LABORATORY MEDICINE

FATTY ACID CONTENT OF THE SMOKED, FRESH-WATER FISH *CLAIRAS GARIEPINUS (WANKA HARWADA,* HAUSA) IN NORTHERN NIGERIA*

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ABSTRACT

Since the healthful n-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are underrepresented in the diets of many people who live in the hot semi-arid regions of West Africa, we were interested in knowing the fatty acid content and composition of the oil of *Clairas gariepinus* (*wanka harwada*, Hausa), a dried, fresh-water fish that is consumed widely in northern Nigeria. Dried *C. gariepinus*, purchased in the central market in Jos, Nigeria was divided into the head, mid-section, and tail sections, vacuum-dried to constant weight, and extracted with chloroform-methanol to provide the lipid fraction. After methylation of the lipid fraction, the individual fatty acid methyl esters were separated and quantified by gas-liquid chromatography. The fatty acid content and fatty acid composition of each of the three sections of the fish were very similar. Overall, fatty acids accounted for 9.43-11.5% of the true dry weight of *C. gariepinus*. The amounts of EPA and DHA were relatively low, 1.14 and 3.78 mg/g dry weight, respectively. The arachidonic acid, linoleic acid and α-linoleic acid contents were 4.52, 9.00, and 3.76 mg/g dry weight, respectively. Compared to marine fish such as salmon and herring that are rich sources of n-3 polyunsaturated fatty acids, *C. gariepinus* appears to be unsuitable as a source of healthful quantities of EPA or DHA.

INTRODUCTION

Several recent studies have shown that certain populations in northern Nigeria would likely benefit from an increase in the content of n-3 and n-6 polyunsaturated fatty acids in their diet. One such group is lactating women. In our investigations of the nutrient quality of the milk of nursing women in the Jos Plateau of Nigeria (1,2) we found that the milk triglycerides of particular ethnic groups contained relatively low proportions of docosahexaenoic acid (DHA) and the essential n-6 fatty acid, linoleic acid. The growth and development of the central nervous system of the fetus, especially during the third trimester, and that of the infant during the first two years of life (3) are highly dependent on arachidonic acid and DHA. Arachidonic acid and DHA account for as much as 60% of the fatty acids that comprise the phospholipids of the retina and brain of the newborn (4). Arachidonic acid can be formed from linoleic acid by elongation and desaturation of the latter.

A second population in northern Nigeria whose DHA status is marginal is the Fulani. The Fulani of the Jos Plateau are primarily a semi-nomadic people whose life style and culture are based on cattle. Furthermore, their diets are rich in saturated fatty acids and provide little in the way of polyunsaturated fatty acids (5,6). In a recent study in which we determined the fatty acid composition of the serum phospholipids of Fulani herdsmen and based on the assumption that serum phospholipids are surrogates for tissue phospholipids (7), we obtained evidence indicating that the tissue membranes of these adult Fulani males contained less polyunsaturated fatty acids and were considerably less fluid than those of eight other populations worldwide. Since the function of various membrane-associated enzymes and proteins involved in signaling, hormone binding, and solute transport are influenced by the fluidity of the membrane with which they are associated, our observations provide grounds for being concerned about the polyunsaturated fatty acid intake of the Fulanis.

A third group in northern Nigeria whose intake of polyunsaturated fatty acids appears to be sub-optimal are children with sickle cell disease. In several studies conducted in Jos, Nigeria we found that, compared to healthy children who did not have this hematologic disorder, the red cell phospholipids of children with sickle cell disease contained reduced amounts of a number of n-3 and n-6 polyunsaturated fatty acids (8-10). Furthermore, in another study where bioelectrical impedance analysis was used to investigate the body composition (11,12) of children with sickle cell disease, we found that the phase angle of the children with this hematologic disorder was much reduced relative to their healthy counterparts. It is widely accepted that the phase angle is directly proportional to the overall health of an individual and is thought to reflect the quality and integrity of their tissue membranes. In addition, we observed strong positive correlations between the phase angle of healthy children and children with sickle cell disease versus the content of n-3 polyunsaturated fatty acids in the phospholipids of their red cell membranes (13).

On the basis of these observations and in light of the knowledge that the anti-thrombotic and anti-inflammatory n-3 fatty acids DHA and eicosapentaenoic acid (EPA) have prophylactic and therapeutic benefits in humans vis-à-vis cardiovascular disease (14,15), we were interested in identifying dietary sources of these particular fatty acid in northern Nigeria. EPA and DHA are present in fish oils but not in most plant oils. Furthermore, marine oils are among the richest sources of these polyunsaturated n-3 fatty acids. However, since the indigenous populations of northern Nigeria are located far from the ocean and because refrigeration is not widely available, marine fish such as haddock, hake, kingklip and sole that are good sources of DHA and EPA (16) are not widely available to these people. We were therefore interested in the fatty acid composition of *Clairas gariepinus* (*wanka harwada*, Hausa), one of the most common of the dried fish in the market-place and one that is within economic reach of many people in northern Nigeria. Although the oils of most fresh-water fish do not contain large proportions of DHA or EPA, there are some exceptions to this generalization (17). We therefore extracted the oil from the flesh of smoked *C. gariepinus* purchased in a local market in Jos, Nigeria and determined its fatty acid content and composition.

MATERIALS AND METHODS

Fatty acid analysis. Smoked *C. gariepinus* was purchased in the central market in Jos, Nigeria and dried to constant weight at 25 °C in a vacuum. The dried specimens of head, tail and mid-section were extracted with chloroform:methanol (2:1, vol/vol) as described elsewhere (18) and the solid, non-lipid material was removed by filtration. The total extracted lipid material was recovered after solvent removal in a stream of nitrogen. The samples were then redissolved in anhydrous chloroform/methanol (19:1, v:v) and clarified by centrifugation at 10,000 x g for 10 min. Transmethylation was performed using 14% (w/v) boron trifluoride (BF_3) in methanol (19). A one-ml aliquot of each sample containing 50 nanograms of heptadecanoic acid (internal standard) was transferred to a 15-ml Teflon-lined screw-cap tube. After removal of solvent by nitrogen gassing, the sample was mixed with 0.5 ml of BF_3 reagent (15%, w/v), placed in a warm bath at 100°C for 30 min and cooled. After the addition of a saline solution, the methylated fatty acids were extracted into hexane. A calibration mixture of fatty acid standards was processed in parallel.

Aliquots of the hexane phase were analysed by gas chromatography. Fatty acids were separated and quantified using a Hewlett-Packard gas chromatograph (5890 Series II) equipped with a flame-ionization detector. One or two microliter aliquots of the hexane phase were injected in split-mode onto a fused-silica capillary column (Omegawax; 30 m x 0.32 mm I.D., Supleco, Bellefonte, PA). The injector temperature was set at 200 °C, detector at 230 °C, oven at 120 °C initially, then 120-205 °C at 4 °C per min, 205 °C for 18 min. The carrier gas was helium and the flow rate was approximately 50 cm/sec. Electronic pressure control in the constant flow mode was used. The calibration standards (NuCheck, Elysian, MN) were used for quantitation of fatty acids in the lipid extracts. The fatty acids reported represent the average of three determinations.

RESULTS

Approximately 10% of the dry weight of *C. gariepinus* was oil . Compared to marine fish such as salmon (16), the fish we analyzed for fatty acids contained not only relatively low percentages of EPA (1.1%) and DHA (3.5%), but low absolute amounts of these fatty acids as well (Table 2). In addition, there were only small differences in both the amounts and the percentages of these two n-3 fatty acids in the oil extracted from the three sections of the fish (e.g., head, mid-section, tail). On the other hand, the percentages of arachidonic acid and the two essential fatty acids linoleic acid and α-linolenic acid were substantially greater in *C. gariepinus* than they are for the oil extracted from salmon. The percentages of arachidonic acid, linoleic acid, and α-linolenic acid we found in *C. gariepinus* were 4.2%, 8.3%, and 3.5%, respectively as compared to the corresponding percentages that have been reported for salmon, which are 0.2%, 4.8%, and 0.4% (Table 1)(16).

Saturated fatty acids accounted for 35-40% of the fatty acid total in the oil of *C. gariepinus* and palmitic acid was the dominant saturated fatty acid. The most abundant monounsaturated fatty acid in *C. gariepinus* was oleic acid (18:1n-9), accounting for 20-25% of the fatty acid total. Oleic acid is widely regarded as having beneficial effects on the cardiovascular system.

Table 1. Percentages of selected fatty acids in the flesh of *C. gariepinus* and several marine and other cold-water fish

	Percent				
Fatty acid	*C. gariepinus*	Mozambique Tilapia*	Largemouth Bass*	Salmon*	Herring*
	Mean ± (SD)	Mean	Mean	Mean	Mean
10:0	ND	NR	NR	NR	NR
12:0	0.1 (0.03)	NR	NR	NR	NR
14:0	1.7 (0.3)	NR	NR	NR	NR
14:1	0.02 (0.02)	NR	NR	NR	NR
15:0	1.2 (0.02)	NR	NR	NR	NR
16:0	24.7 (0.4)	23.4	20.0	29.4	15.4
16:1	7.1 (0.2)	3.1	5.9	2.1	10.0
18:0	10.4 (0.3)	7.3	7.8	8.7	1.9
18:1n-9	20.5 (0.6)	14.0	21.2	6.1	13.2
18:1n-7	6.6 (0.07)	NR	NR	NR	NR
18:2n-6	8.3 (0.3)	0.8	5.0	4.8	2.6
18:3n-6	0.5 (0.03)	NR	NR	NR	NR
18:3n-3	3.5 (0.3)	1.4	3.2	0.4	0.6
20:0	0.6 (0.04)	ND	ND	NR	NR
20:1n-9	0.7 (0.07)	ND	ND	2.2	18.4
20:2n-6	0.5 (0.05)	ND	ND	NR	NR
20:3n-6	0.8 (0.06)	ND	ND	NR	NR
20:4n-6	4.2 (0.31)	2.3	11.4	0.2	0.4
20:3n-3	0.3 (0.03)	NR	NR	NR	NR
20:4n-3	0.4 (0.1)	NR	NR	NR	NR
20:5n-3	1.1 (0.1)	4.6	2.5	7.0	7.9
22:0	0.3 (0.03)	NR	NR	4.8	20.8
22:1n-9	0.04 (0.07)	NR	NR	NR	NR
22:4n-6	0.7 (0.06)	NR	NR	NR	NR
22:5n-6	0.8 (0.08)	NR	NR	NR	NR
22:5n-3	1.2 (0.08)	6.7	2.6	2.3	2.0
24:0	0.2 (0.02)	NR	NR	NR	NR
22:6n-3	3.5 (0.3)	35.5	18.6	11.4	6.8
24:1	0.1 (0.08)	0.9	1.0	NR	NR

ND, not detected; NR, not reported.

*Data from Van der Westhuyzen et al. (16).

Table 2 Fatty acid content of *C. gariepinus* (mg/g dry weight)

Fatty acid	Total (n =9) Mean ± sd	Head (n =3) Mean ± sd	Mid-section (n =3) Mean ± sd	Tail (n =3) Mean ± sd
10:0	- (-)	- (-)	- (-)	- (-)
12:0	0.13 (0.05)	0.09 (0.02)	0.16 (0.03)	0.16 (0.05)
14:0	1.89 (0.54)	1.56 (0.07)	2.24 (0.86)	1.87 (0.28)
14:1	0.03 (0.03)	- (-)	0.03 (0.04)	0.05 (0.02)
15:0	1.33 (0.18)	1.16 (0.04)	1.41 (0.16)	1.41 (0.19)
16:0	26.6 (3.30)	23.6 (0.43)	28.2 (2.94)	28.1 (3.76)
16:1	7.70 (1.24)	6.58 (0.27)	8.38 (1.30)	8.14 (1.22)
18:0	11.2 (1.65)	9.79 (0.07)	12.2 (2.06)	11.8 (1.32)
18:1n-9	22.1 (3.14)	18.9 (0.54)	23.4 (1.83)	24.1 (3.33)
18:1n-7	7.15 (0.96)	6.21 (0.15)	7.56 (0.87)	7.68 (0.98)
18:2n-6	9.00 (1.24)	7.71 (0.26)	9.46 (0.69)	9.82 (1.33)
18:3n-6	0.49 (0.09)	0.41 (0.02)	0.55 (0.10)	0.52 (0.09)
18:3n-3	3.76 (0.73)	3.22 (0.15)	4.14 (1.05)	3.93 (0.57)
20:0	0.61 (0.10)	0.51 (0.01)	0.62 (0.05)	0.69 (0.13)
20:1n-9	0.70 (0.14)	0.58 (0.03)	0.72 (0.09)	0.82 (0.17)
20:2n-6	0.56 (0.08)	0.50 (0.06)	0.55 (0.06)	0.64 (0.09)
20:3n-6	0.85 (0.11)	0.76 (0.03)	0.86 (0.06)	0.94 (0.12)
20:4n-6	4.52 (0.30)	4.29 (0.09)	4.60 (0.39)	4.67 (0.27)
20:3n-3	0.37 (0.04)	0.35 (0.01)	0.36 (0.03)	0.41 (0.06)
20:4n-3	0.43 (0.15)	0.34 (0.01)	0.53 (0.25)	0.42 (0.05)
20:5n-3	1.14 (0.21)	1.00 (0.05)	1.25 (0.34)	1.17 (0.12)
22:0	0.28 (0.04)	0.25 (0.01)	0.29 (0.04)	0.31 (0.03)
22:1n-9	0.04 (0.09)	0.02 (0.01)	0.1 (0.16)	0.01 (0.01)
22:4n-6	0.78 (0.09)	0.70 (0.02)	0.78 (0.09)	0.85 (0.08)
22:5n-6	0.91 (0.06)	0.87 (0.07)	0.90 (0.06)	0.95 (0.05)
22:5n-3	1.29 (0.13)	1.17 (0.04)	1.32 (0.10)	1.39 (0.12)
24:0	0.21 (0.04)	0.18 (0.03)	0.24 (0.03)	0.21 (0.02)
22:6n-3	3.78 (0.27)	3.59 (0.15)	3.83 (0.39)	3.91 (0.18)
24:1	0.10 (0.11)	0.06 (0.01)	0.17 (0.18)	0.06 (0.01)
Total	108.0	94.3	114.8	115.1

DISCUSSION

The main objective of this study was to inquire if *C. gariepinus*, one of the most widely available fresh-water fishes of northern Nigeria, might contain amounts of the n-3 polyunsaturated that could be sufficient to ameliorate the marginal EPA and DHA status that seems to prevail among some of the indigenous populations of that part of the world. Our main finding was that although *C. gariepinus* does have a fatty acid content in the 10-12% range that is comparable to that of other fresh-water fish that are consumed in sub-Saharan Africa (17) and while it does represent a good source of arachidonic acid and the two essential fatty acids (Table 2), its EPA and DHA content is so low as to render it a poor and impractical source of these important n-3 fatty acids for humans. Assuming that the smoked *C. gariepinus* one finds in the marketplace contains 50% water, from the data contained in Table 2, we estimate that 10 grams of the exhaustively, vacuum-dried specimens we analyzed corresponds to 50 grams of commercial fish, which is the quantity of fish an adult is likely to consume in one meal. This quantity of fish would provide approximately 0.06 grams of EPA and 0.19 grams of DHA (approximately 0.25 grams total n-3 fatty acid). In most human studies involving fish oil supplementation aimed at improving human health, the daily administration of gram quantities per day of combined EPA and DHA was required to obtain a significant effect on one or another cardiovascular disease risk factor in adults (18). Thus, the fatty acid data in Table 2, together with the fact that dried fish in general is relatively costly in northern Nigeria, indicate that *C. gariepinus* is probably unsuitable as a significant source of n-3 polyunsaturated fatty acids for the populations who reside there. The anti-thrombotic and anti-inflammatory effects of EPA and to a lesser extent DHA are thought to be due to the replacement of these two n-3 fatty acids for arachidonic acid in the pathway of prostaglandin biosynthesis. Whereas the n-6 fatty acid arachidonic acid is metabolized to the 2-series of prostaglandins, EPA and DHA are converted by cyclo-oxygenases into prostaglandins of the 3-series. Clot-promoting thromboxane TXA_3 is less potent than TXA_2, whereas prostacyclin I_3 and prostacyclin I_2 are about equally potent when it comes to reducing the tendency of platelets to adhere to one another or to the vascular endothelium. Furthermore, since the vascular endothelial cells produce relatively more prostacyclin than platelets synthesize thromboxane, the prostaglandin profile is shifted away from thrombosis (20).

The EPA/arachidonic acid ratio of *C. gariepinus* oil was about 0.25, which is 70 to 130-times lower than the corresponding ratio one finds in salmon (32.0) or herring (17.8)(see ref. 16). Thus, it is unlikely that consumption of *C. gariepinus* would have any of the salutary effects of marine fish on the human cardiovascular system. On the other hand, it should be acknowledged that *C. gariepinus* could provide the human diet useful quantities of arachidonic acid and the two essential fatty acids, linoleic acid and α-linolenic acid.

Since *C. gariepinus* and probably the other species of fresh-water fish that are also consumed in northern Nigeria cannot significantly increase the levels of n-3 polyunsaturated fatty acids in the tissue phospholipids of the indigenous populations, It would be worthwhile considering alternative sources of these nutritionally significant fatty acids. One wonders if it would it be wise to encourage nutritionists, public health officials, and bioengineers in Nigeria and other Sahelian regions to consider growing n-3 polyunsaturated-rich algae commercially as a means of providing a source of these fatty acids for human consumption as is being done elsewhere in the world (21).

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