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Quantitative determination of polycyclic aromatic hydrocarbons and fatty acids in commonly consumed dried fish samples in Owerri Imo State

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Abstract

The need to assess and evaluate the concentrations of toxicants such as Polycyclic Aromatic Hydrocarbons (PAHs) and their possible impact/effects on the nutritional property of foods consumed has continued to attract research interests for environmental biomonitoring. Five dried fish samples: Bonga fish (*Ethmalosa fimbriata*), Cray fish (*Palaemon hastatus*), Stock fish (*Gadus morhua*), Cat fish (*Clarias gariepinus*) and River African Catfish (known as Round fish) sold and consumed in Owerri Imo State, Nigeria were investigated in this study. Concentrations of PAHs and fatty acid profiles of dried fish samples were respectivelydetermined using Gas Chromatography. Concentrations of total PAHs in the dried fish samples were: Cat fish (11.84±10.00 µ/kg), Round fish (10.32±8.74 µ/kg), Bonga fish (8.04±3.00 µ/kg), Cray fish (3.92±0.54 µ/kg) and 3.53±0.08 µ/kg in Stock fish. The total Fatty acid concentrations are 139.42±12.12 mg/kg for Bonga fish >66.15±4.80 mg/kg for Cray Fish > 60.68±2.22 mg/kg for Cat fish >59.13 mg/kg for Stock fish> 58.28 ±10.19 mg/kg for Round Fish. From results, Cray fish was identified to possess highest concentration of Arachidonic acid (Omega 6 fatty acid) of 3.84±0.26 mg/kg and docohexaenoic acid (Omega 3 fatty acid) of 9.90±0.43 mg/kg, while Stock fish contains the highest concentration of Linoleic acid (Omega 6 fatty acid) at 11.82±2.15 mg/kg.Study findings indicated that healthier and quality fatty acids were present in dried fish not processed by open flame (crayfish and stock fish) compared to Bonga Fish, Cat fish and Round fish. Furthermore, deductions from the study attributes the loss of essential fatty acids, reduction of nutritional value and induction of PAHs in fish samples to the method used to process and preserve the dietary item.

Keywords: Polycyclic Aromatic hydrocarbons, Fatty acids, dried fish, carcinogens, health risk

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1. Introduction

The aquaculture industries are exposed to many chemicals, such as polycyclic aromatic hydrocarbons (PAHs). PAHs are widespread organic contaminant with some of its constituents that instigate mutagenic and carcinogenic effects that bioaccumulate in animal and human tissues. PAHs are incorporated into the environment from natural and anthropogenic sources, and majorly from incomplete combustion of organic materials, fossil fuels and petroleum [1]. Food processing methods such as grilling, roasting, smoking, and barbecuing produces PAHs [2]. Fish smoking, grilling, roasting and barbecuing are practiced as methods that prolong shelf life, enhance flavour, and increase utilization [3]. It is reported that Nigeria produces 194,000 metric tons of dried fish yearly with smoked fish accounting for 61%of the reported figure [4,5,6,7]. Studies have shown that some food processing methods such as smoking is a major source of PAHs contamination in fish and the impact of the smoking techniques on the amount and type of PAHs that are generated varies [8]. The wide and unregulated use of the smoking technique as a method of fish processing in Nigeria has been implicated in many public health disorders. Some studies have associated high health risks with the consumption of smoked fish in Nigeria [5,7,9]. Dried fish is a common source of protein and essential fatty acids and it is used for local soups and stews with its unique indigenous aroma and taste. Although, fish consumption is a good source of nutrient, it is also one major exposure route of toxicants to humans. Few data exist that relate theconsumption of smoked fishtypes in Owerri Imo State, Nigeria and prevalent genotoxic-linked medical disorders. Result from this study may provide relevant data which can be useful for environmental biomonitoring and assessment.

2. Materials and methods

2.1. Collection of fish samples

Dried fish samples of Bonga fish (*Ethmalosa fimbriata*), Cray fish (*Palaemon hastatus*), Stock fish (*Gadus morhua*), Cat fish (*Clarias gariepinus*) and River African Cat fish (alsoknown as Round fish) sold and consumed in Owerri Imo State Nigeria, were purchased from two popular markets: Ekeonunwa market and Relief Market. Investigated fish samples were purchased respectively from five different outlets in Ekeonunwa Market and subsequently purchased from five different outlets at the Relief Market. The samples were labelled accordingly in sterile polythene bags and transported to the laboratory. Samples were identified by Dr. C. Ezeafulukwe of the Department of Aquaculture and Fishery in the School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Imo State Nigeria. In the laboratory, samples were dried at 70° C for 1 hour and homogenized using a stainless-steel micro hammer mill. The milled samples were stored in a glass vials for digestion and further analyses.

2.2. Determination of polycyclic aromatic hydrocarbons

The Gas Chromatography technique was used to determine the PAHs content of respective pulverised fish samples, as described by AOAC [10]. The fat content was extracted by Soxhlet extraction method. A measured 20 g of the homogenized sample was mixed with 60g anhydrous sodium sulphate in an agate mortar to absorb moisture. The homogenate was then placed in 500 ml beaker and extracted with 300 ml of n-hexane. Subsequently, the crude extract was dried at 40^{o}C with a rotary vacuum evaporator. A measured 1 g of the n-hexane extract was dissolved in 50 ml chloroform and transferred into a 100 ml volumetric flask, which was then diluted to the mark. Most of the chloroform was evaporated off at room temperature. Afterwards, 1 ml of a mixture of 20 vol% of benzene and 55 vol% of methanol was added. The flask was sealed and placed in a water bath for 30 min at 40^{o}C. The organic phase was extracted with hexane and water, to a final mixture of: reagent, hexane and water in proportions of 1:1:1 (i.e., adding 1ml each of hexane and water to the reaction mixture). The mixture was agitated vigorously for 2 min and about half of the top hexane phase was transferred to a small test tube for injection into the GC column of Buck 530 gas chromatograph equipped with an on-column, automatic injector, Electron capture detector, and HP 88 capillary column with dimension100m x 0.25µm film thickness (CA, USA) with the following parameters: Detector Temperature A:280°C; Column temperature 210°C; Injector temperature 250°C; Integrator chart speed: 2cm/min. The GC oven temperature was set at 180°C and allowed to warm up after which 1 µl n-hexane extract was injected onto column A of GC using proper injection technique.

2.3. Determination of Fatty Acid Profile

Fatty acid profile was determined by Buck 530 Gas Chromatography as described by AOAC [10]. The homogenized fish sample (20 g) of respective fish samples were subjected to oil extraction by soaking in 300 ml of n-hexane for 48 hrs. Afterwards, the setup was filtered into a round bottom-flask using Whatman filter paper. After filtration the oil was separated from the sample with the aid of soxhlet apparatus and the oil was collected using a beaker. Finally, the extracted oil was concentrated at 40°C with rotary vacuum evaporator. Cotton wool was placed in a separating funnel, 1 g and 0.5 g of sodium sulphate and magnesium silicate were respectively added into the separating funnel. The mixture was made wet by using 5 ml of n-hexane after which the extracted oil was introduced into the mixture and allowed to stand for 20 min. The tap of the separating funnel was unlocked, and solvent was allowed to go into the gas chromatography sample container. With the aid of a 2 ml syringe, the solvent was taken and injected into the GC. The oven temperatures were programmed from an initial temperature of 180°C (held for 4 min) rising to 200°C in 10 min. Identification was made by comparison of retention time to those of authenticated standards for 45 min per parameter after which the result was printed out.

2.4. Statistical Analysis

The obtained data were expressed as mean ± standard deviation and the significant difference among the group was assessed using the One-way analysis of variance (ANOVA).

3. Results and Discussions

The results of Table 1 shows that total PAHs concentration in the dried fish samples ranged as thus 11.84±10.00 µ/kg> 10.32±8.74 µ/kg> 8.04±3.00 µ/kg> 3.92±0.54 µ/kg> 3.53±0.08 µ/kg for Cat fish, Round fish, Bonga fish, Cray fish and Stock fish respectively. The concentrations of BaP (Benzo(a) pyrene), which is the most potent PAH carcinogenic [5] was identified andranged as thus: 0.22±0.30 µ/kg>0.11±0.16 µ/kg> 0.08±0.02 µ/kg> 0.02±0.02 µ/kg> 0.01±0.00 µ/kg for Round fish, Cat fish, Cray fish, Stock fish and Bonga fish respectively. Furthermore, the other important PAHs: benz(a)anthracene, benzo(b)fluoranthene and chrysene were also detected in appreciable amount.

From lipid profile result (Table 2), Bonga fish showed significantly high amount of Lauric acid (C-12) (9.74±0.00 mg/kg) when compared to Stock fish and Cat fish. Lauric acid was not detected in Cray fish and Round fish. The concentration of Myristic acid (C-14) present in all investigated fish samples were not significantly differentt (P<0.05), however the highest concentration of 6.27±5.67 mg/kg was observed in Stock fish. Palmitic acid (C-16) was significantly high in Cat fish (5.86±0.04 mg/kg) and Cray fish (4.98±0.23 mg/kg) when compared to other fish samples. Palmitic acid was not detected in Bonga fish. Stearic acid (C-18) was identified at 18.16±2.28 mg/kg in Bonga fish and was significantly high compared to 7.36±0.00 mg/kg in Cray fish, 3.24±1.57 mg/kg in Round fish and 1.02±0.02 mg/kg in Stock fish. Oleic acid concentration (C-18:1) was found at81.93±9.62 mg/kg in Bonga fish > 33.66±2.54mg/kg in Cat fish > 30.39±4.61 mg/kg in Cray fish > 23.65±4.87 mg/kg in Round fish > 17.89±1.54 mg/kg in Stock fish. Furthermore, linoleic acid (C-18:2) at 11.82±2.15 mg/kg and alpha-Linolenic acid (C-18:3) at 4.12±0.00 mg/kg were detected highest in Stock fish and Bonga fish respectively. Ssignificantly high levels of Icosadienoic acid (C-20:2) and Icosatrienoic acid (C-20:3) were present at 9.33±0.72 mg/kg in Cat fish and 13.57±0.35 mg/kg in Bonga fishrespectively. Arachidonic acid (C-20:4), Tetracosanolpentaenoic acid (C-

24:5) and Docosahexaenoic acid (C-22:6) were detected at varying concentrations in investigated fish samples. Generally, total fatty acid concentrations in the fish samples were 139.42±12.12 mg/kg in Bonga fish > 66.15±4.80 mg/kg in Cray fish > 60.68±2.22 mg/kg in Cat fish > 59.13±4.79 mg/kg in Stock fish > 58.28±10.09 mg/kg in round fish.

Discussion

In vitro and in vivo studies have implicated a number of PAHs as potentialmutagenes and carcinogenes in experimental animals. Polycyclic aromatic hydrocarbons (PAHs) usually contaminate or end up in food as a result of environmental contamination of the raw materials, or formed during manufacturing or processing. Furthermore, PAHs have presented adverse haematological, reproductive, developmental and immunological effects in experimental animals [5,11,12]. Some PAHs such as benzo(a)pyrene are among the most potent [13,14] and best documented carcinogen. It is classified as carcinogenic to humans (1A). While PAHs such as benz[a]anthracene and dibenz[a,h]anthracene are classified as probable carcinogenes to humans (Group 2A), and others as potential carcinogens to humans (Group 2B), the other PAHs are not classified in relation to carcinogenicity to humans [5,15].

In the present study, the total PAHs concentrations in the fish samples processed by smoking and/or by heat were observed to contain the following quantities of PAHs: Cat fish (11.84±10.00 µ/kg), Round fish (10.32±8.74 µ/kg), and Bonga fish (8.04±3.00 µ/kg) and were significantly higher compared to 3.92±0.54 µ/kg in Cray fish and 3.53±0.08 µ/kg in Stock fish processed by sun drying or freeze drying. These varying concentrations of PAHs in these fish samples implicate the method of processing as the major source of PAHs. Exposure of the fish samples to smoke generated by the firewood can cause considerable contamination with various PAHs because the process is usually poorly controlled [5,16]. High concentration of the PAHs in food samples processed by smoke/heat can also be attributed to its fat content, and pyrolysis resulting from increased amount of melted fat dropped on heat source [5,16,17]. The result of this study showed presence of 16 PAHs in all the fish samples studied. However, toxicological attention should be focused on the presence of high molecular weight PAHs such as benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene and indeno[*1,2,3-cd*]pyrene, benzo[g,h,i]perylene which are largely carcinogenic [5]. The presence of PAHs classified as probable human carcinogens (Group 2A) such as benzo[a]pyrene, benz[a]anthracene and dibenz[a,h]anthracene should be a public health concern to consumers. Benzo[a]pyrene is the most potent PAHs and metabolism in human system activates its toxicity [5,18]. On ingestion, the living system attempts to degrade Benzo[*a*]pyrene, however this metabolic process activates the formation of reactive metabolites benzo[*a*]pyrene 7,8-dihydrodiol, oxidized to 7,8- diol-9,10-epoxide and this bind to DNA generating disruption to normal genetic activities and invariable inducing a genotoxic effect [5,19,20].The highest concentrations of benzo[*a*]pyrene(0.22±0.30 µ/kg) and 0.11±0.16 µ/kg benzo[*a*]pyrene were obtained in Round fish and Cat fish respectively. The reported levels in this study were less than 1.757 µ/kg obtained in freshly heat processed pork but more than 0.05 µ/kg contained in long heat processed fish by Ujowundu *et al* [5]. Furthermore, the concentration of dibenz[a,h]anthracene a probable human carcinogen at 1.48±1.92 µ/kg in Round fish and 1.12±0.56 µ/kg in Stock fish were higher than the concentrations reported by Ujowundu *et al* [5]. There was also significant presence of Indeno (1,2,3-cd) pyrene in all the samples. It is important to note that the concentrations of benzo[*a*]pyrene which ranged from 0.01±0.00 µ/kg in Bonga fish to 0.22±0.30 µ/kg in stock fish was less than 2 µg/kg and 5µg/kg Maximum permissible levels of benzo[a]pyrene in food, by European Union (EU) [21].

Some of the major dietary sources of PAHs are meat and fish cooked over an open flame. The lipophilic nature of PAHs facilitates the contamination of fats and oils. Fatty acids are naturally occurring compounds of fats formed by the combination of fats with glycerol. Fatty acids, are important compound in metabolism, involved in providing metabolic fuel (storage and transport of energy), as essential components of all membranes, and as gene regulators [22,23]. Fish and other sea food have shown as important source of fatty acids with significant physiological effects such protection against coronary heart disease and capacity to reduce platelet aggregation [24]. In this study, twelve fatty acids (Table 2) were detected in varying concentrations in five dried fish samples of Bonga fish (*Ethmalosa fimbriata*), Cray fish (*Palaemon hastatus*), Stock fish (*Gadus morhua*), Cat fish (*Clarias gariepinus*) and River African Cat fish (known as Round fish) sold and consumed in Owerri Imo State Nigeria. Appreciable amount of Lauric acid were observed in three fish samples with Bonga fish showing the highest value at 9.74±0.00 mg/kg. Lauric acid are easily digestible, and a good source of direct energy. The chemical structure of lauric acid allows the body to absorb them whole [25]. Lauric acid increases total serum lipoproteins more than many other fatty acids, but mostly high-density lipoprotein (HDL). Therefore, lauric acid is regarded as having a more favorable effect on total HDL than other fatty acids [26]. Generally, a lower total/HDL serum lipoprotein ratio correlates with a decrease in atherosclerotic incidence [27]. Myristic acid was detected in appreciable amount in all the fish samples except cray fish with stock fish presenting the highest value of 6.27±5.67 mg/kg. Myristic acid is a saturated fatty acid, involved in the stabilization of many different proteins, such as proteins of the immune system and some antitumour proteins [28]. This function is called myristoylation; it occurs when myristic acid is attached to the protein in a specific position where it functions usefully. This highlights the importance of dietary myristic acid to enable anti-cancer and immune system optimal function [29]. Stearic acid at 18.16±2.28 mg/kg in Bonga fish was the most abundant fatty acid after Oleic acid at 81.93±9.62 mg/kg in Bonga fish in all the fish samples evaluated. Stearic acid is a saturated fatty acid and has been implicated in making absorption of essential nutrient by the digestive system difficult. It can also cause damage to the immune system. However, investigations showed that stearic acid does not raise serum cholestrol (hyercholestrolemic),and may not have the capacity to raise LDL-cholesterol [30].Oleic acid at concentrations of 81.93±9.62 mg/kg in Bonga fish to 17.89±1.54 mg/kg in Stock fish presented the highest values of fatty acids in all the fish samples studied.

Table 1: Concentrations (µg/kg) of Polycyclic Aromatic Hydrocarbons in dried fish samples consumed in Owerri Imo State Nigeria

PAHs µg/kg	Bonga fish (*Ethmalosa fimbriata*)	Cat fish (*Clarias gariepinus*)	Cray fish (*Palaemon hastatus*)	Round fish (River African Cat)	Stock fish (*Gadus morhua*)
Naphthalene	1.49±1.52[a]	0.39±0.48a	0.02±0.00a	0.07±0.09a	0.01±0.01a
2-methyl naphthalene	0.08±0.02[a]	0.46±0.49[a]	0.05±0.03[a]	0.08±0.09[a]	0.04±0.03[a]
Acenaphthylene	1.66±0.14[a]	1.12±1.03a	0.16±0.15a	0.87±1.00a	0.26±0.04a
Fluorene	0.69±0.24[b]	0.27±0.33[ab]	0.01±0.00[a]	0.04±0.03[a]	0.01±0.00[a]
Acenaphthene	0.03±0.03[a]	0.07±0.09[a]	0.03±0.01[a]	0.04±0.05[a]	0.01±0.00[a]
Phenanthrene	0.05±0.06[a]	0.15±0.16[a]	0.01±0.00[a]	0.48±0.65a	0.07±0.00[a]
Anthracene	0.06±0.05[a]	0.90±1.26[a]	0.00±0.003[a]	0.16±0.21[a]	0.02±0.01[a]
Fluoranthene	0.05±0.03[a]	0.26±0.37[a]	0.00±0.002[a]	0.11±0.14[a]	0.03±0.01[a]
Pyrene	0.02±0.01[a]	1.02±0.32[a]	0.65±0.30[a]	2.65±0.85[b]	0.02±0.02[a]
Benzo (a) anthracene	0.04±0.04[a]	0.02±0.00[a]	0.05±0.03[a]	0.68±0.68[a]	0.10±0.06[a]
Chrysene	0.77±1.06[a]	0.06±0.02[a]	0.05±0.04[a]	0.67±0.81[a]	0.18±0.03[a]
Benzo (b) fluoranthene	0.02±0.00[a]	0.27±0.37[a]	0.00±0.00[a]	0.22±0.29[a]	0.05±0.01[a]
Benzo (k) fluoranthene	0.68±0.08[b]	0.16±0.14[a]	0.05±0.02[a]	0.31±0.21[a]	0.07±0.04[a]
Benzo (a) pyrene	0.01±0.00[a]	0.11±0.16[a]	0.08±0.02[a]	0.22±0.30[a]	0.02±0.02[a]
Dibenz (a, h) anthracene	0.03±0.03[a]	0.44±0.41[a]	0.16±0.08[a]	1.48±1.92[a]	1.12±0.56[a]
Indeno (1,2,3-cd) pyrene	2.06±0.04[a]	5.09±3.80[a]	1.71±0.53[a]	1.25±0.33[a]	0.59±0.29[a]
Benzo (g, h, i) perylene	0.29±0.04[a]	1.04±1.21[a]	0.89±0.64[a]	1.01±1.09[a]	0.95±0.89[a]
Total	8.04±3.00b	11.84±10.00b	3.92±0.54a	10.32±8.74b	3.53±0.08a

Rows represent mean ± standard deviation of triplicate determinations. Values bearing different superscripts per row are significantly ($p<0.05$) different

Table 2: Concentrations (µg/kg) of Fatty acids in dried fish samples consumed in Owerri Imo State Nigeria

Sample (mg/kg)	Bonga fish (*Ethmalosa fimbriata*)	Cat fish (*Clarias gariepinus*)	Cray fish (*Palaemon hastatus*)	Round fish (River African Cat)	Stock fish (*Gadus morhua*)
Lauric acid (C 12)	9.74±0.00^{c}	2.70±0.01^{a}	ND	ND	3.58±0.04^{b}
Myristic acid (C 14)	0.05±0.05a	3.43±0.04^{a}	ND	2.49±0.12^{a}	6.27±5.67^{a}
Palmitic acid (C 16)	ND	5.86±0.04^{b}	4.98±0.23^{b}	3.62±1.69^{ab}	2.44±0.90^{a}
Stearic acid (C 18)	18.16±2.28^{c}	ND	7.36±0.00^{b}	3.24±1.57^{a}	1.02±0.02^{a}
Oleic acid (C 18:1)	81.93±9.62^{c}	33.66±2.54^{b}	30.39±4.61^{ab}	23.65±4.87a^{b}	17.89±1.54^{a}
Linoleic acid (C 18:2)	6.38±0.63^{a}	ND	6.43±0.21^{a}	6.53±0.07a	11.82±2.15^{b}
alpha-Linolenic acid (C 18:3)	4.12±0.00^{b}	ND	1.67±0.021^{a}	1.19±0.47^{a}	3.82±0.04^{b}
Icosadienoic acid (C 20:2)	ND	9.33±0.72^{b}	ND	3.51±0.06^{a}	3.52±0.07^{a}
Icosatrienoic acid (C20:3)	13.57±0.35^{c}	ND	ND	0.07±0.02^{a}	6.05±0.01^{b}
Arachidonic acid (C 20:4)	ND	ND	3.84±0.26^{a}	2.90±1.1.58^{a}	2.71±0.65^{a}
Tetracosanolpentaenoic acid (C 24:5)	1.06±0.01^{b}	1.18±0.14^{b}	1.59±0.21^{c}	1.64±0.0.14^{c}	0.01±0.00^{a}
Docosahexaenoic acid (C 22:6)	4.36±0.00^{b}	4.52±0.28b	9.90±0.43^{c}	9.44±0.36^{c}	0.01±0.00^{a}
Total	139.42±12.12^{b}	60.68±2.22^{a}	66.15±4.80^{a}	58.28±10.09^{a}	59.13±4.79a

Rows represent mean ± standard deviation of duplicate determinations. Values bearing different superscripts per row are significantly ($p<0.05$) different

Oleic acid is a monounsaturated fatty acid and presents beneficial effects such as reduction of inflammation and may have beneficial effects on genes linked to cancer. Oleic acid has other functions such as improving insulin sensitivity, lowering blood pressure, lowers cholesterol levels, decrease of chronic nerve pain, slowing of ageing process [31].Linoleic acid at 11.82±2.15 mg/kg and alpha-Linolenic acid at 4.12±0.00 mg/kg showed highest concentrations in Stock fish and Bonga fish respectively. However, both were not detected in Cat fish. Linoloeic acid and Linolenic acid are polyunsaturated fatty acids (PUFA). They are involved in lowering serum cholesterol levels, slowing the development of atherosclerosis, and formation of arachidonic acid [24,32]. These PUFA have modulatory potentials and are important in blood pressure reduction [33]. Other PUFAs such as Eicosadienoic acid, Eicosatrienoic acid,Arachidonic acid, Tetracosanolpentaenoic acid and Docosahexaenoic acid are regarded as essential fatty acids and important for brain development and function and might be vital for facilitating the transmission of signals between neurons [34]. Many studies have positively correlated essential fatty acids with reduction of cardiovascular morbidity and mortality, infant development, cancer prevention, optimal brain and vision functioning, arthritis, hypertension, diabetes mellitus and neurological/neuropsychiatric disorders [35,36].

4. Conclusions

With high consumption of dried fish processed over open flame, it becomes a major dietary source of PAHs in Owerri Imo State Nigeria. The monitoring of the PAHs and fat content of open flame processed fish become important aspects of human health risk assessment. This is so because PAHs tend to bioaccumulate in fatty foods such as fish, meat, oil and milk, and are extremely toxic to humans even at low concentrations. Foods containing high amount of lipids such as fish are excellent delivery systems for PAHs since lipid contents acts as carriers and allows passive absorption by the gastrointestinal tract. Several countries including Nigeria have insufficient or no legislation to enforce acceptable levels of PAHs in foods, however, data on levels of PAHs

contamination in foods and risk exposure are essential to aid nutritional planning.

Conflicts of Interest: The authors declare no conflict of interest.

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