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Association of exposure to polycyclic aromatic hydrocarbons with inflammation, oxidative DNA damage and renal-pulmonary dysfunctions in barbecue makers in Southern Nigeria

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Abstract

Background: Multiple organ dysfunctions have been linked to exposure to polycyclic aromatic hydrocarbons (PAH) and oxidative stress (OS), oxidative DNA damage, and inflammatory response to PAH have been implicated. The biomarkers of OS (malondialdehyde (MDA), total plasma peroxide (TPP), total antioxidant capacity (TAC), glutathione (GSH), nitric oxide (NO), oxidative stress index (OSI)); 8-hydroxy-2-deoxyguanosine (8-OHdG)); tumor necrosis factor-alpha (TNF-α)); 1-hydroxy pyrene (1-HOP)), serum and urine creatinine, uric acid (UA), estimated glomerular filtration rate (eGFR) and peak expiratory flow rate (PEFR) were assessed in barbecue makers.

Methods: One hundred barbecue makers and 50 controls were enrolled into the study. Serum and urine creatinine, UA, TAC, MDA, GSH, NO and TPP were estimated by colorimetry, 8-OHdG and TNF-α by ELISA, PEFR using peak flow meter, 1-HOP by HPLC, eGFR and OSI by calculation.

Results: Barbecue makers had lower TAC, PEFR, and higher TNF-α and OS compared to controls ($p<0.05$). Higher TNF-α, lipid peroxidation, and lower antioxidants were observed in barbecue makers who had worked for >5years compared to <5years ($p<0.05$). Increasing number of working hours was associated with higher NO, lipid peroxidation, OS and lower antioxidants in barbecue makers ($p<0.05$). Positive associations were observed between 1-HOP and TPP ($r=0.570$, $p=0.000$), OSI ($r=0.299$, $p=0.035$) and negative association between TAC and TNF-α ($r=-0.209$, $p=0.037$), MDA ($r=-0.265$, $p=0.008$) in barbecue makers.

Conclusion: Increased lipid peroxidation, OS, inflammation and depressed antioxidants and lung function observed in barbecue makers suggest increased risk of chronic lung conditions which may be associated with exposure to PAH in barbecue fumes.

Keywords: Inflammation, Kidney, Lipid Peroxidation, Lung, Oxidative Stress, Polycyclic Aromatic Hydrocarbon.

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Introduction

Environmental and occupational exposure to toxic chemicals including PAH have been associated with deleterious health effects including cardio-pulmonary diseases, hepato-renal toxicities, and various forms of cancers (1). PAHs are organic compounds with fused benzenoid ring derived from incomplete combustion of organic matter. Sources of PAH include tobacco smoke, waste incineration, residential and industrial heating, and motor vehicle exhaust gas. Major route of human exposure to PAH includes inhalation of polluted air, dietary intake and dermal contact (2).

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In mammalian cells, PAHs undergo metabolic activation to form free radical intermediates which can undergo redox cycling to generate reactive oxygen species (ROS) that bind covalently to biomolecules as lipids, proteins, and DNA, leading to lipid peroxidation, formation of protein and DNA adduct, oxidative stress and DNA mutations (3). These processes have been implicated in PAH induced multiple organ dysfunctions including immunologic, renal and lung dysfunctions (4). Exposure to PAH has been associated with increased plasma levels of lipid peroxidation products (5, 6), biomarkers of oxidative stress and oxidative DNA damage (3) and decline in eGFR and lung functions (7, 8).

Processing of meat and other food products by smoking, grilling and broiling lead to increase formation of PAHs, hence barbecue makers are therefore exposed to higher-than-normal levels of PAHs via inhalation which may predispose to increased risk of developing PAH induced systemic toxicities including cancers (4). Increasing incidence of lung and colon cancers has been reported in the study area (9), though their causes are still uncertain, it may be speculated that unregulated proliferation of barbecue making ventures may be implicated. The level of exposure to PAH and the cellular oxidative insults and multiple organ dysfunctions accruing from such exposures among barbecue makers is still uncertain. This study assessed the biomarkers of OS, inflammation, oxidative DNA damage and renal-pulmonary functions among barbecue makers.

Materials and Methods

Ethical Clearance

One hundred and fifteen adult men and women is the actual number of the participants in the current study. The study protocol was approved ethically by the Cross River State Ministry of Health Research Ethics Committee (REC No. RP/REC/2017/405), and every participant has signed a written consent form after listening to a brief presentation about the project. The conduct of the study was in compliance with the ethical principles guiding medical research involving human subjects as declared in Helsinki in 1975 and subsequent revisions of the declaration.

Study design

This comparative cross-sectional study assessing the lung and renal functions, biomarkers of OS, oxidative DNA damage and inflammatory response to exposure to PAH in barbecue makers was conducted in Calabar, Southern Nigeria.

Selection of subjects

The population of the study was made up of 100 apparently healthy barbecue makers aged 18-40 years and 50 age and sex matched control group of non-barbecue makers residing in Calabar. The barbecue makers were made up of individuals that prepare and sell barbecued food daily as means of livelihood without the use of personal protective devices for the past one year and above. They were recruited based on simple random sampling method from selected major barbecue outlets in the area of study. The control group were individuals who are not involved in barbecue making, have not consumed barbecued food, or been subjected to prolonged exposure to smoke of any form in the past 12 weeks. A semi-structured questionnaire was administered to all subjects of study to obtain information on socio-demographic characteristics, health status, family, employment and medical history and lifestyle habits. Anthropometric indices and blood pressure were obtained using standard methods. Individuals with a history of cigarette smoking, alcoholic addiction, drug, or substance abuse or suffering from any chronic organ or systemic illness and long- term medication were excluded from the study.

Sample collection

Random urine samples (10 ml) were collected from all subjects into a sterile universal bottle for the estimation of creatinine, 1-HOP and 8-OHdG. Whole blood samples (5 ml) were also collected aseptically by venipuncture from the cubital fossa into a gel separator vacutainer.

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The blood samples were spun at 500g for 5 minutes at room temperature to extract sera for the estimation of creatinine, uric acid, TPP, MDA, NO, GSH, TAC and TNF-α. Samples were collected after close of work at the workplace.

Laboratory methods

The PEFR of the study population were determined using the peak flow meter (10); PAH metabolite (1-HOP) were estimated using high performance liquid chromatography (11); renal function indices (urine creatinine, serum creatinine, uric acid and eGFR) were estimated using modified Jaffe's reaction method, enzyme colorimetric method and Cockcroft-Gault equation respectively (12-14). Biomarkers of inflammation (TNF-α) and oxidative DNA damage (8-OHdG) were determined by enzyme linked immunosorbent assay (15,16); while biomarkers of oxidative stress (TAC, TPP, NO, GSH, MDA) were determined by colorimetric methods and OSI by calculation respectively (17- 21).

Statistical analysis

Results were presented as mean±standard deviation and analyzed using the SPSS version 23.0. Student's t-test was used to determine mean differences between groups, analysis of variance was used to determine variations within and among groups, and Pearson's correlation analysis were employed for determination of associations between variables. Results were considered significant at $p< 0.05$.

Results

Table 1 shows comparison of age, BMI, systolic and diastolic blood pressure, biomarkers of oxidative stress (MDA, TPP, TAC, OSI, NO, GSH), oxidative DNA damage (8-OHdG) and inflammation (TNF-α), 1-HOP, PEFR, uric acid, serum, and urine creatinine and eGFR in barbecue makers and non-barbecue makers. The diastolic blood pressure, OSI and TNF-α were significantly higher and TAC and PEFR lower in barbecue makers compared to non-barbecue makers ($p< 0.05$). There were no significant differences in the levels of other indices between the 2 groups ($p> 0.05$).

Table 1. Comparison of age, BMI, BP, indices of OS, inflammation and oxidative DNA damage, 1-hydroxy pyrene, lung, and renal functions in barbecue makers and non-barbecue makers.

Parameter	Barbecue makers n= 100	Non-Barbecue makers n= 50	p-value
Age (years)	26.24±5.21	25.90±5.19	0.745
BMI (kg/m^2)	23.84±4.19	23.92±4.26	0.925
SBP (mmHg)	123.00±14.25	119.44±10.75	0.162
DBP (mmHg)	80.60±11.85	76.60±7.52	0.047*
MDA (mmol/L)	50.76±17.78	49.00±17.34	0.618
TPP (mmol/L)	20.02±6.52	18.51±5.74	0.222
TAC (mmol/L)	73.61±21.49	87.59±19.82	0.001*
OSI (%)	30.24±15.20	22.56±9.32	0.003*
NO (nmol/L)	26.32±6.24	26.70±6.30	0.763
GSH (mmol/L)	77.61±9.27	80.11±9.59	0.189
8-OHdG (ng/ml)	59.99±43.12	56.20±49.27	0.683
TNF-α (pg/ml)	8.64±16.08	2.42±0.67	0.009*
1-HOP (µg/g Cr)	1.34±1.84	0.78±0.85	0.056
PEFR (L/min)	382.00±93.27	424.70±108.13	0.037*
UA (mmol/l)	0.51±0.11	0.52±0.09	0.825
sCr (µmmo/L)	126.14±29.20	117.09±15.00	0.055
uCr (mg/L)	811.77±601.22	716.96±434.21	0.368
eGFR (ml/mins)	75.77±15.17	80.07±16.40	0.176

Result presented as mean±SD, *, significant at $p< 0.05$; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MDA, malomdialdehyde; TPP, total plasma peroxides; TAC, total antioxidant capacity; OSI, oxidative stress index; NO, nitric oxide; GSH, reduced glutathione; 8-OHdG, 8-hydroxy-2-deoxyguanosine; TNF-α, tumour necrosis factor alpha; 1-HOP, 1-hydroxy pyrene; PEFR, peak expiratory flow rate; UA, uric acid; sCr, serum creatinine; uCr, urine creatinine; eGFR, estimated glomerular filtration rate.

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Figure 1 shows effect of years at work on BMI, MDA, TPP, OSI, GSH and TNF-α in barbecue makers. Significantly higher BMI, MDA, TPP, OSI and TNF-α and lower GSH) were observed in barbecue makers who had worked for>5 yrs compared to< 5 yrs ($p< 0.05$). No significant differences were observed in the levels of other indices studied with the number of years at work ($p> 0.05$).

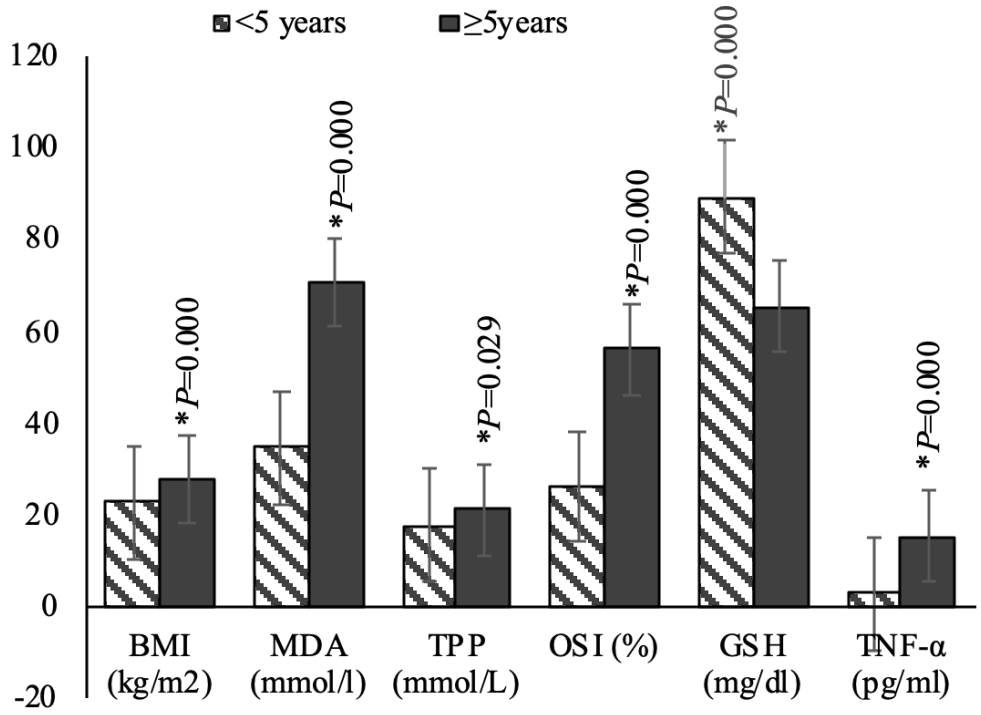

Fig. 1. Effect of years at work on BMI, MDA, TPP, OSI, GSH and TNF-α in barbecue makers. Result presented as mean±SD, *, significant at $p< 0.05$; BMI, body mass index; MDA, malondialdehyde; TPP, total plasma peroxides; OSI, oxidative stress index; GSH, reduced glutathione; TNF-α, tumour necrosis factor alpha;

The effect of hours at work on systolic and diastolic blood pressure, biomarkers of oxidative stress (MDA, TPP, TAC, OSI, NO, GSH), oxidative DNA damage (8-OHdG) and inflammation (TNF-α), 1-HOP, PEFR, uric acid, serum, and urine creatinine and eGFR in barbecue makers were depicted in Table 2. Significant variations were observed in the BMI, SBP, MDA, TPP, TAC, OSI, NO and GSH levels among barbecue makers who work for < 5 hours, 5-10 hours and >10 hours daily. Lower BMI, SBP, MDA, TPP, OSI and NO and higher TAC and GSH were observed in those who work for < 5 hours daily compared to those working for > 5 hours ($p< 0.05$) while those working for 5-10 hours had lower BMI, NO and GSH compared to those working for > 10 hours. No significant differences were observed in the levels of other indices studied with the number of hours at work ($p> 0.05$).

Table 3 shows the correlation of biochemical indices in barbecue makers studied. Significant positive correlations were observed between 1-HOP and TPP ($r= 0.570$, $p=<0.001$), 1-HOP and OSI ($r=0.299$,

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p=0.035) and negative correlations between TAC and TNF-α (r= -0.209, p= 0.037), TAC and MDA (r= -0.265, p= 0.008) only in barbecue makers.

Table 2. Effects of hours at work on BMI, BP, indices of OS, inflammation and oxidative DNA damage,1-hydroxy pyrene, lung, and renal functions in barbecue makers.

Parameter	1-<5 hours n= 12	5-10 hours n= 54	>10 hours n= 34	p-value
BMI (kg/m^2)	23.17±2.86^{b}	22.00±2.88^{c}	27.00±4.62	0.000*
SBP (mmHg)	108.00±22.77^{ab}	122.62±11.35	128.88±11.35	0.006*
DBP (mmHg)	75.33±17.45	79.37±10.45	84.41±11.38	0.201
MDA (mmol/L)	35.00±7.87^{b}	49.52±13.78	53.59±22.88	0.081
TPP (mmol/L)	12.42±2.03^{ab}	20.52±7.13	19.78±5.85	0.023*
TAC (mmol/L)	111.71±26.97^{ab}	78.32±18.89	68.56±25.92	0.001*
OSI (%)	11.39±1.21^{ab}	28.24±13.99	33.08±15.74	0.008*
NO (nmol/L)	25.00±2.00^{b}	24.03±4.41^{c}	30.41±7.71	0.002*
GSH (mmol/L)	98.04±4.14^{ab}	75.11±7.54^{c}	83.26±10.51	0.000*
8-OHdG (ng/ml)	34.67±39.91	59.16±39.25	70.26±48.38	0.222
TNF-α (pg/mL)	1.80±0.56	8.21±16.70	11.57±17.73	0.444
1-HOP(µg/gCr)	0.54±0.58	1.61±2.27	1.17±1.21	0.401
PEFR (L/min)	340.83±77.61	407.59±98.03	355.88±81.74	0.102
UA (mmol/l)	0.57±0.10	0.50±0.09	0.53±0.10	0.269
uCr (mg/L)	786.78±224.70	908.06±131.61	667.65±112.30	0.441
sCr (µmmo/L)	128.53±27.97	120.52±29.44	134.19±28.88	0.318
eGFR (ml/mins)	72.87±16.42	77.92±15.10	73.36±15.26	0.561*

Result presented as mean±SD, *, significant at p< 0.05; a=significant difference between 1-<5hrs and 5-10hrs, b= significant difference between 1-<5hrs and >10hrs, c= significant difference between 5-10hrs and >10hrs, BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; MDA= malondialdehyde; TPP= total plasma peroxides; TAC= total antioxidant capacity; OSI= oxidative stress index; NO= nitric oxide; GSH= reduced glutathione; 8-OHdG=8-hydroxy-2-deoxyguanosine; TNF-α= tumour necrosis factor alpha; 1-HOP=1-hydroxy pyrene; PEFR= peak expiratory flow rate; UA= uric acid; sCr= serum creatinine; uCr= urine creatinine; eGFR= estimated glomerular filtration rate

Table 3. Correlation of 1-hydroxypyrene, indices of inflammation (TNF-α) and oxidative stress (MDA, TPP & OSI) in barbecue makers.

Parameter		R	p-value
1-HOP versus	TPP	0.570	0.001*
	OSI	0.299	0.035*
TAC versus	TNF-α	-0.209	0.037*
	MDA	-0.265	0.008*

*= significant difference at p< 0.05; 1-HOP= 1-hydroxypyrene, TPP= total plasma peroxides, OSI= oxidative stress index, TAC= antioxidant capacity, MDA= malondialdehyde, TNF-α= tumour necrosis factor alpha.

Discussion

In this study, the barbecue makers studied had higher levels of 1-HOP (biomarker of PAH exposure) compared to controls though not statistically significant. Comparable levels of PAH between barbecue makers and controls may be related to the observation that majority of barbecue makers recruited into the study work in open spaces hence air current dilute and distribute the fumes generated, thus reducing the concentration of PAH they would have been exposed to if they were working in confined spaces. Our finding is in consonance with a study which also reported comparable levels of urinary PAH metabolites concentration in kitchen workers exposed to cooking fumes containing PAHs compared to unexposed controls (22).

The PEFR was significantly lower and diastolic blood pressure higher in barbecue makers compared to the controls. The decline in

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PEFR in barbecue makers may be attributed to reduced air flow rate in the bronchial airways as a result of smoke inhalation and exacerbated by high concentration of PAH in the air (23). Deposition and retention of PAH and inflammatory response to PAH in the airways has been implicated in PAH induced decline in respiratory functions (6). Significant reduction in PEFR has also been reported among kitchen workers exposed to PAH compared to controls by a previous study (23). Higher diastolic blood pressure observed in barbecue makers may be attributed to exposure to PAH present in barbecue fumes. Unfavorable changes in BP associated with exposure to PAH may be related to OS induced structural and functional changes in vascular smooth muscle leading to blood pressure disorder. Diastolic blood pressure has been positively associated with exposure to PAH in chimney sweeps (7).

Barbecue makers had lower TAC and higher OSI compared to controls. Lower TAC levels with increased OSI in barbecue makers may be related to their increased exposure to PAH in barbecue fumes generated from incomplete combustion of meats used in barbecue making via inhalation. Inhaled PAHs are actively metabolized to redox-reactive intermediates that can induce generation of radical species that upset the redox balance leading to oxidative stress, lipid peroxidation and depression of antioxidants (4). The end result will be preponderance of indices of lipid peroxidation (TPP, MDA and OSI) and depression of antioxidants; (TAC and GSH). Consistent with our findings, higher levels of biomarkers of OS and lipid peroxidation have been observed in adults occupationally exposed to PAH compared to controls (8). The negative correlations observed between TAC and MDA in the barbecue makers are in line with the postulation that increasing levels of lipid peroxidation products may be associated with a corresponding decrease in antioxidants as a result of increased consumption to maintain redox balance (24, 25). Positive associations were observed between urine levels of 1-OHP and TPP and OSI in barbecue makers. Previous studies have demonstrated strong positive correlation between urinary 1-OHP and total oxidant status as evidence for PAHs induced lipid peroxidation and oxidative stress (26). Urinary PAH levels have also been positively associated with serum markers of OS (8).

Increasing number of working hours and years at work were associated with higher NO, lipid peroxidation (MDA, TPP, OSI) and lower antioxidants (TAC, GSH) in barbecue makers studied. Adverse health effects associated with exposure to environmental toxicants including PAH have been shown to be a function of the chemical composition of the toxicant, route of exposure, the dose and the duration of exposure to the substance (5). Thus, increase in number of working hours and years at work implies increased duration of exposure to increasing doses of PAH and consequently progressive PAH induced health deterioration. Higher NO, lipid peroxidation (MDA, TPP, OSI) and lower antioxidants (TAC, GSH) observed with increasing number of working hours and years at work may be a consequence of accumulated oxidative stress and lipid peroxidation accruing from exposure to PAH and enhanced consumption of antioxidants associated with such accumulations over time. Chronic exposure to PAH has been shown to saturate cell xenobiotic metabolic pathways, promoting intercellular ROS production and accumulation (27). The OS accruing from such ROS production and accumulations may account for higher lipid peroxidation (MDA, TPP, OSI) observed in barbecue makers with longer working hours and years at work experience. The neutralization of accumulated ROS by cellular antioxidants in order to maintain redox balance leads to depression in the levels of these molecules (28, 29). However, barbecue makers working for > 10 hours had higher GSH compared to those working for 5-10 hours. Contrary to our findings, reduced GSH levels have been reported across all lengths of duration of exposure to PAH on the job among photocopier operators (30). Higher NO levels were observed with increasing number of working hours. Exposure to PAH has been shown to induce the activities of inducible nitric oxide synthase (iNOS) in a dose- and time-

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dependent manner. The induction of iNOS results in a high, sustained level of nitric oxide production which has been shown to exert a cytoprotective effect against PAH induced cell death (31).

The TNF-α levels of barbecue makers were significantly higher than the controls, increased with increasing number of years at work and correlated negatively with TAC levels. Our observation is consistent with the findings of a previous study that reported higher TNF-α (a potent modulator of immune and inflammatory response) in coke oven workers exposed to PAH compared to unexposed controls (4). Exposure to PAHs has been shown to promote the release of inflammatory cytokines as TNF-α (8). Inflammatory response has been shown to be associated with increased ROS generation and consequently reduction in antioxidants which were consumed in ROS neutralization to maintain redox balance. This may be responsible for the negative association observed between TAC and TNF-α in barbecue makers studied. Oxidative stress-induced lipid peroxidation has been implicated in PAH induced toxic responses in immunological systems (5).

Comparable levels of urinary 8-OHdG a biomarker of oxidative DNA damage was observed in both barbecue makers and their control counterparts. Cellular ability to effect rapid damage repair and recovery when exposed to toxicants including PAH may be responsible for this observation (32). Comparable levels of 8-OHdG have also been reported between smokers exposed to PAH in cigarette smoke and non-smokers (2). However, the levels of urinary 8-OHdG in coke oven workers exposed to PAH was reported to be 1.38 times higher than those of unexposed controls (3).

The eGFR of barbecue makers though lower than those of their control counterparts were not statistically significant. Similar eGFR observed between barbecue makers and controls may be related to the observation that the exposure level to PAH in barbecue makers as reflected by their urinary 1-HOP excretion were below the no-biological effect level for 1-HOP and therefore exerts minimal effect on eGFR. Moreover, the eGFR though widely used as a good index of renal function has been shown to be limited by the observation that obvious changes in eGFR value might only be observed when there is severe kidneys damage. However, decreased eGFR has been reported in individuals exposed to PAH with higher urinary 1-HOP levels compared to those with lower levels and controls. Exposure to B[a]P has been shown to elicit a nephropathic response that result in renal injury (33).

The findings of depressed antioxidants and lung function with increased lipid peroxidation, oxidative stress and inflammation observed in barbecue makers may be associated with their exposure to polycyclic aromatic hydrocarbons in barbecue fumes and may be implicated as possible mechanisms of development of lung impairment. Assessment of these indices may therefore be useful in predicting those at increased risk of PAH induced organ toxicities while antioxidant supplementation may aid in averting future health issues among barbecue makers.

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