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# Plasma antioxidant vitamins, chronic hepatitis B virus infection and urinary aflatoxin $B_1$–DNA adducts in healthy males

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**Epidemiological evidence indicates that aflatoxin $B_1$ ($AFB_1$) intake is associated with an increased risk of hepatocellular carcinoma (HCC). The hepatocarcinogenesis is initiated by covalent binding of $AFB_1$ to cellular DNA. To determine whether nutritional factors and hormonal status may influence the binding of $AFB_1$ to hepatic DNA, a cross-sectional study was performed on a total of 42 male asymptomatic hepatitis B surface antigen (HBsAg) carriers and 43 male non-carriers in a cohort study on the multistage development of HCC in Taiwan. The major $AFB_1$–DNA adduct *in vivo*, $AFB_1$-$N^7$-guanine, was measured by high-performance liquid chromatography in urine. Urinary $AFB_1$-$N^7$-guanine was detectable in 40% of the subjects. HBsAg carriers had a higher detection rate of urinary $AFB_1$–DNA adducts than non-carriers and the difference was statistically significant after multivariate adjustment. After taking into account the total $AFB_1$ urinary metabolite level, chronic HBsAg carrier status, and other potential confounders, plasma levels of cholesterol, α-tocopherol, and α- and β-carotene were positively associated with the detection rate of the $AFB_1$–DNA adducts in a dose-dependent manner, whereas plasma lycopene level was inversely related to the presence of the adducts in urine. The association of urinary $AFB_1$–DNA adducts with the plasma levels of cholesterol, α-tocopherol, lycopene, and α- and β-carotene was observed at both low and high exposure levels of $AFB_1$. There was a synergistic interaction of plasma α-tocopherol with α- and β-carotene on the adduct levels. No association with the adducts was found for plasma levels of retinol and testosterone. This study demonstrated different associations of antioxidant vitamins with $AFB_1$–DNA adduct formation. The data consistent with our previous finding in cultured woodchuck hepatocytes that α-tocopherol and β-carotene enhanced $AFB_1$–DNA adduct formation suggest that prospective investigation of the relationship between plasma micronutrients and risk of $AFB_1$-related HCC is warranted.**

## Introduction

Ingestion of aflatoxin $B_1$ ($AFB_1$*) and chronic infection with hepatitis B virus (HBV) have been identified as major risk factors for the development of hepatocellular carcinoma (HCC) in Taiwan and other parts of southeastern Asia and Africa (1–5). $AFB_1$ is carcinogenic in many animal species and its covalent binding to hepatic DNA has been shown to be a critical step in hepatocarcinogenesis (6).

The major $AFB_1$–DNA adduct formed *in vivo* is $AFB_1$-$N^7$-guanine (7). Treatment of $AFB_1$ to rats resulted in the urinary excretion of $AFB_1$-$N^7$-guanine adducts. The adduct excretion following $AFB_1$ administration was shown to be proportional to the total amount of $AFB_1$-$N^7$-guanine initially formed in the hepatic DNA (8). Although the presence of $AFB_1$–DNA adducts in liver specimens is readily detected by immunohistochemical methods (3), liver tissues are usually difficult to obtain. Measurement of the adduct levels in urine provides a non-invasive means of estimating the degree of $AFB_1$ binding to hepatic DNA. It has been used as an intermediate endpoint in cancer prevention trials for assessing the efficacy of chemopreventive agents that may have an inhibitory effect at early stages of $AFB_1$-induced hepatocarcinogenesis (6,9). The relationship between urinary $AFB_1$-$N^7$-guanine adducts and HCC risk was recently investigated in a prospective study and the presence of the DNA adduct in urine has been shown to be a significant predictor for the cancer risk (4).

In addition to chronic HBV infection and aflatoxin exposure, our previous epidemiological studies have linked many risk factors, including hepatitis C virus (HCV), alcohol drinking, cigarette smoking, elevated serum level of endogenous testosterone, low serum retinol level and/or vegetable consumption, as well as genetic susceptibility, to the development of HCC (10–15). There may be a complex interaction among multiple HCC risk factors at various stages of hepatocarcinogenesis. We previously examined the effect of chronic HBV infection, sociodemographic characteristics, and habits of cigarette smoking and alcohol drinking on $AFB_1$–DNA adduct formation (16). Age and habits of cigarette smoking and alcohol drinking were found to be positively associated with the adduct levels. Whether other factors may influence the binding of $AFB_1$ to hepatic DNA have yet to be identified. Synthetic antioxidants have been reported to inhibit $AFB_1$–DNA adduct formation and thus the tumorigenicity of $AFB_1$ in rats (17,18). Experimental studies have also suggested that dietary antioxidants such as vitamin C, vitamin E and several carotenoids may suppress chemically induced carcinogenesis through a variety of mechanisms, including reduction of oxidative DNA damage, modulation of carcinogen-metabolizing enzymes, and/or trapping of electrophilic forms of carcinogens (19–21). However, vitamin E and β-carotene were observed to enhance $AFB_1$–DNA adduct formation in our previous *in vitro* study on cultured woodchuck hepatocytes (22). In addition to the environmental agents, hormonal status may also influence the $AFB_1$ binding to DNA. The pathway leading to metabolic activation of $AFB_1$ was reported to be more active in male than in female rats (23). The role of endogenous testosterone in $AFB_1$ metabolism in humans

***Abbreviations:** HCC, hepatocellular carcinoma; HBV, hepatitis B virus; $AFB_1$, aflatoxin $B_1$; $AFB_1$-$N^7$-guanine, 8,9-dihydro-8-($N^7$-guanyl)-9-hydroxy-aflatoxin $B_1$; HBsAg, hepatitis B surface antigen; HPLC, high-performance liquid chromatography; OR, odds ratio; CI, confidence interval.

remains to be explored. This study was carried out to elucidate the potential roles of endogenous testosterone and nutritional factors including common antioxidants at the early stage of $AFB_1$-induced hepatocarcinogenesis by investigating the covalent binding of $AFB_1$ to hepatic DNA. Urinary $AFB_1$-$N^7$-guanine was used as a biomarker for assessing $AFB_1$–DNA binding in the liver.

## Materials and methods

*Study subjects*

The subjects of the present study have been described in a previous publication that examined associations of multiple factors with urinary levels of $AFB_1$–DNA adducts (16). Initially, a total of 43 male asymptomatic HBsAg carriers and 43 male HBsAg non-carriers were included in the study. HBsAg non-carriers were matched with the carriers on age (±5 years) and time of biospecimen collection (±3 months). They were randomly selected from the cohort members without HCC in a prospective study on the multifactorial etiology of multistage hepatocarcinogenesis in Taiwan. The cohort characteristics and method of follow-up have been described in a previous study (15). The urine and blood samples from study subjects were consecutively collected between August 1988 and June 1992. Urine samples were kept frozen at –30°C and blood samples were kept at –70°C until they were used in the present analyses. At the time of urine and blood collection, each study subject was also personally interviewed to obtain information related to habits of cigarette smoking and alcohol drinking, dietary pattern and health history. In the present analysis, study subjects were 42 HBsAg carriers and 43 HBsAg non-carriers whose plasma samples were sufficient for analysis of micronutrients and testosterone. All the chronic HBsAg carriers included in this study remained asymptomatic throughout a 5-year follow-up period, except two who were subsequently affected with liver cirrhosis. The mean duration of biospecimen storage prior to testing is 4.4 years (range: 2.4–6.3 years). There was no significant difference in seasons of biospecimen collection between HBsAg carriers and non-carriers.

*Laboratory analyses*

Serum HBsAg was assayed using a radioimmunoassay (Abbott Laboratories, North Chicago, IL). All but six study subjects were also tested for antibodies against HCV (anti-HCV). Anti-HCV was examined by a second-generation enzyme immunoassay (Abbott Laboratories, North Chicago, IL). The urine samples were assayed for levels of $AFB_1$, two of its main metabolites ($AFM_1$ and $AFP_1$) and the $AFB_1$-$N^7$-guanine adducts. Urinary $AFB_1$ metabolites were quantified by high-performance liquid chromatography (HPLC) as described previously (16). In short, aflatoxin metabolites were extracted from a 5-ml aliquot of urine. Analysis of various aflatoxin metabolites was done on an HPLC gradient liquid chromatograph with a Waters model 470 fluorescence detector (Waters Associates, Milford, MA). The HPLC column was a $C_{18}$ 10-μm (3.9 mm inside diameter × 30 cm length) μBondpak column (Waters). For analysis of $AFM_1$, $AFB_1$-$N^7$-guanine, and $AFB_1$, chromatographic separation was achieved by elution for 12 min with 15% acetonitrile followed by 22% acetonitrile in water. The flow rates were 1.5, 0.8, 0.3 and 1.0 min/ml at 0–12, 13–21, 22–35 and 36–41 min, respectively. Elutes were measured by fluorescence detection with 365 nm excitation and 430 nm emission wavelength for $AFM_1$, and 500 nm emission for $AFB_1$ and $AFB_1$-$N^7$-guanine. The HPLC analysis for $AFP_1$ was done with a 30-min elution with 12% acetonitrile followed by a 12–22% acetonitrile linear gradient generated over 12 min, then elution at 22% acetonitrile. The flow rates were 1.5, 0.3, 1.5, 0.3, 1.0 and 0.3 ml/min at 0–17, 18–19, 20–29, 30–41, 42–44 and 45–52 min, respectively. $AFP_1$ was measured by monitoring the fluorescence emission at 500 nm with the excitation wavelength at 365 nm. Authentic aflatoxin standards were used to determine chromatographic retention times. All aqueous mobile phases before use were adjusted by orthophosphoric acid and triethylammonium formate buffer to pH 3.0. Plasma micronutrients were also measured by HPLC using a modification of the method by Miller and Yang (24). Plasma testosterone levels were determined by a radioimmunoassay kit (Biomerieux, Marcy l'Etoile, France).

*Statistical methods*

Odds ratios (ORs) and their 95% confidence intervals (CIs) were computed to examine the associations of the positivity of urinary $AFB_1$-$N^7$-guanine with various variables. Mantel's chi-square test for a trend was performed to examine the dose–response relationship for the odds ratios. Logistic regression was used to estimate ORs when adjusting covariates.

## Results

The age range of the subjects was 33 to 66 years, with a mean age of 51.5 years (±9.8 SD). Only two HBsAg carriers and two HBsAg non-carriers were positive for anti-HCV. $AFM_1$ was observed as the most abundant $AFB_1$ metabolite excreted. The mean level of total $AFB_1$ urinary metabolites (including $AFB_1$, $AFM_1$, $AFP_1$, and $AFB_1$-$N^7$-guanine) was 9.47 ng/ml (±16.89 SD). Forty percent (34/85) of the urine samples contained a detectable level of $AFB_1$-$N^7$-guanine adducts. The mean level of $AFB_1$-$N^7$-guanine adduct in the positive urine samples was 0.52 ng/ml (±1.02 SD). More than 90% of the study subjects had a urinary $AFB_1$-$N^7$-guanine adduct level <10% of the total $AFB_1$ metabolites excreted in the urine.

In univariate analysis, the odds ratios associated with the positivity of urinary $AFB_1$-$N^7$-guanine were elevated with increasing plasma levels of α-tocopherol ($P$ for trend = 0.005) and β-carotene ($P$ for trend = 0.018). No significant association with the $AFB_1$–DNA adducts in urine was observed for chronic HBsAg carrier status and plasma levels of testosterone and other nutrients. Since there were correlations among various plasma nutrients, logistic regression analysis was adopted to estimate multivariate-adjusted ORs associated with the positivity of urinary $AFB_1$–DNA adducts. After taking into account other potential confounders including the total $AFB_1$ urinary metabolites, plasma α-tocopherol level retained its significance. Chronic HBsAg carrier status and plasma cholesterol level were also significant factors associated with the positivity of urinary $AFB_1$–DNA adducts. Increase in the detection rate of the adducts was related to the plasma α-carotene at a borderline significant level (high versus low tertile, $P = 0.0548$). Although a positive association was also observed between plasma β-carotene levels and urinary $AFB_1$–DNA adducts, this association was not statistically significant. Plasma lycopene level was inversely associated with the positivity of the adducts. No significant association with urinary $AFB_1$–DNA adducts was found for plasma retinol and testosterone. Because plasma levels of α-carotene and β-carotene were highly correlated ($r = 0.64$) and the two carotenoids associated with urinary $AFB_1$–DNA adducts in a similar manner, the levels of the two carotenoids were therefore combined in the analysis. This made almost no change in the ORs of the positivity of urinary $AFB_1$–DNA adducts with other variables. There was a significant positive association between the adducts and plasma levels of α- and β-carotene (Table I).

Table II shows the detection rate of $AFB_1$-$N^7$-guanine in urine by levels of total $AFB_1$ urinary metabolites and plasma nutrients. Due to the sample size not being large, study subjects were categorized into only two groups according to the median of their distribution. The association of urinary $AFB_1$–DNA adducts with the plasma levels of cholesterol, α-tocopherol, and α- and β-carotene was observed at both low and high exposure levels of $AFB_1$. The multivariate-adjusted ORs associated with the positivity of the $AFB_1$–DNA adducts in urine suggested a strong synergistic interaction of $AFB_1$ exposure with plasma levels of these nutrients. In contrast, lycopene appeared to have an inhibitory effect on $AFB_1$–DNA adduct formation at low and high $AFB_1$ exposure. Interactive effect between plasma α-tocopherol and α- and β-carotene on the adduct levels is depicted in Table III. All the ORs associated with the positivity of urinary $AFB_1$–DNA adducts in relation to various variables in this study were not materially changed when adjustment was also made for storage time of biospecimens and seasons of collecting biospecimens.

**Table I.** Associations of the positivity of urinary $AFB_1$-$N^7$-guanine adducts with HBsAg carrier status and plasma levels of testosterone and various nutrients

| Variable | $AFB_1$-$N^7$-guanine | | Crude OR | Multivariate-adjusted OR (95% CI)[a] | *P* |
|---|---|---|---|---|---|
| | Negative | Positive | | | |
| HBsAg carrier status | | | | | |
| Negative | 28 | 15 | 1.0 | 1.0 | |
| Positive | 23 | 19 | 1.54 | 7.52 (1.51–37.53) | 0.0138 |
| Testosterone (ng/ml) | | | | | |
| ⩽4.7[b] | 17 | 11 | 1.0 | 1.0 | |
| 4.8–5.9 | 16 | 12 | 1.16 | 1.89 (0.33–10.77) | 0.4739 |
| >5.9 | 18 | 11 | 0.94 | 0.93 (0.16–5.50) | 0.9342 |
| Cholesterol (mg/dl) | | | | | |
| ⩽178.7[b] | 19 | 10 | 1.0 | 1.0 | |
| 178.8–216.7 | 20 | 9 | 0.86 | 0.75 (0.15–3.71) | 0.7287 |
| >216.7 | 12 | 15 | 2.38 | 6.99 (1.24–39.44) | 0.0276 |
| Retinol (μg/dl) | | | | | |
| ⩽43.0[b] | 18 | 10 | 1.0 | 1.0 | |
| 43.1–58.0 | 19 | 9 | 0.85 | 0.57 (0.10–3.13) | 0.5169 |
| >58.0 | 14 | 15 | 1.93 | 1.86 (0.32–10.91) | 0.4911 |
| α-Tocopherol (μg/dl) | | | | | |
| ⩽621.0[b] | 22 | 7 | 1.0[c] | 1.0 | |
| 621.1–829.0 | 17 | 9 | 1.66 | 1.88 (0.28–12.71) | 0.5162 |
| >829.0 | 12 | 18 | 4.71 | 11.20 (1.47–85.59) | 0.0199 |
| Lycopene (μg/dl) | | | | | |
| ⩽14.3[b] | 20 | 8 | 1.0 | 1.0 | |
| 14.4–21.7 | 15 | 13 | 2.17 | 0.22 (0.02–2.68) | 0.2343 |
| >21.7 | 16 | 13 | 2.03 | 0.04 (0.002–0.79) | 0.0342 |
| α-Carotene + β-carotene (μg/dl) | | | | | |
| ⩽13.4[b] | 21 | 7 | 1.0[c] | 1.0 | |
| 13.5–22.7 | 17 | 10 | 1.76 | 7.77 (0.65–93.56) | 0.1064 |
| >22.7 | 13 | 17 | 3.92 | 33.12 (1.58–695.66) | 0.0243 |

[a]All the variables shown in the table, total $AFB_1$ urinary metabolites, age and habits of cigarette smoking and alcohol drinking were also included in the multiple logistic regression model.
[b]Categorized according to the tertile distribution of subjects.
[c]Test for trend was statistically significant.

**Table II.** Detection rate of urinary $AFB_1$-$N^7$-guanine adducts by total $AFB_1$ metabolites in urine and plasma levels of selected nutrients

| Variable | Total no. | Detection rate of $AFB_1$-$N^7$-guanine (%) | Crude OR | Multivariate-adjusted OR (95% CI)[a] | *P* |
|---|---|---|---|---|---|
| Total urinary $AFB_1$/α-tocopherol | | | | | |
| Low/low[b] | 22 | 4.5 | 1.0[c] | 1.0 | |
| Low/high | 20 | 45.0 | 17.18 | 22.56 (1.64–309.74) | 0.0197 |
| High/low | 21 | 47.6 | 19.09 | 41.83 (2.99–584.57) | 0.0055 |
| High/high | 22 | 63.6 | 36.75 | 73.19 (4.56–1173.76) | 0.0024 |
| Total urinary $AFB_1$/α-carotene + β-carotene | | | | | |
| Low/low | 24 | 12.5 | 1.0[c] | 1.0 | |
| Low/high | 18 | 38.9 | 4.45 | 8.63 (1.03–72.34) | 0.0470 |
| High/low | 19 | 47.4 | 6.30 | 8.10 (1.25–52.29) | 0.0280 |
| High/high | 24 | 62.5 | 11.67 | 27.68 (3.33–230.41) | 0.0021 |
| Total urinary $AFB_1$/lycopene | | | | | |
| Low/high | 21 | 28.6 | 1.0[c] | 1.0 | |
| Low/low | 21 | 19.0 | 0.59 | 3.02 (0.27–34.35) | 0.3725 |
| High/high | 21 | 57.1 | 3.33 | 3.45 (0.62–19.02) | 0.1556 |
| High/low | 22 | 54.5 | 3.00 | 32.55 (3.22–329.40) | 0.0032 |
| Total urinary $AFB_1$/cholesterol | | | | | |
| Low/low | 23 | 21.7 | 1.0[c] | 1.0 | |
| Low/high | 19 | 26.3 | 1.29 | 1.54 (0.21–11.31) | 0.6701 |
| High/low | 21 | 47.6 | 3.27 | 4.04 (0.69–23.52) | 0.1204 |
| High/high | 22 | 63.6 | 6.30 | 11.03 (1.78–68.16) | 0.0098 |

[a]Age, HBsAg carrier status, habits of cigarette smoking and alcohol drinking and plasma levels of testosterone and other nutrients were also included in the multiple logistic regression model.
[b]High and low level was categorized according to the median of study subjects.
[c]Test for trend was statistically significant.

**Table III.** Detection rate of urinary $AFB_1$-$N^7$-guanine adducts by plasma levels of α-tocopherol and α-carotene plus β-carotene

| α-Tocopherol/α-carotene + β-carotene | Total no. | Detection rate of $AFB_1$-$N^7$-guanine (%) | Crude OR | Multivariate-adjusted OR (95% CI)[a] | *P* |
|---|---|---|---|---|---|
| Low/low[b] | 25 | 20.0 | 1.0[c] | 1.0 | |
| Low/high | 18 | 33.3 | 2.00 | 8.19 (0.74–90.77) | 0.0867 |
| High/low | 18 | 38.9 | 2.55 | 7.30 (0.96–55.30) | 0.0543 |
| High/high | 24 | 66.7 | 8.00 | 29.43 (2.94–294.27) | 0.0040 |

[a]Age, total $AFB_1$ urinary metabolites, habits of cigarette smoking and alcohol drinking, HBsAg carrier status and plasma levels of testosterone and other nutrients were also included in the multiple logistic regression model.
[b]High and low level was categorized according to the median of study subjects.
[c]Test for trend was statistically significant.

## Discussion

The data on urinary aflatoxins were based on spot urine samples. Although no adjustment was made for urine concentration in the calculation of $AFB_1$-$N^7$-guanine excretion in this study, this may not pose significant problems for investigation of the associations between various variables and the positivity of urinary $AFB_1$–DNA adducts, because total quantity of $AFB_1$ metabolites excreted in urine was included in the multivariate analyses as a covariate for adjusting its effect.

Epidemiological studies suggest that HBV and $AFB_1$ may exert a synergistic effect on the development of HCC (4). The mechanisms responsible for this interaction remain to be elucidated. In woodchucks, chronic infection with woodchuck hepatitis virus, a virus similar in characteristics to HBV, produced an enhanced metabolic activation of chemical carcinogen, including $AFB_1$ (25). However, epidemiological studies of the relationship between HBsAg carrier status and the formation of $AFB_1$–DNA adducts, using $AFB_1$-albumin adduct or urinary $AFB_1$-$N^7$-guanine as the surrogate dosimeter for estimating hepatic DNA binding by $AFB_1$, have been inconsistent (16,26–28). We have previously demonstrated that the detection rate of urinary $AFB_1$-$N^7$-guanine adducts was higher in HBsAg carriers than in non-carriers, but this association was not statistically significant in univariate analysis or in a multivariate analysis including the total $AFB_1$ urinary metabolites, age, and habits of cigarette smoking and alcohol drinking, as covariates (16). In the present study, HBsAg carrier status was strongly associated with urinary $AFB_1$–DNA adduct levels after adjustment was made for plasma nutrients and other potential confounders. The discrepancy between our two studies may be due to the difference in the control of plasma nutrients that were significantly associated with the $AFB_1$–DNA adduct levels. The possible biological mechanisms for HBV involvement in the genesis of HCC have been extensively reviewed (1). This study provides evidence that chronic HBV infection may interact with $AFB_1$ intake in the initiation process of hepatocarcinogenesis and thus result in an increased risk of $AFB_1$-related HCC.

HCC is two to three times more frequent in men than in women (1). The marked sex difference in susceptibility to hepatocarcinogenesis was also observed in various animal models (29–31). A relationship between elevated serum testosterone level and HCC risk in humans has been documented (12). In experimental studies, the mechanism for the action of testosterone in hepatocarcinogenesis may be through its effects on promotion of the growth of tumor and/or modulation of the activity of enzymes involved in metabolism of hepatocarcinogens (23,30). For a given level of $AFB_1$ exposure, male rats were found to produce more $AFB_1$-epoxide, which can form adducts with DNA, than female rats (23). However, variation in plasma testosterone levels was not correlated with $AFB_1$–DNA adducts in this study.

Low serum retinol level has been associated with the development of various human cancers, including HCC (14). Liver fractions from rats with vitamin A-deficiency formed a higher level of DNA adducts by $AFB_1$ (32). Our previous *in vitro* study with cultured woodchuck hepatocytes demonstrated a potent inhibitory effect of retinol on $AFB_1$–DNA adduct formation (22). However, we failed to find a significant association of $AFB_1$–DNA adducts with plasma retinol levels in this cross-sectional study. The reason for the discrepancy between our *in vitro* and human study is unclear but may be explained by the difference in the effect of retinol between species. On the other hand, since this study was conducted in a well-nourished population with low prevalence of vitamin A-deficiency, whether a more striking association between urinary $AFB_1$–DNA adducts and plasma retinol level may be shown up in malnourished populations requires further investigations.

Lycopene, α-carotene and β-carotene are carotenoids with a similar chemical structure. They are antioxidant contents of vegetables and fruits (21). Experimental and epidemiological studies on the potential role of α-carotene and lycopene in carcinogenesis are limited (33–37). The influence of β-carotenoids on susceptibility to various forms of cancers have been evaluated in a number of epidemiological studies (38–40). Although the majority of results have shown that high intake and/or high serum level of β-carotene were associated with a reduced risk of cancer at several sites (38,39), some data are conflicting and the significance of β-carotene in carcinogenesis remains unclear (40). The most marked finding of this study was the strong and extremely diverse associations of various carotenoids with $AFB_1$-$N^7$-guanine adducts in urine. α-Carotene and β-carotene were positively associated with the DNA adducts, while the adducts appeared to reduce with increasing plasma levels of lycopene. Difference in the ability of diverse carotenoids to suppress the development of spontaneous HCC and chemically-induced neoplastic transformation, enhance gap junctional communication and regulate gene expression have been shown in experimental studies (34–37). Although the underlying mechanism for the molecular specificity of each of the carotenoids to influence $AFB_1$–DNA adduct formation is unclear, this study provides additional insight into the complexity of the biological function of carotenoids.

α-Tocopherol is among the most potent antioxidants from natural source (21,36). Several experimental and epidemiological suggest that α-tocopherol may reduce the risk of cancer

(41). The potential use of α-tocopherol in cancer chemoprevention has been evaluated in lung cancer, oral leukoplakia and colorectal polyps. However, epidemiologic evidence for its chemopreventive action is inconsistent (40,42,43). In this study, the detection rate of the $AFB_1$–DNA adducts in urine was significantly elevated with increasing plasma levels of α-tocopherol. There was a synergistic interaction between α-tocopherol and α- and β-carotene on the DNA adduct levels.

The data demonstrating a positive association between urinary $AFB_1$–DNA adducts and the plasma levels of α-tocopherol and β-carotene are in accordance with our previous *in vitro* study in which α-tocopherol and β-carotene enhanced the adduct formation in cultured woodchuck hepatocytes (22). Urinary $AFB_1$–DNA adduct levels can be influenced by carcinogen metabolism and DNA repair. However, it was shown that a supplement with antioxidant vitamins had no significant effect on DNA repair activity (44,45). Thus, our findings cast doubts on the potential role of certain dietary antioxidants, such as α-tocopherol, α-carotene and β-carotene, in prevention of $AFB_1$-induced DNA damage. However, it is premature to conclude that these antioxidant vitamins have an adverse effect on the development of HCC. The pathogenesis of cancer is a multistage process including DNA alteration and cell proliferation. Vitamins may have multiple biological mechanisms to inhibit or retard one or more stages of carcinogenesis. The association of HCC risk with plasma levels of various antioxidant vitamins remains to be elucidated. On the other hand, our prospective study has demonstrated that an increased risk of HCC is associated with low vegetable intake (14). Vegetables contain a wide variety of phytochemicals with the potential to modulate carcinogenesis (21). Whether constituents in vegetables other than α-carotene and β-carotene may be important protective factors for $AFB_1$-induced HCC required further studies.

To date we have not found any reports on the effect of cholesterol on $AFB_1$–DNA adduct formation. The finding of the positive association of plasma cholesterol with urinary $AFB_1$–DNA adducts in this study warrants further investigation.

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