The "Basic" Fatty Acid Composition of Atlantic Fish Oils: Potential Similarities Useful for Enrichment of Polyunsaturated Fatty Acids by Urea Complexation[1]

R.G. Ackman*, W.M.N. Ratnayake and **B. Olsson**
Canadian Institute of Fisheries Technology, Technical University of Nova Scotia, P.O. Box 1000, Halifax, Nova Scotia B3J 2X4

It is known that the 20:1 and 22:1 fatty acids of fish oils from temperate and northern latitudes are of exogenous origin. By discounting these two fatty acids, calculation shows that the remaining fatty acids are a "basic" composition for these fish oils, with similar totals for saturated (14:0, 16:0), monounsaturated (16:1, 18:1) and polyunsaturated (primarily n-3) fatty acids. Thus, any of these oils are potential raw materials for urea complexing of acids or esters to give concentrates enriched in eicosapentaenoic and docosahexaenoic fatty acids.

The current interest in the biochemical effects of fish oils in human health and nutrition (1,2) has placed renewed emphasis on the apparent differences in the compositions of fish oils (3,4). Our interest in large-scale urea complexing of the fatty acids of fish oils to produce concentrates significantly enriched in polyunsaturated fatty acids (PUFA) included fish oils of diverse types such as Atlantic redfish (*Sebastes sp.*), Pacific salmon (mixed, but probably mostly from sockeye (*Oncorhynchus nerka*), Atlantic herring (*Clupea harengus*), Pacific dogfish (*Squalus acanthias*) and Atlantic menhaden (*Brevoortia tyrannis*). Although the yields of n-3 PUFA varied, it soon became apparent that the concentrates generally were about 70% total n-3 PUFA. We conclude that the mystique attached to differences among fish oils is generally a misapprehension. There is only one basic fish oil from temperate and polar latitudes. This basic oil is in general represented by the oil from menhaden *B. tyrannis*, a species which appears sporadically in Canadian waters (5,6), but is otherwise the mainstay of U.S. Atlantic coast and Gulf of Mexico fisheries (4,7).

EXPERIMENTAL PROCEDURES

All oils were treated in the same way after the addition of 10 g of commercial antioxidant mix (60% BHA + BHT) or the equivalent of ethoxyquin. The procedure developed with redfish oil consisted of 20 kg being saponified under nitrogen in a stirred, steam-jacketed, 400-l kettle with 96% ethanol (27 l), H_2O (17 l), and reagent grade KOH (5 kg), under reflux, for 2 hr. To the soaps were added H_2O (50 l) and conc. HCl (6.9 l). After a brief stirring period further cooling was accelerated by passing tap water through the kettle jacket. After about 6 hr of standing the bottom layer was drained off until a small amount of emulsion appeared. The fatty acids (19.3 kg) were left in the kettle, 96% ethanol (180 l) and urea, Canadian Industries Limited fertilizer grade (50 kg), were added, and the contents heated until a clear homogenous solution was obtained. This solution was dumped into as many stainless steel pots of 40-l capacity as necessary, covered, and let stand at room temperature for 24 hr. These pots were then moved to a 5° ambient chill room for 24 hr. The ethanolic solution of non-urea complexing fatty (NUCF) acids was then decanted and mixed with H_2O (360 l) and conc. HCl (4.4 l). After sitting for 24 hr and draining off the bottom layer, the yield of fatty acid layer (NUCF-PUFA) was usually 3.5 - 4.0 kg.

Samples of oil and NUCF-PUFA were converted to methyl esters by heating at 100 C in a nitrogen-flushed screw-cap centrifuge tube for 60 min (oils) or 30 min (fatty acids) with 10% BF_3-MeOH (1 ml) and benzene or hexane (1 ml). Gas liquid chromatography was executed with a Perkin-Elmer Sigma-3B GLC unit and a Supelcowax-10 column, flexible fused silica, 30 m x 0.25 mm i.d., operated either isothermally (8) or with programmed temperatures (9).

RESULTS AND DISCUSSION

Redfish oil was chosen in Halifax as the raw material for production of polyunsaturated fatty acids by urea complexing of the nonesterified fatty acids. Table 1 gives the fatty acid changes resulting from the enrichment achieved by this process for redfish oil produced recently. Most of the saturated and mono-ethylenic fatty acids, except small amounts of 16:1 and 18:1,

TABLE 1.

Urea Complex Concentration of EPA and Other Fatty Acids From a Redfish (*Sabastes sp.*) Oil

Fatty acid	Starting oil	Concentrate
14:0	2.5	1.0
16:0	5.2	0.1
18:0	1.3	—
16:1	7.5	6.5
18:1	9.4	0.3
20:1	22.0	0.1
22:1	28.9	—
18:2n-6	0.4	1.2
18:3n-3	0.2	0.7
18:4n-3	0.8	5.1
20:4n-6	0.6	0.9
20:4n-3	0.3	1.6
20:5n-3	5.4	32.5
22:5n-3	0.8	3.0
22:6n-3	3.9	29.2
16:PUFA	2.1	10.4

*To whom correspondence should be addressed.

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were removed. The 20:1 and 22:1, if present are almost totally removed from all oils once split into acids or esters (10).

The urea process has only a modest influence on the relative proportion among 18:4n-3, 20:4n-3, 20:5n-3, 22:5n-3 and 22:6n-3. There is, however, discrimination favoring a reduction in the dienoic and trienoic fatty acids relative to 20:5n-3, and also in 20:4n-6. Fortunately, all of these latter types of fatty acids occur in low proportions in fish oils from temperate and cold waters (3).

In most fish oils eight fatty acids give an accurate picture of the fatty acid components (3,11). These are 14:0, 16:0, 16:1, 18:1, 20:1, 22:1, 20:5n-3 and 22:6n-3. To these one should add five others, 18:0, 18:2n-6, 18:3n-3, 18:4n-3 and 20:4n-6, to satisfy those interested in nutritional issues, especially of the n-6 fatty acids, and also to clarify fatty acid elongation biochemistry in the n-3 family. In commercial seal oils one also adds 22:5n-3, which usually is half the proportion of 22:6n-3 (12). It is a potentially important intermediate in retroconversion of 22:6n-3 to 20:5n-3 (13).

In Table 2 are four examples of how these relatively few fatty acids make up about 90% of the fatty acids. In menhaden oils (3,4,7) there are also usually 3–5% of C_{16} polyunsaturated fatty acids (14) which originate in the phytoplankton diet of menhaden but are largely screened out in other fish oils where there are one or more smaller animals intermediate between the phytoplankters and the fish yielding the oil.

Of the fatty acids in the menhaden oil of Table 2, saturated, monounsaturated and polyunsaturated fatty acids are roughly one-third each. These proportions have very little to do with there being three glycerol hydroxyls available for esterification. Brockerhoff did show that generally in fish oils there was a polyunsaturated acid in the 2-position, and often a monounsaturated 16:1 or 18:1 acid in the 1-position, together with a tendency for the very long chain monounsaturated acids to be in the 3-position, but these are rather sweeping generalities (15,16).

To some extent the latter specificity for 20:1 and 22:1 reflects the exogenous origin of these two fatty acids (17), whereas both 16:1 and 18:1 can also be biosynthesized by fish along with 14:0 and 16:0. To demonstrate these relationships among the other 11 fatty acids of Table 2, the 20:1 and 22:1 were deleted from the compositions of three different North Atlantic fish oils and the percentages of the remainder adjusted to give the same totals. Two of the three "calculated" oils thus generated have saturated acid totals of about 30%, close to that of the menhaden oil. It is typical of very high iodine value marine oils produced from fish bodies (menhaden, anchovy, sardine, pilchard), with the "basic" fatty acid composition, that they include high proportions of saturated fatty acids (3), presumably biosynthesized in situ in adipocytes as part of the process leading to the incorporation of exogenous 20:5 and 22:6 into depot fat triglycerides. The cod liver oil from *Gadus morhua* is

TABLE 2.

Comparison of Menhaden Oil Fatty Acids With the "Basic" Fish Oil Composition Calculated for a Very Low Iodine Value Herring Oil, Cod Liver Oil and Redfish Oil by Eliminating 20:1 and 22:1

	Menhaden oil	Herring oils		Cod liver oils		Redfish oils	
	Chesapeake[a]	Very low iodine value[b]	Calculated	Average[a]	Calculated	Commercial[c]	Calculated
Wijs IV	167	101	—	163	—	125	—
Fatty acid							
14:0	11.0	6.0	12.6	3.3	3.8	2.5	4.1
16:0	19.9	9.3	19.5	13.4	15.5	13.2	21.4
18:0	3.3	0.9	1.9	2.7	3.1	2.2	3.6
Total saturated[d]	34.2	16.2	34.0	19.4	22.4	17.9	29.1
16:1	13.7	7.2	15.1	9.6	11.1	13.3	21.5
18:1	10.9	12.1	25.4	23.4	27.1	13.3	21.5
20:1	1.3	19.4	—	7.8	—	17.2	—
22:1	—	29.7	—	5.5	—	18.9	—
Total monounsaturated[d]	25.9	68.4	40.5	46.1	38.2	62.7	43.0
18:2n-6	1.1	0.8	1.7	1.4	1.6	0.9	1.5
20:4n-6	0.6	0.2	0.4	1.4	1.6	0.3	0.5
18:3n-3	1.1	0.4	0.8	0.6	0.7	0.5	0.8
18:4n-3	2.6	1.0	2.1	0.2	0.2	1.5	2.4
20:5n-3	14.6	4.4	9.2	11.5	13.3	7.3	11.8
22:6n-3	7.5	2.1	4.4	12.5	14.5	3.3	5.3
Total polyunsaturated[d]	27.5	8.9	18.6	27.6	31.9	13.8	22.3
Total these acids	87.6	93.5	93.1	93.1	92.5	94.4	94.4

[a]Ref. (3).
[b]Ref. (19).
[c]Ref. (20).
[d]Ignoring minor components.

rather more highly unsaturated than the herring and redfish oils to start with, with less 20:1 and 22:1. Removal of these by the "calculation" procedure leaves a lower proportion of saturated acids than for the other two oils. All three "calculated" oils have about 40% monoethylenic fatty acids. The redfish oil, from *Sebastes sp.*, is from an analysis of production dating to 1967.

Although the proportions of 20:5 and 22:6 in the original redfish oil are quite modest compared to oils of much higher iodine value and general unsaturation, such as menhaden (3), the underlying fish oil composition for the "calculated" oils is such that the total polyunsaturated acids of Table 2 are nearly the same as menhaden oil when the exogenous 20:1 and 22:1 are discounted. Even the particular herring oil, selected for its extremely low iodine value, is revealed as an alternative source of a concentrate of omega-3 fatty acids. From actual urea complexing trials it is also known that the fatty acids recovered from dogfish (*Squalus acanthias*) liver oil (also with an iodine value of about 100) can yield a satisfactory omega-3 concentrate despite the glyceryl ether content (Ackman et. al., unpublished). Thus, most fish oils of northern waters will produce an omega-3 concentrate, normally with 18:4n-3, 20:5n-3, 22:5n-3 and 22:6n-3, and other but minor n-3 fatty acids, totalling about 70% of fatty acids. We believe that this should also apply to fish oils from similar Antarctic, South Atlantic and South Pacific latitudes.

Stansby (18) has addressed some of the reasons for the variability in fish oil fatty acid composition within a given species. In addition to biological and biochemical variability from seasonal and geographical effects, the part of the fish used and mode of recovery must be included. This brief summary is an attempt to show that the enormous amount of work suggested by Stansby is perhaps not necessary if a few representative analyses of each oil can be shown to be related to the proposed "basic" fish oil composition, typically that of menhaden oil, supplemented by exogenous 20:1 and 22:1. There will always be some exceptions to any set of rules in the oils and fats field, but the acute contemporary interest in concentrating omega-3 fatty acids requires a means for simple and rapid assessment of commercially available fish oils. Total polyunsaturated fatty acids in a fish oil can easily be estimated (21) from the iodine value by the formula:

$$w/w\% \text{ PUFA} = 10.7 + 0.337 \text{ (Oil IV-100)}$$

Since n-6 acids are invariably present in a constant and low proportions (3), the yield of n-3 acids can be projected.

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REFERENCES

1. Ackman, R.G., in *n-3 News: Perspectives on Eicosapentaenoic Acid [EPA], Vol. 1,* No. 4. (1986).
2. Lands, W.E.M., *Fish and Human Health,* Academic Press, 1986.
3. Ackman, R.G., in *Nutritional Evaluation of Long-Chain Fatty Acids in Fish Oil,* edited by S.M. Barlow and M.E. Stansby, Academic Press, London, 1982, pp. 25–88.
4. Bimbo, A.P., *J. Am. Oil Chem. Soc. 64*:706 (1987).
5. Ackman, R.G., C.A. Eaton and J.H. Hingley, *J. Sci. Food Agric. 27*:1132 (1976).
6. Ackman, R.G., W.M.N. Ratnayake and C.A. Eaton, *Proc. N.S. Inst. Sci. 31*:207 (1981).
7. Joseph, J., *Mar. Fish. Rev. 47*:30 (1985).
8. Ackman, R.G., in *Analysis of Oils and Fats,* edited by R.J. Hamilton and J.B. Rossell, Elsevier Appl. Sci. Publishers, London, 1986, pp. 137–206.
9. Ackman, R.G., *Acta Med. Scand. 222*:99 (1987).
10. Ackman, R.G., R.D. Burgher and P.M. Jangaard, *Can. J. Biochem. Physiol. 41*:1627 (1963).
11. Lambertsen, G., *Fisk. Dir. Skr. Ser. Ernaering. 1(4)*:105 (1978).
12. Ackman, R.G., S. Epstein and C.A. Eaton, *Comp. Biochem. Physiol. 40B*:683 (1971).
13. von Schacky, C., and P.C. Weber, *J. Clin. Invest. 76*:2446 (1985).
14. Ackman, R.G., and P.M. Jangaard, *J. Am. Oil Chem. Soc. 40*:744 (1963).
15. Brockerhoff, H., *Comp. Biochem. Physiol. 19*:1 (1966).
16. Brockerhoff, H., and R.J. Hoyle, *Arch. Biochem. Biophys. 102*:452 (1967).
17. Ackman, R.G., J-L. Sebedio and M.I.P. Kovacs, *Mar. Chem. 9*:157 (1980).
18. Stansby, M.E., *J. Am. Oil Chem. Soc. 58*:13 (1981).
19. Ackman, R.G., C.A. Eaton and P.J. Ke, *J. Fish. Res. Bd. Canada 24*:2563 (1967).
20. Ackman, R.G., and P.J. Ke, *Ibid. 25*:1061 (1968).
21. Ackman, R.G., *J. Am. Oil Chem. Soc. 43*:385 (1966).

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