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Effect of consumption of charcoal-broiled beef on body weight and antioxidant status in rats

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Abstract
In this study, the effects of sun-dried and charcoal-broiled beef compounded diets and their vitamin A supplemented versions on the antioxidant status in the liver were examined. Also examined was the effect of the same diets on the body and organ weights of the rats. The rats were in five experimental groups according to various diets and vitamin A supplemented version of the diets. The rats were given 0.8 g feed/g average group body weight in terms of feed for 14 days. The vitamin A supplements were administered to the rats by gavage at a dose of 400 IU units daily for 14 days. After 14 days, charcoal-broiled beef compounded diet only produced significant ($P < 0.05$) increase in malondialdehyde (MDA) in the liver compared to rats given sun-dried beef compounded diet only. Administering vitamin A supplements in addition to the charcoal-broiled beef compounded diet led to a significant decrease in MDA levels compared to rats given just the compounded diet. Rats which received broiled beef diet only, had a higher liver-to-body weight ratio relative to other treatment groups. Rats given charcoal-broiled beef compounded diet only also showed retarded growth in the mean group weight, with the growth improved in rats given supplemented version of the diet. The results presented here show that regular consumption of broiled beef leads to the production of bioactive metabolites or compounds which increases lipid peroxidation in the liver in rats, and also leads to retarded growth with vitamin A supplements reversing these effects.
Keywords: charcoal-broiled beef, vitamin A, lipid peroxidation, gravimetry

Introduction
The World Health Organization (WHO) has determined that dietary factors account for at least 30 percent of all cancers in Western countries and up to 20 percent in developing countries. When cancer researchers started to search for links between diet and cancer, one of the most noticeable findings was that people who avoided meat were much less likely to develop the disease (1). A number of hypotheses have been advanced to explain the connection between meat consumption and cancer risk. First, meat is devoid of fibre. Meat also contains animal protein, saturated fat, and, in some cases, carcinogenic compounds such as heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAH) formed during the processing or cooking of meat. HCAs, formed as meat is cooked at high temperatures, and PAHs, formed during the burning of organic substances, are believed to increase cancer risk (2).

The term polycyclic aromatic hydrocarbons (PAHs) refer to a ubiquitous group of several hundred chemically-related, environmentally persistent organic compounds of various structures and varied toxicity. Most of them are formed by a process of thermal decomposition (pyrolysis) and subsequent recombination (pyrosynthesis) of organic molecules (3). Benzo(*a*)pyrene is a polycyclic aromatic hydrocarbon (PAH) that has been identified in ambient air, surface water, drinking water, and waste water, and in char-broiled foods (4). Benzo[*a*]pyrene is transformed into (+)-BP-7-8-epoxide by the monooxygenase enzyme, into (-)-trans-BP-7,8-dihydrodiol by the epoxide hydrolase enzyme and thereafter into (+)-anti-BP-7,8-diol-9,10-epoxide by the monooxygenase enzyme (5,6). Thus, this pro-carcinogen epoxide derivation is formed by B(a)P, which is metabolised in the liver. Like every other reactive PAH derivative, this product can modify carbohydrate, proteins, lipids and nucleic acids leading to various diseases.

Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. Whenever the balance between reactive oxygen species (ROS) production and antioxidant defense is lost, 'oxidative stress' results which through a series of events deregulates the cellular functions leading to various pathological conditions (7,8).

Vitamin A (retinol) is an essential, fat-soluble nutrient stored in body organs, mainly the liver. It is released, as needed, into the bloodstream, becoming available for use by cells throughout the body (9). The human body does not make vitamin A, so intake of vitamin A from external sources is necessary. Vitamin A is naturally present in some foods such as milk fat, breast milk, butter, cheese, liver, and fish liver oils. Carotenoids, generally found in plants, are converted into vitamin A in the body. Carotenoids are found in dark green leafy vegetables, deeply colored yellow and orange fruits and vegetables, and egg yolk (10). β-carotene is one of many hundreds of food carotenoids, relatively few of which have been studied in relation to their impact on

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human physiology. β-carotene is the most abundant form of provitamin A in fruits and vegetables (11). Function of vitamin A in mammals can be broadly grouped under five headings: (i) vision, (ii) bone growth, (iii) reproduction, (iv) maintenance of epithelia, (v) antioxidant role and (vi) overall growth (12).
There are indications that consumption of charcoal-broiled beef increases lipid peroxidation, and at the same time reduces the activity of antioxidant enzymes (13). There have also been reports that charcoal-broiled beef affects the body weight and organ morphology (14). Hence, the aims of this study were;

(i) To investigate the effect of regular consumption of charcoal-broiled beef on gravimetry, lipid peroxidation in the liver and anti-oxidant enzyme activity in the liver of the rats.
(ii) To see the effect(s) vitamin A supplements would have on the changes caused by the charcoal-broiled beef.

Materials and Methods

Materials

Meat samples: Lean beef meat samples were purchased from Uselu Market, Benin city.
Animals: Forty albino rats (Wistar strain) weighing between 90-140g were used for this study. They were obtained from Biochemistry Department, University of Benin. They were kept in wooden cages with free access to food and water, and allowed to acclimatize to our laboratory conditions for four weeks. They were maintained on growers mash (Bendel Feed and Flour Mill, Ewu, Edo State).

Methods

Preparation and proximate analysis of meat samples
Sun-dried: Lean beef meat samples were dried directly under the sun in small metal cages. The sun-drying was done between 9:00 hr and 17:00 hr daily for 5 days until they were thoroughly dried. The average daily temperature during this period was 35^0C and relative humidity was 82%. They were then ground with a manual blender to obtain finer slices which were dried further. The dried slices of the meat samples were then thoroughly pulverized with a 40W blender to give powdery form, which was stored in dark containers at 0^OC until required.
Charcoal-broiled: Beef samples from the same stock were used to prepare the charcoal-broiled barbecue beef as described by Aygun and Kabadayi, (15) with slight modifications to the time used for grilling in order to obtain properly barbecued samples. The meat samples were grilled under open flame charcoal grill (~ 12g and 0.5cm thick) with the distance between fire and sample meat measured 15.0cm ± 0.5cm. The meat samples were grilled for 15 minutes and thereafter thoroughly pulverized with a 40W blender to obtain fine powdery samples. This was stored in dark containers at 0^OC until required.

Figure 1: Charcoal grill

Proximate analysis was then immediately done on the meat samples, and the samples were stored for 3 days until required to make diets used for this study.

Determination of the Proximate Composition of the Meat Samples

Proximate compositions of the sun-dried and charcoal broiled beef which include moisture content, ash content, fat content and crude fibre were determined using the AOAC methods (16) while protein was quantified using standard Kjeldahl method (17). The difference method was used to determine the carbohydrate content (18).

Table 1: Proximate analysis of meat samples

	Charcoal-broiled beef	**Sun-dried beef**
Crude Protein (%)	57.75	61.25
Moisture content (%)	12.00	16.00
Lipid (%)	26.00	17.00
Ash (%)	3.00	5.00
Crude Fibre (%)	1.25	0.75
Carbohydrate content (%)	0.00	0.00

Food Composition and Pelleting

Based on the proximate composition of the meat samples, compounded diets of the charcoal-broiled beef and sun-dried beef samples were made. The feed composition is as shown in Table 2. After all the components of the diet were thoroughly mixed to form a uniform blend, a little distilled water was added to form a solid paste. A cork borer was then used to form fine divided pellets from the paste. The pellets were dried under the sun and stored at 0^{O}C to prevent fungal growth.

Table 2: Food composition table

	Sun-dried compounded diet (%)	**Charcoal-broiled beef compounded diet (%)**
Ground sun-dried beef	10.0	0.0
Ground charcoal-broiled beef	0.0	10.0
Corn starch	63.0	63.0
Sugar	5.0	5.0
Palm oil	7.0	7.0
Dried peanut husk	10.0	10.0
*Multivitamins/minerals	5.0	5.0
Total	**100.0**	**100.0**

* **multivitamin composition** - vitamin A: 5000IU, vitamin D2: 400IU, vitamin B1: 5mg, vitamin B2: 5mg, vitamin B6: 1.5mg, vitamin B12: 5mcg, nicotinamide: 50mg, calcium pantothenate: 5mg, folic acid: 1mg, vitamin C: 75mg, vitamin E: 15mg, vitamin K: 0.1mg

Experimental Design

At the end of acclimatization, the rats were re-weighed and thereafter separated into five groups of 8 rats each. The mean weights of the rats in the various groups ranged from 154-184g.

The treatment administered to each rat in each group is categorized as follows:

Group 1 – The rats received 0.8g/g avr. gp. wt. sun-dried beef compounded diet daily for 14 days.

Group 2 – The rats received 0.8g/g avr. gp. wt. charcoal-broiled beef compounded diet daily for 14 days.

Group 3 – The rats received 0.8g/g avr. gp. wt. growers mash* and 400 IU units of vitamin A daily for 14 days by gavage.

Group 4 – The rats received 0.8g/g avr. gp. wt. sun-dried beef compounded diet and 400 IU units of vitamin A daily for 14 days by gavage.

Group 5 – The rats received 0.8g/g avr. gp. wt. charcoal-broiled beef compounded diet and 400 IU units of vitamin A daily for 14 days by gavage.

* growers mash (Protein: 12.0%, Fat: 6.0%, Fibre: 8.5%)

Sample collection and preparation

Each rat was weighed and then anaesthetized in a chloroform fume saturated jar and blood collected through cardiac puncture and kept in plain containers without anti-coagulant. The liver was excised and placed in a universal container. The liver was weighed and left standing on ice. Two grammes of liver from each sample were thoroughly homogenized in 4ml saline solution using a mortar and pestle. The homogenate was then centrifuged at 3000 x g for 10 minutes. The supernatant was collected in plain containers and stored at -20^{O}C until required for the assays.

Biochemical analysis

Malondialdehyde level (MDA) was determined based on the method of Guttridge and Wilkins (19). The principle that underlies this assay is that MDA- a product of lipid peroxidation when heated with thiobarbituric acid (TBA), in acidic medium, forms a pink or reddish complex that is measured spectrophotometrically at 532nm. Superoxide dismutase (SOD) assay was determined by an indirect method which is based on the inhibitory effect of SOD on the initial rate of epinephrine auto-oxidation which is derived from the reaction proposed by Misra and Fridovich (20), for the base catalysed auto-oxidation of epinephrine. Catalase activity was estimated based on the method of Cohen *et al.*, (21). This estimation is based on the measurement of the rate of decomposition of hydrogen peroxide (H_2O_2) after the addition of the material containing the enzyme.

Statistical analysis

Statistical analyses carried out are student's –test and analysis of variance (ANOVA) using Microsoft Excel data analysis (2007 version) at 5% confidence level.

Results

Lipid peroxidation and anti-oxidant enzyme activity in the liver.

Lipid peroxidation and anti-oxidant enzyme activities in the liver of rats fed with the compounded diets and vitamin A supplemented version of the diets are shown in table 3. Relative to the rats fed with sun-dried beef compounded diet only (group 1), rats which received the charcoal-broiled beef compounded diet only (group 2) had statistical significant ($P < 0.05$) increase in the malondialdehyde (MDA) level in the liver. Vitamin A supplements in addition to charcoal-broiled beef compounded diets administered to rats in group 5 led to a significant decrease ($P < 0.05$) in MDA levels in the liver when compared to rats in group 2. Rats maintained on growers mash and vitamin A supplements (group 3) had MDA levels significantly lower ($P < 0.05$) than that of rats which were treated with sun-dried beef compounded diet and vitamin A (group 4), and charcoal-broiled beef compounded diet and vitamin A supplements (group 5).

Superoxide dismutase (SOD) activity but not catalase in the liver of the rats was significantly different among the various groups after treatment. When compared to rats in group 4 and group 5, the SOD activity in the liver of group 3 was significantly lower ($P < 0.05$). Rats given charcoal-broiled beef compounded diet supplemented with vitamin A had a statistical significant increase ($P < 0.05$) in SOD activity compared to rats administered sun-dried beef compounded diet and vitamin A supplements.

Table 3: Lipid peroxidation and anti-oxidant enzyme activity in the liver.

Grps	Treatment	MDA (X 10^{-6} Unit/g tissue)	Catalase (K/min)	SOD (Unit/ g wet tissue)
1	Sun-dried beef compounded diet (A)	6.02 ± 0.78	1.22 ± 0.07	17.27 ± 2.16
2	Charcoal-broiled beef compounded diet (B)	8.52 ± 1.03 [a]	1.29 ± 0.13	20.54 ± 4.56 [b]
3	Normal feed (Growers mash) + vitamin A (C)	3.78 ± 1.07 [a,c]	1.24 ± 0.23	13.68 ± 2.73 [a,c]
4	Sun-dried beef compounded diet + vitamin A (D)	6.75 ± 0.98 [b,c,d]	1.20 ± 0.10	19.12 ± 2.28 [b,d]
5	Charcoal-broiled beef compounded diet + vitamin A (E)	6.99 ± 1.09 [b,c,d]	1.18 ± 0.07	24.49 ± 2.13 [a,b,d,e]

Values expressed as mean ± SD; n = 6

a – value significantly different ($P < 0.05$) from group 1 within the same column

b – value not significantly different ($P > 0.05$) within the same column

c – value significantly different ($P < 0.05$) from group 2 within the same column

d – value significantly different ($P < 0.05$) from group 3 within the same column

e – value significantly different ($P < 0.05$) from group 4 within the same column

Gravimetry

Table 4 shows the mean group body weight at the start of the treatment and the group mean body weight just before the rats were sacrificed.

Table 4: Mean body weight of the rats before and after treatment.

Groups	Treatment	Mean body weight before treatment commenced (g)	Mean body weight after treatment (g)
1	Sun-dried beef compounded diet (A)	154 ± 30.19	174 ± 34.36[a]
2	Charcoal-broiled beef compounded diet (B)	156 ± 12.79	146 ± 18.60[a]
3	Normal feed (Growers mash) + vitamin A (C)	184 ± 23.01	193 ± 36.63[a]
4	Sun-dried beef compounded diet + vitamin A (D)	180 ± 14.10	195 ± 25.76[a]
5	Charcoal-broiled beef compounded diet + vitamin A (E)	163 ± 20.62	174 ± 18.76[a]

Values expressed as mean ± SD; n = 7

a – value not significantly different (P > 0.05) after treatment

Rats in group 2, which were given charcoal-broiled beef compounded diet only, had growth retardation after treatment. This is evident in the decrease in the mean post-treatment weight when compared to the mean pre-treatment weight.

Table 5: Liver weight and liver-to-body weight ratio.

Groups	Treatment	Mean liver weight after treatment (g)	Liver weight/ body weight
1	Sun-dried beef compounded diet (A)	7.63 ± 1.47	0.044 ± 0.003
2	Charcoal-broiled beef compounded diet (B)	6.84 ±1.01[c]	0.047 ± 0.002
3	Normal feed (Growers mash) + vitamin A (C)	6.54 ±1.06[c]	0.034 ± 0.003[a,b]
4	Sun-dried beef compounded diet + vitamin A (D)	6.95 ± 0.87[c]	0.036 ± 0.001[a,b]
5	Charcoal-broiled beef compounded diet + vitamin A (E)	6.43 ± 0.33[c]	0.037 ± 0.002[a]

Values expressed as mean ± SD; n = 7

a – value significantly different (P < 0.05) from group 1 within the same column

b – value significantly different (P < 0.05) from group 2 within the same column

c – value not significantly different (P > 0.05) within the same column

Table 5 shows the mean liver weight of the rats in the various groups at the end of the treatment. Also shown in Table 5 is the liver-to-body weight ratio at the end of the treatment. Relative to rats in other treatment groups, the rats given charcoal-broiled beef compounded diet only (group 2) had a higher liver-to-body weight ratio.

Discussion

Polycyclic aromatic hydrocarbons (PAHs) are found in foods as a result of certain industrial methods such as smoke curing, boiling, roasting and grilling over open fires or charcoal which permit the direct contact between food and combustion products (22). Grilling meat leads to the formation of benzo[α]pyrene (BaP) and this formation is temperature dependent. Direct contact of the fatty meat surfaces during the open flame charcoal grilling at high temperatures and prolonged times increases the formation of, and accumulation of BaP in cooked meats (15).

In this study, the effect of char-broiled meat on lipid peroxidation, antioxidant enzyme activity, body and organ weights was further studied using sun-dried beef compounded diet and charcoal-broiled beef compounded diets and vitamin A supplemented versions of the diet. The results presented in table 3 show that charcoal-broiled beef compounded diet, which has been extensively studied and shown to have high levels of benzo[α]pyrene (15), caused significant increase in lipid peroxidation. This is in agreement with findings that rats given benzo[α]pyrene had an increase in malondialdehyde (MDA) levels which signify lipid peroxidation (23). The result also corroborates earlier reports of Pan et al., (24) who studied the effects that benzo[α]pyrene exposure had on Scallop chlamys farreri; and reports of Emre et al., (25) that showed that benzo[α]pyrene led to higher levels of malondialdehyde in rats. The increase in lipid peroxidation is also in agreement with previous study of the effect of palclitaxel (a known anti-carcinogen) and benzo[α]pyrene on rats (26). Vitamin A supplements significantly reduced the level of lipid peroxidation as a result of a decrease in MDA levels of rats given vitamin A supplements along with charcoal-broiled beef compounded diet. This suggests that vitamin A has free radical scavenging activity as is previously believed (27) and mops up reactive oxygen species (ROS) in the rat liver.

The effect of the different compounded diets and vitamin A supplemented versions of the diets on antioxidant enzymes in rat liver was also studied under experimental conditions. The two most important antioxidant enzymes are superoxide dismutase (SOD) and catalase (CAT) that acts against free radicals such as superoxide (O_2^-) and hydroxyl ions (OH^-). SOD is an enzyme containing copper (Cu^{2+}) and zinc (Zn^{2+}) that converts superoxide radical into hydrogen peroxide (28,29) and molecular oxygen and thus protects the cells from oxidative damage caused by H_2O_2 and OH^- (30). CAT is a hemoprotein, localized in peroxisomes or microperoxisomes, that also catalyses the decomposition of H_2O_2 to H_2O and O_2 thus protecting the cells from oxidative damage caused by H_2O_2.

Superoxide dismutase but not catalase activity in the liver of the rats was significantly higher among the various groups which received vitamin A supplements when compared to the rats that received the corresponding diets only after treatment. No statistical change was observed in the effect of the various treatments on catalase activities during this study, a result which is in consonance with the findings of Sheng and Bao, (31) with *Boleophthalmus pectinirostris* liver exposed to different concentrations of benzo[α]pyrene. Table 3 also show that the activity of SOD in liver of rats given charcoal-broiled beef compounded diet was higher than that of rats given sun-dried beef compounded diet. The mechanism behind this variation is unclear as benzo[α]pyrene usually cause an increase in lipid peroxidation either by producing the ROS or decreasing the level of endogenous antioxidant enzymes (32, 33). However, similar increase in SOD activity agrees with the findings of Sheng and Bao (31) whose study showed that *Boleophthalmus pectinirostris* exposed to benzo[α]pyrene had SOD activities that did not change on exposure to benzo[α]pyrene in low concentration but was increased significantly when exposed to higher concentration of benzo[α]pyrene. Similar result was also observed in study by Cosan *et al*., (26) who reported increase in SOD activity. Supplementing charcoal-broiled beef compounded diet with vitamin A also resulted in an increase in SOD activity in the liver of rats when compared to rats given charcoal-broiled beef compounded diet only. The significant increase in SOD in the vitamin A supplemented group of animals appear to facilitate removal of superoxide anions and H_2O_2 (27).

Charcoal-broiled beef compounded diets elevated lipid peroxidation in the liver. Lipid peroxidation is an important event to cell death and has been reported to cause serve impairment of membrane functions through increased membrane permeability and membrane damage, cytotoxicity and eventually cell death. The free radicals reacts with lipids and generates lipid peroxidation, which is involved in the formation of tumours. (34). Rats fed with charcoal-broiled beef showed a significant increase in lipid peroxidation. The free radical scavenging efficiency of vitamin A supplements thus might be playing an important role in the anti-mutagenic activity. The present investigations reveal that vitamin A supplement is able to decrease the free radical effect of the charcoal-broiled beef on the liver system. Antioxidants alleviates the effect of charcoal-broiled beef by reducing lipid peroxidation and by acting on antioxidant response elements and thereby increasing the synthesis of enzymes involved in detoxification.

Tables 4 and 5 show the effect the compounded diets and vitamin A supplemented versions of the diet had on gravimetric parameters (whole body weights, post-treatment liver weight and liver-to-body weight ratio) in the rats. The animals which received charcoal-broiled beef compounded diets showed retarded growth (6.4% decrease in the mean group weight). Charcoal-broiled beef has sufficient amount of PAHs, especially benzo[α]pyrene. The growth retardation observed in animals which received charcoal-broield beef compounded

diet may thus be as a result of the PAHs in the diet. This is similar with the finding that rats given dibenzathracene, a PAH, had decreased body weight (35). This is also in agreement with a study by Essien and Akpan (14); on pregnant rats fed graded amounts of charcoal-fire roasted meat (charred meat) during gestation and lactation for three weeks resulting in dose-related weight retardation and growth retardation of their offspring. Rats which received vitamin A supplements in addition to charcoal-broiled beef compounded diet however had improved growth with a 6.75% increase in mean body weight at the end of the treatment. The quantity of food spilled at the end of the treatment was approximately the same across the groups and thus the growth rate pattern observed at the end of the experiment occurred due to the effect of the various treatments. Rats also given charcoal-broiled beef compounded diet also had a higher liver-to-body weight ratio relative to other treatment groups which agree with the findings of Abels *et al*., (35) who studied the effect of dibenzanthracene, another polycyclic aromatic hydrocarbon, on rat liver as well as that of Knuckles *et al*., (36) who studied the effect of acute and subchronic oral toxicities of benzo[α]pyrene in rats.

Conclusion: From the observations, it can be concluded that regular consumption of broiled beef leads to the production of bioactive metabolites or compounds which increases lipid peroxidation in the liver. Regular consumption of charcoal-broiled beef also leads to retarded growth in animals. Charcoal-broiled beef has been reported to be contaminated by polycyclic aromatic hydrocarbon especially benzo[α]pyrene, and this may be the bioactive compound responsible for the effects of charcoal-broiled beef in rats. Vitamin A supplements alleviates the effects of the broiled beef by reducing lipid peroxidation in rats.

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