PROC. N.S. INST. SCI (1986)
Volume 37, pp. 1-7

VITAMINS A, D_3 AND E IN NOVA SCOTIAN COD LIVER OILS

S. F. O'KEEFE and R. G. ACKMAN*
Canadian Institute of Fisheries Technology
Technical University of Nova Scotia
P.O. Box 1000, Halifax, N.S.
B3J 2X4

There has been no production of cod liver oil in Nova Scotia for decades and in this time fish stocks and landings have been altered by the overfishing and resultant quotas and restrictions. Accordingly individual livers from cod, *Gadus morhua*, taken in three Nova Scotia fishing areas were examined for vitamins A, D_3 and E. Average contents for fish landed in Yarmouth, Lower Prospect and Canso respectively were: -Vitamin A, 1100, 420 and 850 retinol equivalents (R.E.) g^{-1} oil; vitamin D_3, 1.56, 0.88 and 0.88 $\mu g\ g^{-1}$ oil; vitamin E, 259, 286, 320 $\mu g\ g^{-1}$ oil. In terms of meeting current pharmaceutical standards for commercial oils there is a reasonable prospect of vitamin A content being at or near requirements, but vitamin D_3 would likely have to be supplemented. Vitamin E contents correlate with other oil soluble vitamin values, suggesting that the content of this oil-soluble material may be related to the age and size of fish as is the case with vitamins A and D_3.

L'huile de foie de morue n'as pas été produite en Nouvelle Ecosse depuis des décennies et en cette période les réserves et la prise de poisson ont été changés par le surpêchage et les quotas et les restrictions imposés par la suite. Les foies de morues *Gadus morhua* ont été pris de poissons attrapés dans trois régions de pêche de la Nouvelle Ecosse et ils ont été examinés pour la présence des vitamines A, D_3 et E. Les taux moyens pour des poissons pris a Yarmouth, Lower Prospect et Canso étaient respectivement : Vitamine A, 1100, 420 et 850 équivalents de retinol (E.R.) g^{-1} d'huile; vitamine D_3, 1.56, 0.88 et 0.88 $\mu g\ g^{-1}$ d'huile; vitamine E, 259, 286 et 320 $\mu g\ g^{-1}$ d'huile. Il est possible que les taux en vitamine A atteindraient les normes pharmaceutiques actuelles pour les huiles commerciales mais il faudrait probablement ajouter de la vitamine D_3. Les taux en vitamine E correspondent aux taux des autres vitamines soluble à l'huile. Ceci suggère que le taux de cette vitamine pourrait être lié à l'âge et à la taille du poisson ce qui est le cas des viamines A et D_3.

Introduction

Cod liver oil has been used for over two centuries as a food supplement (Möller, 1895; Chabré, 1936). Demand for medicinal cod liver oil produced much research and the emergence of an extensive Atlantic coast industry in the first half of the 20th century (Bailey et al., 1952). Replacement by synthetic vitamins in the 1950's caused the fish liver oil industry to die out. In recent years, there has been a resurgence of interest in pharmaceutical cod liver oil as a natural source of vitamins, thus promoting an industry which, in Nova Scotia, has not existed for at least two decades. Retail cod liver oil is imported and is usually identified on the label as "Norwegian".

Methods of vitamin analysis available to early researchers were limited to biological and chemical (colorimetric) techniques. These methods suffered from lack of precision and, in the case of colorimetry, interference without extensive clean-up procedures. Recently high performance liquid chromatography (HPLC) has been employed to determine vitamins A (Stancher and Zonta, 1984), D (Egaas and Lambertsen, 1979; Elton-Bott and Stacey, 1981; Pask-Hughes and Calam, 1982; Takeuchi et al., 1984; Stancher and Zonta, 1983) and E (Hung et al., 1980; Syvaoja et al., 1985) in fish oils.

It is apparent, from the older literature, that the fat soluble vitamin content of Atlantic cod liver oil varies greatly. The major factor influencing the liver oil potency is the spawning cycle (Drummond and Hilditch, 1930; Bailey, 1952; Cruickshank, 1962; Krassowska, 1969). The vitamin concentration in the liver oil increases during the spring-summer spawning period, when liver triglyceride is depleted, and increases in the fall-winter when lipid reserves are built up (Jangaard et al., 1967). The basic work on cod liver oil was executed over forty years ago and many things have changed. Fish populations are undoubtedly different and more accurate analytical techniques re-

* To whom correspondence should be addressed.

place the older colorimetric and biological methods. For these reasons, the older results for the vitamin contents of cod liver oil are not applicable to the cod being caught today.

World production of pharmaceutical fish liver oils is around 24,000 tonnes (Fisheries and Oceans, 1980). Since in Nova Scotia cod livers are currently being discarded, production of cod liver oil could supplement incomes of fishermen. With that in mind, this study was executed to update literature values of vitamin contents of Atlantic cod liver oils.

Materials and Methods

Cod Livers

Round codfish (*Gadus morhua*) were obtained from three Nova Scotian locations. The locations were Lower Prospect, Halifax Co. (n=11, July, 1982), Yarmouth (n=60, Late August, 1983) and Canso (n=7, September, 1983). The Lower Prospect and Canso fish were received from inshore boats and the Yarmouth sample from an offshore boat fishing the Browns Bank. The length and weight measurements of the cod were recorded. The individual livers were removed, placed in Mylar bags, and stored on ice. On return to the laboratory they were weighed, vacuum sealed in Mylar bags and stored at -35°C until use. Four categories were used to described appearances of the livers. They were 1-creamy white, 2-light brown, 3-dark brown, and 4-dark brown with seal worm infestation. Liver condition and general condition were calculated according to Jangaard et al. (1967).

Extraction of the Liver Oil

The individual livers were blended with anhydrous sodium sulfate and dichloromethane (1:5:3 w/w respectively). The mixture was blended for 5 minutes in a Waring Blendor, filtered under nitrogen (Whatman #1) and solvent removed under vacuum with slight heating (30°C). The oils were stored under nitrogen at -80°C until analysis.

Saponification Procedure

About 2 g oil was accurately weighted into a 50 ml leak-proof Teflon-lined screw-cap centrifuge tube. To this 30 ml ethanol (90%), 1.5 ml KOH (50% w/w aq.), a few grains of EDTA (disodium ethylenediamine tetraacetate) and 1 ml sodium ascorbate solution (1.7 g ascorbic acid in 10 ml 0.1 *N* NaOH) were added. The tube was flushed with nitrogen, tightly capped, and placed in a boiling water bath. The tube was shaken by hand every 2 minutes. After exactly 15 minutes the tubes were removed and cooled under running water. One ml of a BHA (butylated hydroxy anisole) solution (1 mg ml^{-1} in hexane) was added and the unsaponifiables were extracted with peroxide free diethyl ether (1 x 100, 2 x 50 ml). The combined ether phases were washed with water (3 x 50 ml) and then dried with sodium sulfate. The solvent was evaporated at 35°/10 mm. Unsaponifiables were transferred, using ether as solvent, to a 5 ml volumetric flask. The ether was evaporated to near dryness with nitrogen. Methanol was used to bring the volume to 5 ml and undissolved cholesterol was solubilized with sonication. This solution was filtered, using a 0.45 μ Millex filter (Waters Assoc., Milford, Mass.), into a 10 ml screwcap centrifuge tube.

High Performance Liquid Chromatography

The HPLC system used included a model 6000A pump, a model 480 UV absorbance detector, a U6K injector (Waters Assoc., Milford, Mass.) and a strip-chart recorder. Chromatographic separations were carried out on normal phase silica (μ-Porasil, 30 x

0.4 cm, 10 μ; Waters Assoc., Milford, Mass.) or reverse phase octadecane bonded silica (Hybar, 12.5 x 0.4 cm, 5 μ; Merck, Darmstadt, Germany) columns. Guard columns were hand packed with either C-18 Corasil or silica Corasil II chromatographic materials (Waters Assoc., Milford, Mass.).

Vitamin Determinations

Vitamin A, as all-trans retinol, was determined by injecting the unsaponifiable fraction onto the C-18 column and eluting with methanol/water (95:5) at a flow rate of 1.0 ml min^{-1}. The UV absorbance detector was set at 325 nm. The same system was used for determination of d-α-tocopherol except the flow rate was 1.2 ml min^{-1} and UV absorbance was 295 nm. For vitamin D_3, a C-18 column cleanup was used prior to silica column determination. The unsaponifiables were injected onto a C-18 column and eluted with methanol (100%) at 1.0 ml min^{-1}. The area of the chromatogram corresponding to vitamin D_3 elution was collected in a 10 ml screw cap tube. The cholecalciferol from two 200 μl injections of the unsaponifiables was thus collected, dried with a stream of nitrogen, and redissolved in 500 μl hexane/isopropanol (97.5:2.5). This solution was injected onto a silica column eluted with hexane/isopropanal (97.5:2.5) at a rate of 1.5 ml min^{-1} and the detector set at 265 nm. Recoveries for all three vitamins were calculated using authentic standards and the standard additions technique.

Statistical Analysis

Analysis of variance (completely random design), correlation coefficients and multiple liner regression (using the step-down technique) was done using the Statistical Analysis System package (SAS Institute Inc., Cary, N.C., U.S.A.) on a NAS-AS9160 mainframe computer.

Results and Discussion

Mean vitamin recoveries and standard errors are illustrated in Table I. The HPLC methods proved satisfactory for all three vitamins. Several clean-up methods other than saponification were examined but proved unsatisfactory (O'Keefe, 1984). The quantitative transfer of unsaponifiables during the saponification procedure required practice to achieve precise results.

The vitamin contents are expressed as retinol equivalents (R.E.), μg cholecalciferol, and μg d-α-tocopherol for vitamins A, D_3 and E respectively, as suggested by Bieri and McKenna (1981). The minimum vitamin potencies required to reach US Pharmacopoeia standards are 850 and 85 IU per gram of oil for vitamins A and D. This translates to 850 R.E. and 2.13 μg vitamin D. The average vitamin content of the livers would not be expected to equal the value of the pooled oil from the livers (i.e. vitamin concentration and amount of oil from individual livers are both important).

Table I Recoveries of added Vitamins A, D_3, and E from Cod Liver Oil.

Vitamin	Mean Recovery (%)*	Std. Dev.
A	93.1	1.3
D_3	80.1	3.0
E	81.3	2.0

* three replications

The biological data for the cod are summarized in Tables II, III, and IV. Cod liver oil was found to have from 210 to 4180 R.E. g^{-1} liver oil. Bailey et al. (1952) reported a range of 165-3000 R.E. g^{-1} liver oil. The average values for Yarmouth (Y), Lower Prospect (LP), and Canso (C) were 1100, 420, and 850 R.E. g^{-1} oil.

The average vitamin D_3 values obtained were 1.56, 0.88, and 0.88 $\mu g\ g^{-1}$ oil for Y, LP and C. The vitamin potencies ranged from 0.11 - 6.21 $\mu g\ g^{-1}$ oil. Although some of the literature values for vitamin D in cod liver oil are for commercial oils, which may have been blended or spiked to achieve a certain vitamin concentration, most of the values reported are wtihin the range cited by Cruickshank (1962), which is 0.5-0.7 $\mu g\ g^{-1}$ oil. The range of the present study also agrees with the older values.

Vitamin E in fish liver oils has been overlooked by most researchers. There are no standards for vitamin E content of cod liver oil. The range of potencies was 51 to 1669 $\mu g\ g^{-1}$ oil. Literature values for cod liver oil range from 115 to 430 $\mu g\ g^{-1}$ oil (Ackman and Cormier, 1967; Cruickshank, 1962; Stancher and Zonta, 1983) and burbot (the freshwater cod *Lota lota*) liver oil has been reported to contain 340 $\mu g\ g^{-1}$ oil (Syvaoja et al., 1985).

There were differences in the vitamin contents of the oils from the three Nova Scotian locations. The difference for vitamin D but not A or E was significant at the 5% level (F=5.00, 1.28 and 0.28 respectively). Because of the variation in sampling date and location, a cautious interpretation of the data is necessary. There may be a dissimilarity between cod caught inshore and offshore. The offshore cod from Browns Bank would likewise not be expected to accurately represent cod captured in other offshore fishing grounds.

Due to the small number of samples from Canso and Lower Prospect, only Yarmouth data were used for correlation and multiple linear regression. The results for the correlation analysis of Yarmouth data are represented in Table V. It should be noted that, in the cases for all three vitamins, the oil content of the liver by itself explained much of the observed variation (r^2 of 0.58, 0.62, and 0.56 for vitamins A, D_3, and E respectively). The backward multiple regression models contained oil, liver weight and fish length for vitamin D_3, while liver condition was also significant for the other two vitamins and fish weight replaced fish length for vitamin A (Table VI). The importance of oil content is obvious because of the dilution effect the triglycerides have on vitamin concentrations. The importance of fish length, fish weight and liver weight probably reflect the fact that these parameters are elevated in older cod, which have incorporated greater amounts of vitamins in their livers. Very reasonable R^2 values of 0.778, 0.732, and 0.715 for the three vitamins (O'Keefe, 1984) indicate that the variables incorporated in the multiple regression equations explain most of the vitamin variations. This is remarkable in the light of the variations in the physical conditions of the fish studied, but suggests that the three oil-soluble vitamins are not functional in cod. The rapidity with which cod can accumulate fat in the liver has recently been demonstrated (Lie et al., 1986), but despite many years of industrial records (Bailey et al., 1952; Aure, 1967) this is the first comparison interrelating three oil-soluble vitamins.

In this study we have shown that vitamin contents of Nova Scotian cod liver oils are sufficiently high to be important commercially. The low vitamin D content may be of concern but it is unlikely that the data in this study would accurately estimate vitamin potencies in a commercially processed and refined oil. The vitamin contents in the commercial oils would have to be determined before resorting to spiking with concentrates.

Table II Summary of Data for Cod from Yarmouth.

Variable	Mean	Std. Dev.	Minimum	Maximum
Fish: Length cm	63.5	17.4	39.0	120.0
Weight kg	3.3	2.9	0.7	14.5
Liver: Weight g	157.5	159.7	6.9	634.4
Oil content %	52.9	16.1	1.9	77.0
Condition	4.3	2.1	1.0	9.3
Appearance	1.6	1.0	1.0	4.0
General Condition	1.1	0.1	0.8	1.5
Vitamin: A in R.E. g^{-1} oil	1100	650	340	4180
D_3 in $\mu g\ g^{-1}$ oil	1.56	0.95	0.57	6.21
E in $\mu g\ g^{-1}$ oil	259	260	51	1669

Table III Summary of Data for Cod from Lower Prospect.

Variable	Mean	St. Dev.	Minimum	Maximum
Fish: Length cm	69.5	8.9	63	90.0
Weight kg	3.4	1.3	2.27	5.90
Liver: Weight g	115.3	84.4	36.3	295.4
Oil content %	46.8	9.4	32.1	59.4
Condition	3.6	1.6	1.9	6.4
Appearance	1.5	0.8	1.0	3.0
General Condition	0.9	0.1	0.8	1.1
Vitamin: A in R.E. g^{-1} oil	420	150	210	670
D_3 in $\mu g\ g^{-1}$ oil	0.88	0.57	0.11	2.36
E in $\mu g\ g^{-1}$ oil	286	64	198	389

Table IV Summary of Data for Cod from Canso.

Variable	Mean	Std. Dev.	Minimum	Maximum
Fish: Length cm	N.D.*			
Weight kg	N.D.			
Liver: Weight g	71.0	24.1	23.7	101.4
Oil content %	56.0	9.5	42.7	67.6
Condition	N.D.			
Appearance	N.D.			
General Condition	N.D.			
Vitamin: A in R.E. g^{-1} oil	850	780	360	1210
D_3 in $\mu g\ g^{-1}$ oil	0.88	0.27	0.54	1.34
E in $\mu g\ g^{-1}$ oil	320	104	117	415

* not determined

Table V Correlation Coefficients.

	Fish Length	Fish Weight	Liver Weight	Oil %	Appearance	A	D_3	E
Fish Length	1.00	0.96	0.79	-0.02	0.10	0.37	0.30	0.32
Fish Wt.	0.96	1.00	0.77	-0.10	0.15	0.38	0.37	0.39
Liver Wt.	0.79	0.77	1.00	0.32	-0.18	-0.01	-0.08	-0.05
Oil %	-0.02	-0.10	0.32	1.00	-0.87	-0.76	-0.79	-0.75
App	0.10	0.15	-0.18	-0.87	1.00	0.69	0.71	0.64
A	0.37	0.38	-0.01	-0.76	0.69	1.00	0.77	0.75
D_3	0.30	0.27	-0.08	-0.79	0.71	0.77	1.00	0.74
E	0.32	0.39	-0.05	-0.75	0.64	0.75	0.74	1.00

Table VI Multiple Regression Analysis.

Vitamin	R^2	Significant parameters	b
A	0.778		
		intercept	1.13
		oil %	-0.035
		liver weight	-0.002
		fish length	0.024
		liver condition	0.154
D_3	0.732	intercept	3.29
		oil %	-0.038
		liver weight	-0.002
		fish weight	0.167
E	0.715	intercept	613.5
		oil %	-11.625
		liver weight	-1.084
		fish weight	64.294
		liver condition	50.429

Acknowledgements

This work was supported in part by the National Sciences Research and Engineering Council of Canada. The co-operation of Kenny and Ross Ltd., Port Saxon, N.S., with raw materials, and the technical advice of W.M.N. Ratnayake of the CIFT are gratefully acknowledged.

References

Ackman, R.G. and **Cormier, M.G.** 1967. α-Tocopherol in some Atlantic fish and shellfish with particular reference to live-holding without food. *J. Fish. Res. Bd. Can.* 24: 357-373.

Aure, L. 1967. Manufacture of fish liver oil. In Fish Oils, Their Chemistry, Technology, Stability, Nutritional Properties and Uses. Stansby, M.E., ed. Avi Pub. Co., Westport, Conn., pp. 193-205.

Bailey, B.E., Carter, N.M. and **Swain, L.A.** 1952. Marine oils with special reference to those of Canada. *Fish. Res. Bd. Canada Bull.* 89: 413 pp.

Bieri, J.G. and **McKenna, M.C.** 1981. Expressing dietary values for fat-soluble vitamins: Changes in concepts and terminology. *Am. J. Clin. Nutr.* 34: 289-295.

Chabré, P. 1936. Les Huiles de Foie de Morue, Leur Teneur en Vitamines A et D. Masson et Cie., Paris, 207 pp.

Cruickshank, E.M. 1962. Fat-soluble vitamins. In Fish as Food, Borgstrom, G., ed. Vol. 2, Academic Press, New York, N.Y.

Drummond, J.C. and **Hilditch, T.P.** 1930. The relative values of cod liver oils from various sources. Empire Marketing Board Report No. 35, 129 pp.

Egaas, E. and **Lambertsen, G.** 1979. Naturally occurring vitamin D_3 in fish products analysed by HPLC, using vitamin D_2 as an international standard. *Internal. J. Vit. Nutr. Res.* 49: 35-42.

Elton-Bott, R.R. and **Stacey, C.I.** 1981. High-performance liquid chromatographic separation of the fat-soluble vitamins in cod liver oil and feeds. *Anal. Chim. Act 2* 127: 213-218.

Fisheries and Oceans. 1980. Annual Report: Maritimes Region. Information Division. Halifax, N.S., Canada 79 pp.

Hung, S.S.O., Cho, Y.C. and **Slinger, S.J.** 1980. High performance liquid chromatographic determination of α-tocopherol in fish liver. *J. Assoc. Off. Anal. Chem.* 53: 889-893.

Jangaard, P.M., Brockerhoff, H., Burger, R.D. and **Hoyle R.J.** 1967. Seasonal changes in general condition and lipid content of cod from inshore waters. *J. Fish. Res. Bd. Canada* 24: 607-612.

Krassowska, T. 1969. Vitamin value of oil from the liver of the Atlantic cod, and its production at sea, during the working cycle. Fisheries Research Board of Canada Translation No. 1380. *Przemysl Spozywczy* 23: 9-11.

Lie, O., Lied, E. and **Lambertsen, G.** 1986. Liver retention of fat and of fatty acids in cod (*Gadus morhua*) fed different oils. *Aquaculture* 59: 187-196.

Möller, F.P. 1895. Cod-Liver Oil and Chemistry. Peter Möller, Snowhill, London. 508 pp.

O'Keefe, S.F. 1984. Vitamins A, D_3 and E in Nova Scotian cod liver oils. M.Sc. Thesis, Technical University of Nova Scotia, 141 pp.

Pask-Hughes, R.A. and **Calam, D.H.** 1982. Determination of vitamin D_3 in cod-liver oil by high-performance liquid chromatography. *J. Chromatogr.* 246: 95-104.

Stancher, B. and **Zonta, F.** 1983. High-performance liquid chromatography of fat-soluble vitamins. Simultaneous quantitative analysis of vitamins D_2, D_3 and E. Study of percentage recoveries of vitamins from cod liver oil. *J. Chromatogr.* 256: 93-100.

Stancher, B. and **Zonta, F.** 1984. High-performance liquid chromatography of the unsaponifiable from samples of marine and freshwater fish: fractionation and identification of retinol (vitamin A) and dehydroretinol (vitamin A_2) isomers. *J. Chromatogr.* 287: 353-364.

Syvaoja, E.L., Salminen, K., Piironen, V., Varo, P., Kerojoki, O. and **Koivistoinen, P.** 1985. Tocopherols and tocotrienols in Finnish foods: Fish and fish products. *J. Am. Oil Chem. Soc.* 62: 1245-1248.

Takeuchi, A., Okano, T., Ayame, M., Yoshikawa, H., Teraoka, S., Murakami, Y. and **Kobayashi, T.** 1984. High-performance liquid chromatographic determination of vitamin D_3 in fish liver oils and eel body oils. *J. Nutr. Sci. Vitaminol.* 30: 421-430.

(Received 11 May 1987)