Effect of the South African traditional meat, biltong, on cancer-associated enzymes CYP2E1 and CYP1A2

To the Editor: It is now accepted that diet is a significant factor in predisposing man to development of some cancers and that some cytochrome-P450 enzymes are involved. Cytochrome-P450 is a family of enzymes active against a broad spectrum of endogenous and exogenous substrates. Although in most cases the products of metabolism are inactive, at times they are carcinogenic as in cancers induced by heterocyclic aromatic amines (HAAs) and polycyclic aromatic hydrocarbons (PAHs). HAAs and PAHs are a family of pro-mutagens and pro-carcinogens formed during the cooking of meat at high temperatures;1,2 HAAs are produced from high-temperature cooking of animal proteins, while PAHs are produced from incomplete combustion and pyrolysis. In animal studies,3,4 development of colon cancer was associated with consumption of meat that had been well done, browned or barbecued, or just red meat. Most important, however, HAAs and PAHs need to be activated in order to act as carcinogens. Therefore, the risk to a high-risk phenotype. CYP2E1 is of particular interest because it is also involved in the activation of many compounds, particularly pro-carcinogens such as N-nitrosamines, low-molecular-weight chemicals such as benzene and styrene and a number of halogenated carbons.8 Therefore, understanding the effect of meat preparation on these two isoenzymes may be valuable in predicting the risk for development of cancer or drug toxicity.

Of concern to South Africans is that, in addition to ingestion of barbecued meat, many people also eat a traditional meat called biltong. Biltong is prepared by gentle drying of raw meat after pre-treatment with salts and undisclosed ingredients. Since biltong is not cooked and it is not fresh meat, it is bound to be different in HAA and PAH composition. Unfortunately, no study has investigated the content of pro-carcinogens in biltong or its effect on cytochrome-P450 activity. Such a study would help to unveil the risk, if any, of cancer or drug toxicity posed by biltong consumption by millions of South Africans. Here, the composition of two major procarcinogens, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), an HAA, and benzo(a)pyrene (BaP), a PAH in biltong, as well as the effect of biltong on the activity of human cytochrome-P450 enzymes CYP1A2 and CYP2E1 in healthy volunteers, were investigated.

Three identical samples of biltong, 1 g each, were extracted and analysed for MeIQx and BaP using a high-performance liquid chromatography (HPLC) method published by Rivera et al.5 CYP1A2 activity was assessed by the ratio of 17-dimethylxanthine plus 1,7-dimethyluric acid to 1,3,7-trimethylxanthine (17X+1,7U: 137X) in the 12-hour urine. Caffeine and its metabolites were analysed as described by Grant et al.6 CYP2E1 activity was assessed using plasma chlorzoxazone metabolic ratio (MR) of 6-OH-chlorzoxazone to chlorzoxazone (6-OH: CZN) at 4 hours. Chlorzoxazone and 6-hydroxychlorzoxazone were measured using an HPLC method reported by Frye and Stiff.7

Nine healthy volunteers, males and non-pregnant females (HCG test-negative), aged 20 - 30 years were enlisted in a cross-over study during which they were studied on two occasions; 48 hours before start, and then 24 hours after completion of a 5-day biltong-enriched diet. The University of the Free State Ethics Committee approved the study.

On each day of the study subjects came to the clinic having fasted from midnight the previous day. They emptied their bladder before start of the study and then swallowed two tablets of chlorzoxazone (500 mg) with 200 ml of water, followed by a cup of coffee 5 g (154 mg of caffeine), within 5 minutes. Chlorzoxazone tablets (United States Pharmacopoeia) 250 mg (AMIDE Pharmaceutical, Inc., New Jersey, USA) were donated by RB Kim, Vanderbilt University, USA. Liquids were allowed 2 hours after drug ingestion while solids were allowed after 4 hours. A 10 ml blood sample was drawn in heparinised tubes via antecubital venepuncture 4 hours after oral intake of the medicines. The blood sample was immediately placed on ice and sent to the laboratory where it was centrifuged and the plasma extract stored at –20°C until use for assay of chlorzoxazone and 6-hydroxychlorzoxazone. Patients were observed for at least 2 hours after the blood sample withdrawal before they were allowed to be ambulatory. Urine was collected for 12 hours, the total volume measured and a sample (20 ml) was stored for urinalysis and analysis for caffeine (137X) and chlorzoxazone, and their metabolites.

The biltong was finely crumbled in a food processor after which it was weighed and stored in batches of 5 packets of 250 g each. One batch was assigned to each subject, who used 1 packet on each study day. Over the 5 days, subjects consumed approximately 2.5 - 3.0 g/kg/day of biltong, plus other selected foods. Food proportions were calculated per kilogram weight at a normal metabolic rate with the help of a specialist dietician. The daily amount of proteins and fats used in the
food were according to those used in other studies in our clinic; they contained 36% kcal from fat, 44% kcal from carbohydrate, 20% kcal from protein and 7 g of dietary fibre.

During the study all meals were served in the clinic and no external food or self-administered supplements were allowed. The meals were served and closely supervised by the investigators. Evening meals were prepackaged at the facility for home consumption. Subjects were weighed each day before breakfast. Whenever a subject reported getting hungry, he or she was moved to a higher calorie level such that the serving size, but not the nature of the food, on the menu was changed. Also, the amount of biltong eaten was not changed.

Results were evaluated using non-parametric statistics. Metabolic ratios before and after intervention were compared using the Wilcoxon’s signed-rank test with level of significance at \( p < 0.05 \).

The pro-carcinogens MeIQx or B[a]P were not detected in any of the biltong samples. This agrees well with the fact that biltong is not cooked but gently dried, conditions that minimise formation of HAAs and PAHs.

One subject withdrew from the study because he had to move to another city during the study week. The physiological parameters of the remaining 8 subjects were within the normal range before treatment and remained so during treatment. Specifically there was no liver or renal impairment, or haematological abnormality. Fig. 1 illustrates the activity of CYP2E1 before and after the biltong diet. There was no change in the activity of CYP2E1 before and after the biltong diet. Average chlorzoxazone MR was (mean ± standard deviation (S.D)) 0.337 ± 0.16 before, and 0.36 ± 0.14 after, the biltong diet \( (p = 0.414) \). Also, the percentage dose of chlorzoxazone excreted as 6-hydroxy-chlorzoxazone in the 12 hours before and after the biltong diet were similar, viz. 112 ± 64.9% and 99.3 ± 44.2%, respectively, indicating no change in renal function. Of note, a 250% increase in activity of CYP2E1 was observed in 1 subject (P03) while a 226% decrease in activity was observed in another subject (P08). The cause could not be explained.

Fig. 2 illustrates the activity of CYP1A2 before and after the biltong diet. There was no change in the activity of CYP1A2 before and after the biltong diet. The average urinary caffeine MR was 1.77 ± 0.79 before the diet, versus 1.62 ± 0.37 after the diet \( (p = 0.348) \). The corresponding creatinine clearance was 112.4 ± 24.1 ml/min and 95.97 ± 29.04 ml/min, respectively, indicating that renal function was normal before and during the study. Again, there was an 83.3% increase in CYP1A2 activity in subject P03 and a decrease in activity of 69.4% for subject P08.

This study demonstrated that with regard to pro-carcinogen content meat is safer when eaten dried, as in biltong prepared the traditional South African way. The preparation of biltong involves pre-treatment of the raw meat using salts and other chemicals in a process akin to marinating, before gentle drying. The pre-treatment formulas are sold as ‘biltong spices’; there is wide variation in the composition of the different biltong spices, which is probably one of the causes of variation in biltong prepared by different producers. The drying is done gently at low temperatures (25 - 30°C) in a room, with the neatly cut meat hung up to ensure uniform drying by allowing free air flow and draining of the meat juice. The drying process usually takes approximately 3 months. Direct heating is prohibited. The use of different drying methods has been a cause of variation in biltong from different producers. We were satisfied with our biltong because its quality was certified by the department of dietetics which had analysed its nutrient content and method of preparation earlier.

Many HAAs and PAHs are produced during the cooking of meat but in this study only B[a]P and MeIQx were analysed because their standard compounds were easier to obtain. Also,
B[a]P is commonly used as a general measure for the 5-ring PAH compounds. It was envisaged that, as observed in other studies, changes in these 2 compounds would reflect similar trends in their analogues.

It appears that the ‘pre-treatment’ and ‘gentle drying’ steps in the preparation of biltong are the cornerstone to the reduced formation of HAAs and PAHs. This is supported by several reports that grilling and barbecuing of meat increase HAA content several-fold. This was also confirmed in our laboratory where it was found that heating different South African meats in the oven at 250°C for 20 minutes increased their hydrocarbon content several times. Concentrations of MeIQx were increased from 48.9 to 337.6 ng/100 g of fish, 7.8 to 3 372 ng/100 g of beef, 0.0 to 358.9 ng/100 g of pork and 0.0 to 1 493.5 ng/100 g of ostrich. Regarding pre-treatment, it was reported that formation of HAAs and PAHs was prevented when the meat was marinated before grilling. Most probably the tenderiser ingredients prevented oxidation of animal proteins to hydrocarbons or, simply, they interfered with their detection. In this study, interference with the assay was excluded by the feeding of biltong to volunteers, as was done in previous studies with the cooked meat. Whereas increased activity of CYP1A2 was observed in healthy volunteers who consumed the cooked meat, the present study showed that there was no change in CYP1A2 activity in healthy volunteers who were fed biltong. Surely, if there were hydrocarbons in cooking biltong, this would have been demonstrated by their effect on the enzymes.

Although the small sample size could be a limiting factor in this study, better conclusions are unlikely even with a larger sample size until the ‘change in enzyme activity’ at which the risk for cancer is significant has been determined. Our data support the idea that biltong is another traditional food with traditional meat, biltong, does not contain HAAs or PAHs, and has no significant effect on CYP1A2 and CYP2E1 activity, implying that consumption of biltong may not pose any additional cancer risk with regard to activity of these two enzymes. These observations call for a re-evaluation of our traditional recipes with regard to carcinogenesis.

In conclusion, we have demonstrated that the South African traditional meat, biltong, does not contain HAAs or PAHs, and has no significant effect on CYP1A2 and CYP2E1 activity, implying that consumption of biltong may not pose any additional cancer risk with regard to activity of these two enzymes. These observations call for a re-evaluation of our traditional recipes with regard to carcinogenesis.

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