aqua Bimini at 3000 mg/day or 1500 mg/day were analysed for quinic acid (Table 1). Firstly, it has been established that significant levels of quinic acid are present in urine without supplementation of Aqua Bimini. Hence, there must be a natural dietary occurrence of quinic acid in food, since it cannot be synthesized by mammals including humans, and significant levels were found in urine. Moreover, when individuals were supplemented with Aqua Bimini (Quinmax), there was a dose-dependent increase in urinary quinic acid levels compared with the non-supplemented individuals having only dietary sources of quinic acid available. The occurrence of substantial amounts of quinic acid in healthy coloured foods (Beveridge *et al.*, 1999; Engelhardt and Maier, 1985; Jensen *et al.*, 2002; Van Gorsel *et al.*, 1992) confirms these urinary data in the Aqua Bimini-unexposed controls, thus characterizing quinic acid as a naturally occurring component of our diet not just originating from environmental pollution exposures (e.g. organic solvents such as benzene).

Furthermore, the average levels of quinic acid in urine for the entire 9 month evaluation period were found to be about 184 ± 103 mM ($n = 19$) and 65 ± 49 mM ($n = 17$), respectively (Table 1). The standard deviations of these mean values indicate large fluctuations of urinary quinic acid over time. Nonetheless, one subject (RP) still had elevated levels of quinic acid even after 9 months of no treatment. Similar results were also found for the other quinic acid metabolites analysed; e.g. hippuric acid, tryptophan and nicotinamide after 4 months of follow-up (Table 1). These data no doubt reflected metabolic utilization by GI tract microflora when compared with Aqua Bimni-unexposed controls, and in addition, paralleled the Aqua Bimini dose 8 months earlier. Assuming the volume of urine produced per day to be 1.5 L, we could detect about 184 mM after 3000 mg/day oral dose per day quinic acid in urine after 9 months of treatment and follow-up, or 184 mmol/L which converts to 53 039 mg/1.5 L total urine volume per day. A similar calculation for the oral dose of 1500 mg/day which yielded 65 mM quinic acid in urine gave 18 737 mg/1.5 L total urine volume per day. These data are summarized in Table 2 and permit the analysis that 56% and 40% of the total dose administered either by diet or supplementation was excreted as unmetabolized quinic acid after the 9 month evaluation period (Table 2).

Because elevations of quinic acid could be detected in urine during the dry-out period (i.e. for 8 months after treatment), then it was reasoned that quinic acid might accumulate in the GI tract from repeat dosing, otherwise how could increased levels of quinic acid be found excreted into urine for 8 months of follow up (after no oral supplementation, Subject RP, Table 1), unless GI tract metabolism by dietary quinic acid was being induced also. Either of these possibilities have very favourable nutritional implications, because it means any further possible metabolism of quinic acid to aromatic amino acids in the GI tract vi*a* gut bacteria and the shikimate pathway, would allow for a sustained production of aromatic amino acids even in the absence of further supplementation of ether quinic acid or essential amino acids or vitamins.

Pharmacokinetics of hippuric acid

Quinic acid was also evaluated as a source of hippuric acid after oral doses of 1500 mg/day and 3000 mg/day of Aqua Bimini (quinic acid ammonium chelate) for a period of oral administration from April 28 to June 1, and together with samples included from an additional dryout period (after no treatment for 8 months) from June 2, 2006 to January 20, 2007. It is well known that animals including humans can metabolize from 5% to 70% of a dose of quinic acid to benzoic and then to hippuric acid within 1–8 days (Adamson *et al.*, 1970).

Table 2. Metabolic fate of Aqua Bimini (quinic acid ammonium chelate) over a 9 month period in man involving 1 month treatment and 8 months of follow-up (after no treatment)

Oral dose quinic acid	Total dose for 9 months administered	Total dose excreted in 9 months[a]	Dose recovery (excrete/dose)	Dose storage/metabolism (100%-recovery)
Subject RP 3000 mg/day	90 g	53 g	59%	41%
Subject HL 1500 mg/day	45 g	18.5 g	40%	60%
Ratio	2.0	2.86	–	–

[a] The average quinic acid urinary levels were calculated from the total data displayed in Table 1 as the mean for the entire sampling period of 9 months as: RP = 184 mM, $n = 19$ or 53 g/1.5 L; HL = 64.4 mM, $n = 17$ or 18.5 g/1.5 L.
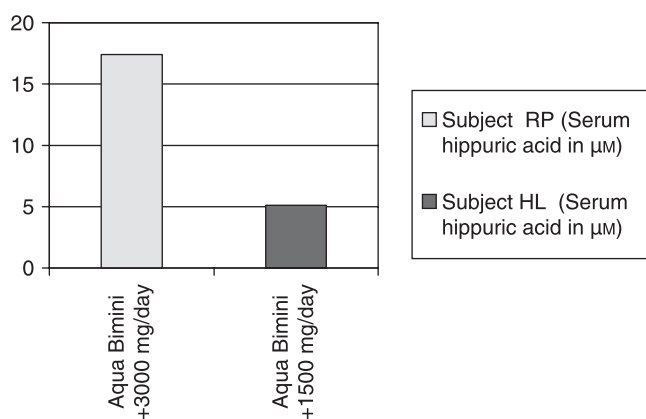
Figure 6. Serum levels of hippuric acid in Subject HL and Subject RP treated daily with 1500 or 3000 mg/day Aqua Bimini (quinic acid ammonium chelate) for 36 days, and then followed-up (no treatment) for 30 days more. Total sampling points for serum analysis of hippuric during the period described were: Subject RP $n = 12$, Subject HL $n = 12$, $p < 0.01$.

Thus hippuric acid has been the only previously known metabolite characterized as originating from quinic acid exposure.

Here both urine and serum were analysed for the presence of hippuric acid. In urine mM amounts of hippuric acid were found (Table 1, Fig. 6). For example, urine yielded up to 12.7 mM hippuric acid at the individual data point collected on 1/6/2006 after trial start-up on 4/28/2006 (individual sampling data points not shown, Table 1), which represented 3412 mg hippuric acid/1.5 L. A total daily dose of 3000 mg Aqua Bimini as quinic acid ammonium chelate/70 kg person is equivalent to 43 mg/kg or equal to about 43 mg/L = 64.5 mg/1.5 L. The total water volume of the human body is estimated to be 0.71 L/kg. Assuming Aqua Bimini is equally distributed in water and urine then 0.71 L × 70 kg = 49 L in which 3000 mg is dissolved that is equivalent to 3000 mg/49 L = 91.8 mg/1.5 L which should have been present if quinic acid had been completely absorbed and not excreted. Hence, these calculations support that 64.5 mg/1.5 L divided by 91.8 mg/1.5 L or about only 70.3% of the daily administered dose of Aqua Bimini (i.e. quinic acid at 3000 mg/ml (Subject RP) was excreted into urine as hippuric acid on a daily basis, and the remaining 29.7% of Aqua Bimini was either absorbed into systemic circulation as hippuric acid or further metabolized to other compounds.

Whereas mM amounts of hippuric were found in urine only extremely low levels <20 µM were found in serum (Fig. 6). Hippuric acid was barely detectable in serum within this dose range, thus supporting the poor absorption of quinic acid into systemic distribution and its conversion therein to hippuric acid. In addition, at least the difference in serum concentration (i.e. <0.02 mM, Fig. 4) and urine levels of hippuric acid after Aqua Bimini administration of 3000 mg/day (0.49–12.7 mM, Table 1 and text calculations) establish that at least some quinic acid was metabolized to other compounds and not just excreted as hippuric acid. Even at these low levels of serum hippuric acid, there was a dose response to Aqua Bimini treatment observed for this material (Fig. 6).

Taken together these data on serum quinic/hippuric acid levels indicate that very little quinic acid was found in systemic circulation (<12.2% of the administered dose, Figs 5–6 and text calculations in pharmacokinetic analy-

sis of quinic acid results section). Although hippuric acid could be detected in both serum and urine proving that quinic acid was indeed subject to systemic uptake metabolism, where about 70.3% of the administered dose was excreted unchanged in the urine (data point 1/6/2006 for hippuric acid = 12.7 mM and text calculations). Of the remaining 29.7% of Aqua Bimini unaccounted for in the urine, only trace amounts were identified as hippuric acid in serum, thus paralleling the pharmacokinetic data of quinic acid and hippuric in serum (Figs 5–6). Because only trace amounts of either quinic acid or hippuric acid were found in serum, then potentially only 29.7% of the daily administered dose of Aqua Bimini could be converted to other compounds in systemic circulation or the GI tract. It was concluded from this pharmacokinetic analysis that the previously documented efficacious effects of quinic acid were likely not due to a direct effect by quinic acid itself, but instead were mediated by a naturally occurring metabolite of quinic acid.

Dependence of hippuric acid levels in urine on the corresponding levels of quinic acid in urine

This study determined baseline urinary values for quinic acid, hippuric acid, tryptophan and nicotinamide in a broad-based reference population ($n = 9$, ages 12–86 years) not receiving Aqua Bimini. The data for urinary quinic and hippuric acids are summarized in Table 1 and Fig. 7 (individual sample point data is available upon request in a supplementary data file). There was a highly significant linear correlation ($p < 0.001$) between the urinary levels of quinic and hippuric acids based on the entire sampled population of controls (unexposed) and repeat sampled Aqua Bimini (quinic acid) treated individuals ($n = 45$, Fig. 7). This was taken as strong evidence that hippuric acid was mainly influenced by dietary factors associated with quinic acid known to occur in coloured fruits and vegetables (Beveridge *et al.*, 1999; Bohm, 1965; Engelhardt and Maier, 1985; Jensen *et al.*, 2002; Van Gorsel *et al.*, 1992) instead of primarily reflecting environmental toxic aromatic exposures such as benzene or toluene (Beer *et al.*, 1951; Bernhard *et al.*, 1955; Borsook and Dubnoff, 1940) as previously thought.

Urinary analysis of metabolites associated with the microbial/plant shikimate pathway

Humans have an intestinal microflora capable of metabolizing quinic acid at least to hippuric acid (Adamson, 1970; Gonthier *et al.*, 2003; Indahl and Scheline, 1973). This fact is well known because if quinic acid is administered orally then hippuric is easily identified in urine samples, but when administered by intraperitoneal injection there is no conversion to hippuric acid. For over 35 years, despite the hippuric acid evidence of a functional shikimate pathway existing in the human GI tract, there have been no studies reported demonstrating that quinic acid could lead to an increased production of the compounds being generated along the metabolic route from shikimate to chorismate to tryptophan to nicotinamide and so on. This study has determined for the first time that the urinary levels of tryptophan and nicotinamide were induced by exposure to quinic acid (Table 1).
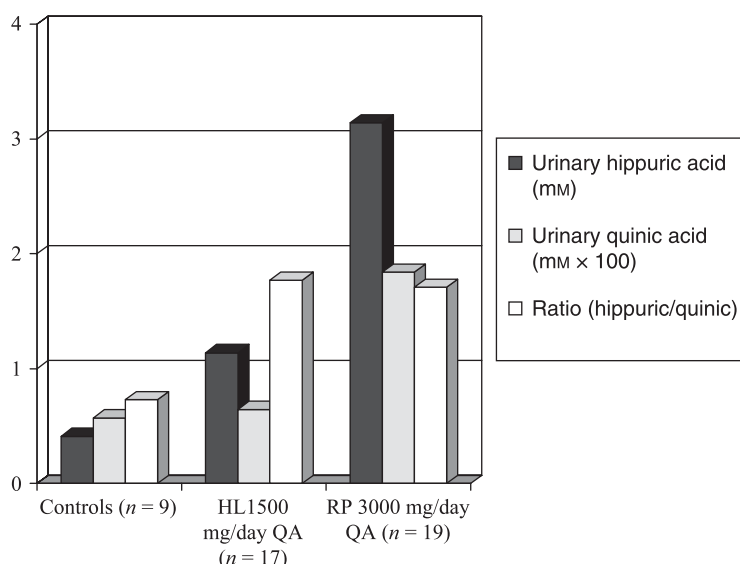
Figure 7. Dependence of urinary levels of hippuric acid, the primary known metabolite of quinic acid, on the urinary level of quinic acid in untreated individuals and in subjects treated with 1500 mg/day and 3000 mg/day Aqua Bimini for 36 days, then followed for an additional 8 months immediately after treatment. The data used here were recorded as the individual values represented by the sample dates and summarized as mean values presented in Table 1. These data were statistically analysed by linear regression of the total sample ($n = 45$) involving both treated ($n = 36$) and controls ($n = 9$), where hippuric acid values versus quinic acid values collected from urine at the same time in the same subject gave $y = 0.0157x + 0.1839$, $r = 0.60$, $p < 0.001$.

The GI tract is one of the most important organ systems of the body, responsible for breakdown and re-synthesis of proteins, fats and sugars necessary to maintain proper nutrition for cellular growth and health maintenance in the rest of the body. Some of the main nutritive sources not synthesized by the body, but much needed for signal transduction (e.g. serotonin and dopamine) and building proteins are the aromatic amino acids tryptophan, phenylalanine and tyrosine. These are all shown to be produced by the shikimate pathway present in the GI tract along with nicotinamide and NAD, a primary energy source.

The data analysis presented in Table 1 show that when urinary levels of tryptophan and nicotinamide were measured in two subjects, one receiving 1500 mg/day and the other 3000 mg/day for 33 consecutive days (i.e. treatment period), there were significantly elevated levels of urinary tryptophan and nicotinamide (Subject HL), when compared with the controls (Aqua Bimini untreated). Even when samples were included in the analysis after 4 months of follow-up after no Aqua Bimini, the tryptophan and nicotinamide levels in urine remained elevated (Table 1). Tryptophan and nicotinamide in urine are well-known strong indicators of the status of these nutrients in the rest of the body (McCreanor and Bender, 1986; Shibata, 1989; Shibata and Matsuo, 1990; Shibata and Toda, 1997; Kimura *et al.*, 2006), and so it was concluded that quinic acid supplementation not only increased the synthesis of hippuric acid in the GI tract but also tryptophan and nicotinamide.

In addition, both tryptophan and nicotinamide remained elevated in urine through a follow-up period of 4 months with no further treatment with quinic acid. This was taken as support for their involvement in mediating the antioxidative systemic effects as evidenced by increased serum thiols during the same period (Figs 2–4). In an effort to better substantiate the relationship between tryptophan/nicotinamide and systemic antioxidant levels assessed by serum thiols, the molar ratios of tryptophan or nicotinamide were analysed in urine in relation to the micromolar levels of serum thiols. Table 3 clearly shows that the ratio of either tryptophan or nicotinamide increased levels in urine (excretion) to the increased micromolar levels of thiols in serum (uptake into systemic circulation), had a strong dose response to quinic acid oral supplementation. Remarkably, these ratios estimated the metabolic control of individual antioxidant status linked to Aqua Bimini (Quinmax) consumption in accordance with the theoretical difference in doses used, calculated as: theoretical 3000 mg per day/1500 mg per day = 2.0 ratio, tryptophan 1.94/0.81 = 2.4 ratio, nicotinamide 4.29/2.32 = 1.85 ratio, respectively (Table 3).

DISCUSSION

The data presented in this study on oral administration of Aqua Bimini (a quinic acid ammonium chelate) confirm earlier reports (Mammone *et al.*, 2006; Pero *et al.*, 2002, 2005; Sheng *et al.*, 2000a, 2000b, 2001, 2005; Åkesson *et al.*, 2003a, 2003b, 2005) that quinic acid or quinic acid-containing water extracts possess DNA repair and/or immune enhancing properties when evaluated using serum thiol analyses as a surrogate DNA repair estimate (Figs 2–4). These data support that serum protein thiols can be increased in humans supplemented with quinic acid (Figs 2–4), and that these effects mirrored DNA repair enhancement as shown previously in other mammals. When these data are combined with the earlier studies, quinic acid or water extracts of Uncaria containing quinic acid, also had similar abilities to inhibit NF-kB (Åkesson *et al.*, 2003a, 2005). Furthermore, because quinic acid has been shown to have an antiinflammatory mode of action, this fact no doubt contributes to the antioxidant properties resulting in DNA repair enhancement (i.e. increased serum thiols) demonstrated here in humans (Figs 2–4).

Table 3. Relationship of nicotinamide and tryptophan to serum protein thiol metabolism. The data are calculated from Figs 2–4 and Table 1. These calculations were intended to minimize any variation originating solely from interindividual differences to metabolize either quinic acid or protein thiols

Oral dose administered for 36 days	Increase in urinary quinic acid (QA) metabolite (ratio)[a]	Increase in serum protein thiols (ratio)[b]	Increase nico. or tryptophan/increase thiols[c]
Nicotinamide analysis (μM/μM)			
HL 1500 mg/day QA (Aqua Bimini)	215/76 = 2.83	1.22	2.32
RP 3000 mg/day QA (Aqua Bimini)	57.9/10.7 = 5.41	1.26	4.29
Tryptophan analysis (μM/μM)			
HL 1500 mg/day QA (Aqua Bimini)	21.3/19.3 = 0.99	1.22	0.82
HL 3000 mg/day QA (Aqua Bimini)	22.7/9.32 = 2.44	1.26	1.94

[a] Ratio of mean nicotinamide or tryptophan urinary levels in μM during treatment +9 months follow-up divided by the mean levels at start of treatment: dates 8/5/2006 and 15/5/2006. Total data are presented in Table 1.
[b] Calculated as the mean. ratio in the after to before (baseline) Aqua Bimini treatment values presented in Figs 2–4.
[c] Baseline Ratio (non-supplemented values) of [a] (increase in tryptophan or nicotinamide) to [b] (increased thiols expressed as ratio of after/ before levels, and thus the no effect is equal to 1.00 instead of 1.22–1.26). Hence the no effect level of the ratio of tryptophan or nicotinamide to serum thiols is about 0.99/1.22 = 0.82.

Because *in vitro* and *in vivo* bioassays did not always give the same results after treatment with quinic acid and the quinic acid ammonium salt (Quinmax™) (Åkesson *et al.*, 2005), then the study also sought to ascertain if quinic acid might be converted to other compounds *in vivo* which might mediate the efficacious effects being observed for quinic acid. Here, our laboratory reports that very little quinic acid could be found systemically in serum (i.e. about 12.2% of the administered dose) although high levels were excreted in the urine unmetabolized (i.e. about 70.3% of the administered dose), thus supporting that quinic acid itself was not the bioactive ingredient itself inhibiting NF-kB or stimulating DNA repair (see text calculations). On the other hand, tryptophan, nicotinamide or their well known metabolites are normal components of serum that are capable of enhancing DNA repair and inhibiting NF-kB (Pero *et al.*, 1999; Virag *et al.*, 2005), and they were elevated in urine (Table 1) in a dose-dependent manner after orally administered quinic acid (Quinmax in Auqa Bimini, i.e. quinic acid ammonium chelate), when evaluated by the antioxidant parameter of serum protein thiol levels (Figs 2–4).

In earlier reported studies when quinic acid was fed in the diet of rabbits, hamsters, guinea-pigs, lemmings, pigeons, dogs, cats, ferrets, hedgehogs, fruit bats, rats, primates or humans 5–70% was converted after 1–8 days of oral administration to hippuric acid (Adamson *et al.*, 1970; Beer *et al.*, 1951; Bernhard Kvon *et al.*, 1955; Gonthier *et al.*, 2003; Indahl and Scheline, 1973). No quinic acid or any of its known intermediate metabolites such as hippuric acid were found in any significant amounts in serum. Thus the previously reported scientific data indicate that quinic acid or its only known recognized metabolite (hippuric acid) were efficiently excreted in urine, and cleared rapidly or metabolized from circulation (serum), thus limiting its ability to modify biological responses to only short periods of time. Primates, including man, had the greatest conversion of quinic acid to hippuric acid, and this metabolism was shown to be completely dependent on the intestinal microflora, because there was no metabolism of quinic acid to hippuric acid when injected intraperitoneally as there was by oral administration.

Elevated urinary levels of tryptophan and nicotinamide are potent indicators of an induced synthesis of these nutrients from oral consumption of quinic acid. Because quinic acid is known to induce aromatic compounds synthesis in the GI tract via resident microflora (e.g. hippuric acid), then a functional shikimate pathway has been indicated for the gut microflora (Herrmann, 1995). Hence, the powerful antioxidant properties observed herein for Aqua Bimini (Figs 2–4), a quinic acid product, might be due to basic nutritional support provided *in situ* in the GI tract resulting in elevated urinary levels of tryptophan and nicotinamide also synthesized by the shikimate pathway in addition to hippuric acid (Fig. 1, Table 1).

Support for this hypothesis can be summarized as (i) Analysis of serum did not identify the presence of any quinic acid, thus eliminating the possibility that quinic acid could directly mediate any systemic efficacious effects, (ii) Urinary levels of tryptophan and nicotinamide were indeed elevated reflecting their dose dependent increased synthesis (Table 1) via the tryptophan-quinolinate-nicotinamide-NAD branch of the shikimate pathway (McCreanor and Bender, 1986; Shibata, 1989; Shibata and Matsuo, 1990), (iii) Because baseline serum protein thiols levels (before treatment) consistently remained lower than after 1 month of treatment plus 8 month of follow-up (after no treatment) then the antioxidant properties of elevated serum thiols were linked to the increased levels of urinary trytophan and nicotinamide, strongly suggesting a cause and effect relationship, (iv) Although tryptophan can be a direct precursor to serotonin, dopamine and other aromatic amino acid syntheses, the data showed that increased urinary tryptophan was paralleled by increased urinary nicotinamide (Table 1), supporting that the primary route of tryptophan metabolism was the tryptophan-quinolinate-nicotinamide-NAD pathway that in turn is regulated by quinolinate production; i.e. the rate limiting enzymatic activity mediated by quinolinate phosphoribosyltransferase (Satyanarayana and Rao, 1977), and (iv) Nicotinamide is known as an NF-kB inhibitor (Pero *et al.*, 1999) and a major antioxidant in part because it increases production of NAD, the energy source required for DNA repair and NF-kB

transcriptional regulation that involves poly (ADP-ribose) polymerase (Virag, 2005; Adams, 2004). Taken together these data support that quinic acid itself is not the efficacious agent enhancing DNA repair and immune function, but rather that it is the increases in tryptophan and nicotinamide that are nutritionally mediated by quinic acid in the GI tract that are responsible for efficacy.

Finally, it has been noted that both tryptophan and nicotinamide are well known therapeutic agents at higher doses than exist naturally. The scientific data pertaining to tryptophan's use as a therapeutic agent, in relation to quinic acid supplementation, as has been detailed previously, and include obesity, anorexia, migraine, depression, insomnia and stress (Brown *et al.*, 1979; Cavaliere and Medeiros-Neto, 1997; Chouinard *et al.*, 1979; Gedye, 2001; Li *et al.*, 2006; Smith, 1982; Weld *et al.*, 1998; Wong and Ong, 2001). Here it can easily be seen that tryptophan doses known to be therapeutic have levels of around 1.5 mg/L tryptophan (calculated from the literature; Smith, 1982) in urine whereas the corresponding tryptophan values after quinic acid supplementation gave about 4.5 mg/mL (calculated from data in Table 1). The implication is very strong that quinic acid nutritional intervention can achieve an even greater efficacious therapy than high dose tryptophan supplementation by itself.

A similar relationship also exists between high dose nicotinamide therapy and therapeutic efficacy. However, the metabolic control of high doses of nicotinamide is strongly regulated so that within 1 day urinary nicotinamide has returned to about non-supplemented levels (Shibata, 1989). Consequently, there were no data available in the literature on urinary levels of high dose nicotinamide over longer periods of time. Nonetheless, high dose nicotinamide has therapeutic value to treat lipid profiles (Handfield-Jones *et al.*, 1988; Takahashi *et al.*, 2004; Zak *et al.*, 2006), diabetes (Rakieten *et al.*, 1976; Wilson and Buchingham, 2001; Yamada *et al.*, 1982; depression (Chouinard *et al.*, 1979), HIV (Murray *et al.*, 2001), renal tumors (Beales *et al.*, 1999), graft failure (Brandhorst *et al.*, 2002), migraines (Gedye, 2001) and as an antioxidant (Kamat and Devasagayam, 1999; Ungerstedt *et al.*, 2003).

Acknowledgements

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