Disproportionately higher levels of myocardial docosahexaenoate and elevated levels of plasma and liver arachidonate in hyperthyroid rats

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ABSTRACT The effects of hyperthyroidism on the metabolism and distribution of polyunsaturated fatty acids in rats were investigated. The animals were fed diets containing an equal amount (1% each) of linoleate and linolenate. Although the hepatic and plasma levels of the linolenate family of acids were not greatly affected by the hyperthyroidism, the heart of the hyperthyroid rat contained 425% more docosahexaenoate than did that of its euthyroid control. The hyperthyroidism was accompanied by accumulations of 85, 105, and 114% more arachidonic acid in the heart, plasma, and liver, respectively. Nevertheless, most of the total increases in plasma and liver fatty acids were due to the greater accumulations of palmitic, stearic, and oleic acids; the hepatic level of oleate was elevated by 204%. Hyperthyroid rats had 106% more total fatty acids in their hearts, this increase being due largely to the greater accumulation of polyunsaturated acids.

The thyroid hormone appears to accelerate the biosynthesis of both arachidonate and docosahexaenoate, and these endogenous polyunsaturated acids are then selectively incorporated into the cardiovascular tissues. Other possible relationships between thyroid action and tissue polyenoic acids in "cold-stressed" animals are discussed.

KEY WORDS hyperthyroidism · tissue uptake of acids · arachidonate · docosahexaenoate · linoleate · linolenate · oleate · heart · plasma · liver · cold adaptation

INCREASED AMOUNTS of esterified and free fatty acids have been reported to accumulate in the heart of the guinea pig (1) and in the liver of the rat (2) as a result of an induced hyperthyroidism. More total fatty acid (TFA) is available for distribution to the tissues because the hyperthyroidism is accompanied by an increase in the biosynthesis and lipolysis of depot triglycerides (3) and an acceleration of the biosynthesis of hepatic fatty acids (4, 5). There is also a rise in level of plasma FFA (6, 7), and this promotes greater uptake and utilization of the FFA by tissues (8–11).

However, the incorporation of specific fatty acids into cardiovascular tissues appears to depend upon factors other than their total availability. The heart, which is largely dependent upon the utilization of fatty acids for its energy expenditure (12, 13), has been reported as selectively removing oleic, linoleic, and certain saturated acids from the plasma lipids of normal (14) and diabetic (15) animals. Linoleate-supplemented rats, when treated with L-thyroxine, accumulated more total fatty acids, but disproportionately greater amounts of palmitic and oleic acids, in their livers; these hyperthyroid rats also had elevated levels of plasma arachidonate (2). When small to trace amounts of arachidonate (20:4) or docosahexaenoate (22:6) were included in the diet of hypercholesterolemic rats, both families[1] of polyunsaturated fatty acids (PUFA) were selectively in-

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Abbreviations: GLC, gas–liquid chromatography; FFA, free fatty acid; TFA, total fatty acids; PUFA, polyunsaturated fatty acids.

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[1] Fatty acids are designated by chain length: number of double bonds in the acid. The linoleate family of acids (ω6 acids) include linoleic acid (18:2) and its metabolic product, arachidonic acid (20:4); all members of this family have their first double bond at C-6 (counted from the methyl end of the acid). The linolenate family of acids (ω3 acids) have their first double bond at C-3 from the methyl end, and these include linolenic acid (18:3) and its metabolic products eicosapentaenoic (20:5), docosapentaenoic (22:5), and docosahexenoic (22:6) acids.

corporated into the different cardiovascular tissues (16, 17). Although little is known about the possible functional significance of the linolenate family of acids, these often represent more than 20% of the total PUFA in cardiovascular tissues of animals that have received only negligible amounts of such acids (16–18). Furthermore, little is known about how both families of PUFA would be distributed to meet the increased fatty acid requirements of the heart and other cardiovascular tissues of hyperthyroid animals.

The high mortality rate caused by a severe thyrotoxicosis in mice or rats is significantly reduced when either linoleate or a concentrate of 20:5 and 22:6 acids is included in the dietary fat (18, 19). This protective effect of the PUFA suggests that there is an increased requirement for polyunsaturated acids, possibly for both families of PUFA, in hyperthyroid animals. Recent studies suggest that the greater need for PUFA is partially met by an accelerated biosynthesis of arachidonate (20) or docosahexaenoate (Peifer and Guzman, data to be published) in the hepatic tissue of hyperthyroid animals. However, in most of the reported studies, linoleate has been the only major source of exogenous PUFA and few data are available concerning the tissue levels of docosahexaenoate and other members of the linolenate family of acids in animals affected with thyroid imbalances.

The present report describes the effects of an induced hyperthyroidism on the metabolism and distribution of fatty acids in hepatic and myocardial tissues and plasma of rats that received equal amounts of linoleate and linolenate in their diets.

MATERIALS AND METHODS

Male Sprague-Dawley rats were fed a diet that contained 18% casein, 62% sucrose, 8% hydrogenated coconut oil, 1% each of ethyl linoleate and ethyl linolenate (Hormel Institute Fatty Acid Preparatory Lab, Austin, Minn.), 4% cellulose powder, and the vitamin and mineral supplements previously described (16, 17). In accordance with recent recommendations (21), the mineral mixture was modified to also supply to each kilogram of diet the following three salts: $ZnCl_2$, 11 mg; $MnCl_2$, 16 mg; $CoCl_2$, 0.7 mg. All of the experimental diets had the following fatty acid (per/100 g of diet): 6:0, trace; 8:0, 0.4 g; 10:0, 0.5 g; 12:0, 4.2 g; 14:0, 1.3 g; 15:0, 0.1 g; 16:0, 0.6 g; 18:0, 0.5 g; 18:1, 0.2 g; 18:2, 1.0 g; 18:3, 1.0 g. All animals were fed the basal diet for 1 month, and then they were divided into two equal groups. During the next 2 months, one group continued to receive the basal diet, while the other was fed a diet containing an additional 0.2% desiccated thyroid powder (Armour Pharmaceutical Co., Kankakee, Ill.). During the last month of the experiment the euthyroid rats ate 14–18 g of diet daily (16 ± 0.4 g/day per rat) (mean ± SEM, n = 10), and the hyperthyroid animals 16–22 g (20 ± 0.5 g/day per rat) (n = 9). Each rat received the equivalent of 53–73 μg of L-thyroxine per day when thyroid powder was included in the diet (22). Each rat was bled as follows: it was held firmly in the left hand, the position of its heart was located by palpation, and the samples of blood were taken directly from the heart with a 21 gauge needle. At the termination of the experiment, each animal was killed by a sudden blow to the base of its skull.

The hearts and livers were immediately removed, freed from extraneous tissues, weighed, and digested in 10 volumes of 2 N KOH–50% CH_3OH at 50°C for 24 hr. The digested mixtures were acidified with 6 N HCl, and the total lipids were extracted three times with petroleum ether (bp 30–60°C); in each extraction, 20 volumes of petroleum ether were used for each 10 volumes of the acidified digestion mixture. The combined petroleum ether extracts were washed free from mineral acid with water, dried over anhydrous Na_2SO_4, and evaporated to near dryness in vacuo. The total lipids were redissolved in $CHCl_3$, made up to volume with the same solvent, and stored at −20°C in glass vials fitted with screw caps lined with aluminum foil. All solvents were distilled before use, and the digestions and extractions were carried out under a blanket of pure nitrogen. The plasma lipids were extracted by the method of Folch, Lees, and Sloane Stanley (23).

Aliquots of the total lipids were fractionated into unsaponifiable and fatty acid fractions by a modification of the procedure of Abell, Levy, Brodie, and Kendall (24). The isolated fatty acids were converted into methyl esters by measure of the BF_3–CH_3OH reagent and the method described by Metcalfe and Schmitz (25). The methyl esters were analyzed by GLC with an instrument (Packard Instrument Co., Inc., Downers Grove, Ill.) equipped with dual, 6 ft × 0.25 inch I.D., coiled glass columns packed with Gas-Chrom P, 80–100 mesh, coated with 25% diethylene glycol succinate polyester (w/w), and treated with phosphoric acid as recommended by Metcalfe (26). The GLC eluates were detected by hydrogen flame detectors; operating conditions included a column temperature of 200°C and 15 psi helium at the head of the columns. Under these conditions, it is possible to complete two GLC analyses of esters of fatty acids ranging from 10:0 to 22:6 within an hour. Quantitative calculations were based on the products of the retention times and peak heights of the individual components and the previously described GLC procedures (16). This analytical procedure was standardized with model mixtures containing 8–15 different fatty acids, including PUFA and other more

saturated acids. By this method, components representing 10%, or more, of the total mixture can be analyzed with an accuracy of approximately 5%. The inclusion of an internal standard, such as the methyl ester of 20:0 acid, permits the quantitative analysis of the absolute quantities of individual or total fatty acids with a similar degree of accuracy.

Milligram quantities of 20:4, 20:5, and 22:6 acids were isolated from the tissue lipid ester preparations by preparative GLC. Only 20:0 was found after hydrogenation of either 20:4 or 20:5; 22:0 was the sole product of hydrogenation of 22:6. The animals had also received ^{14}C-labeled intermediates during the course of the experiment; the hydrogenated products and the originally prepared fractions of PUFA had identical specific activities. The preparative GLC techniques and the metabolic study with ^{14}C-labeled intermediates will be described in a forthcoming publication.

The tissue levels of total fatty acids (TFA) were determined by the addition of an internal standard of arachidic acid (20:0), 0.20–2.00 mg, to aliquots of the total lipids of the tissues prior to methylation of the fatty acids and GLC analysis. The isolated liver fatty acids were also determined gravimetrically; this and the GLC procedure with internal standards gave results that were within 8% of each other. All of these procedures were monitored by thin-layer chromatography on microchromatoplates (27) coated with Silica Gel H (Brinkmann Instruments Inc., Des Plaines, Ill.). Samples were analyzed by GLC only when the thin-layer chromatography technique indicated that the tissue lipids had been completely hydrolyzed, the isolated fatty acids had been completely methylated, and the prepared esters were free from sterols or other unsaponifiable materials. The latter precautions are of particular importance when calculations are based on the GLC procedure with internal standards.

Tissue cholesterol levels were determined by the method of Abell et al. (24) and the other experimental procedures were those described previously (16, 17).

RESULTS

The hyperthyroid rats ceased to grow during the 2 month test period, whereas their euthyroid controls gained 97 g in body weight (Table 1). The hearts of the hyperthyroid rats were hypertrophied, a finding which has also been noted in other animal species (1). However, the most significant difference between the two groups was the increased amount of TFA present in the tissues of the hyperthyroid rats. The livers, plasma, and hearts of these animals contained, respectively, 84, 32, and 106% more TFA than the tissues of their euthyroid controls.

TABLE 1 EFFECTS OF HYPERTHYROIDISM ON GROWTH AND TISSUE LIPIDS OF RATS

	Euthyroid	Hyperthyroid
Body weight (g)	412 ± 8	314 ± 7
Change (g) during experimental period (2 months)	+ 97	− 2
Liver		
Total wet weight (g)	12.1 ± 0.5	10.9 ± 1.0
Weight of liver (g/100 g BW)	3.0	3.5
Total cholesterol (mg)	31 ± 1.2	35 ± 1.6
Total fatty acids (mg)	310 ± 14	570 ± 53
Plasma lipids (mg/100 ml)		
Total cholesterol	88 ± 7	785 ± 6
Total fatty acids	88 ± 5	11 ± 10
Heart		
Total wet weight (g)	1.03 ± 0.02	1.31 ± 0.02
Weight of heart (g/100 g BW)	0.25	0.43
Total fatty acids (mg)	14.7 ± 0.5	30.2 ± 0.5

The euthyroid group included 10 rats, whereas the hyperthyroid group was made up of 9 animals. Where variation is shown, it is the standard error of the mean (SEM). BW, body weight.

The increased amounts of lipids in the tissues were accompanied by major changes in their fatty acid composition (Table 2). Although the hyperthyroidism did not affect the percentages of total PUFA in either the plasma or myocardial lipids, it was accompanied by a reduction of polyunsaturated acids from 43 to 31% in the liver lipids. Furthermore, marked changes occurred in the relative amounts of the linoleic (ω6) and linolenic (ω3) families of acids[1] in these tissues. The ω6/ω3 ratios were significantly increased in the liver and plasma lipids, whereas the opposite was true in myocardial lipids of hyperthyroid rats. The greatest changes were the increased percentages of oleic acid (18:1) in liver lipids and of docosahexaenoate (22:6) in heart lipids of hyperthyroid rats.

The total amounts of specific acids in the tissues of euthyroid and hyperthyroid rats are shown in Table 3. The elevated levels of TFA in the liver and plasma of the hyperthyroid rat were largely due to the greater amounts of palmitic (16:0), stearic (18:0), oleic (18:1), and arachidonic (20:4) acids in these tissues. Although there was a two-fold increase of 20:4 in the tissues of these animals, the induced hyperthyroidism had little effect on the total amounts of ω3 acids in either the liver or plasma. The concentrations of 16:0, 18:0, 18:1, and 20:4 were also doubled in the heart, but the greatest change in the myocardium was the five-fold increase of 22:6. Only the heart of the hyperthyroid rat retained appreciably more 22:6 than 20:4.

Hyperthyroid rats had 114, 105, and 85% more 20:4 in their liver, plasma, and heart, respectively (Fig. 1). However, much less uniform changes were evident in the tissue levels of some of the other acids. The 204% more 18:1 in the liver, 105% more 20:4 in the plasma, and 425% more 22:6 in the heart were disproportion-

TABLE 2 FATTY ACIDS IN TISSUE LIPIDS OF EUTHYROID AND HYPERTHYROID RATS

	14:0	16:0	18:0	22:0	16:1	18:1	18:2	20:4	18:3	20:5	22:5	22:6	Total PUFA	ω6/ω3*
							% of total fatty acids†							
Liver														
Eu-	0.9	21	14	1.8	4	14	11	12	1.4	5	3	11	43	1.08
Hyper-	0.7	25	15	0.9	3	23	7	14	0.9	2	0.8	7	31	2.09
Plasma														
Eu-	2.8	21	10	1.0	6	17	16	10	4.1	5	1	5	41	1.70
Hyper-	1.0	22	12	1.2	4	20	13	16	2.2	3	0.7	6	40	2.47
Heart														
Eu-	0.9	12	18	1.6	2	9	23	18	1.6	1	3	10	57	2.52
Hyper-	0.5	11	18	0.4	1	8	12	16	0.9	1	3	26	58	0.92

* ω6/ω3 is the ratio of the concentration of the linoleate family of acids to that of the linolenate family of acids.[1]

† Mean values of data obtained from hepatic and myocardial lipids from 10 and 9 animals, respectively, in the euthyroid (Eu-) and hyperthyroid (Hyper-) groups. Plasma lipids from five animals in each group were analyzed; plasma lipids of euthyroid and hyperthyroid rats also contained 2.7 and 0.6% of 12:0. Statistical data are included in Table 3.

TABLE 3 TOTAL FATTY ACIDS IN TISSUES OF EU- AND HYPERTHYROID RATS

	14:0	16:0	18:0	22:0	16:1	18:1	18:2	20:4	18:3	20:5	22:5	22:6	Total* ω6	Total* ω3
							mg/liver†							
Euthyroid	2.8	63.9	44.3	5.6	13.0	42.5	32.6	36.6	4.3	16.1	9.0	34.1	69.2	64.1
± SEM	0.4	1.6	1.5	0.2	1.6	2.2	1.6	1.6	0.4	0.9	0.6	1.3		
Hyperthyroid	4.0	142.5‡	87.8‡	5.1	16.5	129.4‡	41.0	78.1‡	5.1	9.1‡	4.6‡	38.2	119.1	57.6
± SEM	0.6	9.8	6.9	1.1	2.1	11.4	3.4	9.7	0.5	1.1	0.6	3.5		
							mg/100 ml of plasma§							
Euthyroid	2.5	18.1	8.8	0.9	5.1	14.5	13.7	8.8	3.6	4.3	1.0	4.3	22.5	13.2
± SEM	0.4	0.7	0.4		0.9	0.8	0.5	0.4	0.4	0.3		0.4		
Hyperthyroid	1.2	25.3‡	14.0‡	1.4	4.0	23.6‡	14.5	18.0‡	2.5	3.1	0.8	6.7‡	32.5	13.1
± SEM	0.5	2.2	1.1		0.2	0.7	0.6	0.7	0.2	0.3		0.3		
							mg/heart†							
Euthyroid	0.13	1.72	2.60	0.12	0.27	1.28	3.40	2.65	0.24	0.18	0.47	1.51	6.06	2.44
± SEM		0.03	0.04			0.06	0.05	0.08			0.01	0.06		
Hyperthyroid	0.15	3.44‡	5.35‡	0.12	0.24	2.48‡	3.60	4.90‡	0.27	0.18	0.76‡	7.94‡	8.62	9.21
± SEM		0.12	0.10			0.06	0.17	0.09			0.03	0.30		

* The total PUFA included small amounts of 22:4 and another isomer of 22:5. The plasma also contained 2.4 and 0.7 mg per 100 ml of lauric acid (12:0) in the euthyroid and hyperthyroid groups, respectively.

† Mean values and SEM of tissue fatty acids from 10 euthyroid and 9 hyperthyroid rats.

‡ Indicates highly significant change ($P < 0.01$).

§ Data obtained on plasmas from five animals in each group.

ately greater than the increase of TFA in these tissues. The overall effects of the hyperthyroidism were an increased accumulation of saturated and monounsaturated acids in the liver, while the heart was accumulating disproportionately greater amounts of total PUFA (20:4 + 22:6) (Fig. 2). A more uniform increase of saturated, monounsaturated, and polyunsaturated acids (20:4) contributed to the elevated levels of TFA in the plasma.

DISCUSSION

A competitive interaction between oleic (28), linoleic, and linolenic (29–31) acids appears to be a major factor affecting the availability of different PUFA for distribution to the tissues. The conversion 18:2 → 20:4 is depressed by 18:1 and 18:3 (28–31), whereas 18:2 inhibits the conversion 18:3 → 22:6 (30). In the study reported here, all diets ingested by the rats contained equal amounts of 18:2 and 18:3 but negligible amounts of 18:1. Under these experimental conditions, euthyroid rats retained equal amounts of 20:4 and 22:6 in their livers; equivalent amounts of the total ω6 and ω3 acids were also found in this tissue (Table 3). Nevertheless, the distribution of ω6 and ω3 acids were apparently dependent upon factors other than their total availability. In contrast to the distribution of PUFA in their livers, the euthyroid rats had approximately twice as much 20:4 as 22:6 in their plasma and hearts (Table 2). The selective uptake of available PUFA by different tissues became even more evident in hyperthyroid rats (Table 3). The disproportionately greater increases of

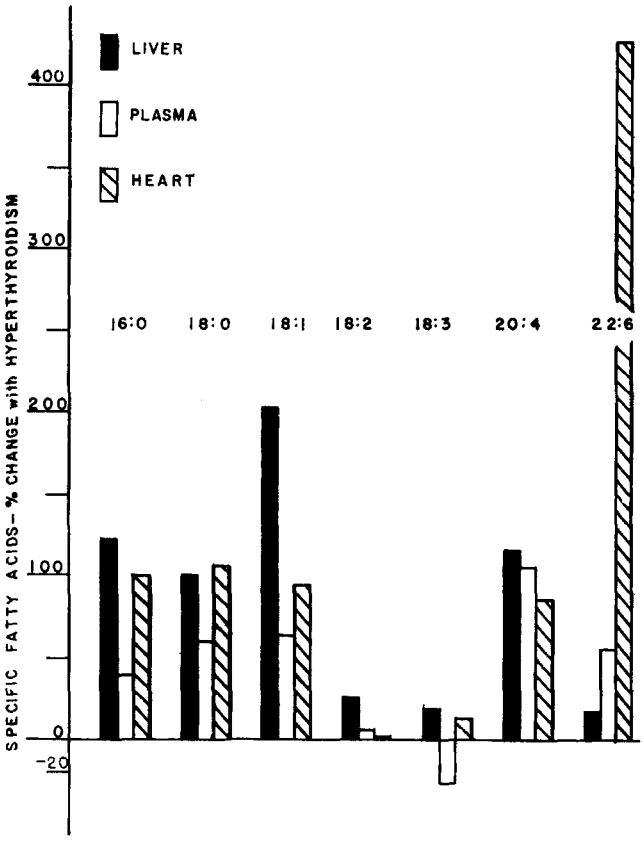

FIG. 1. Percentage changes in individual fatty acids in the liver, plasma, and heart of the hyperthyroid rat. The data for liver and heart are in terms of mg FA per entire organ; those for plasma are per 100 ml.

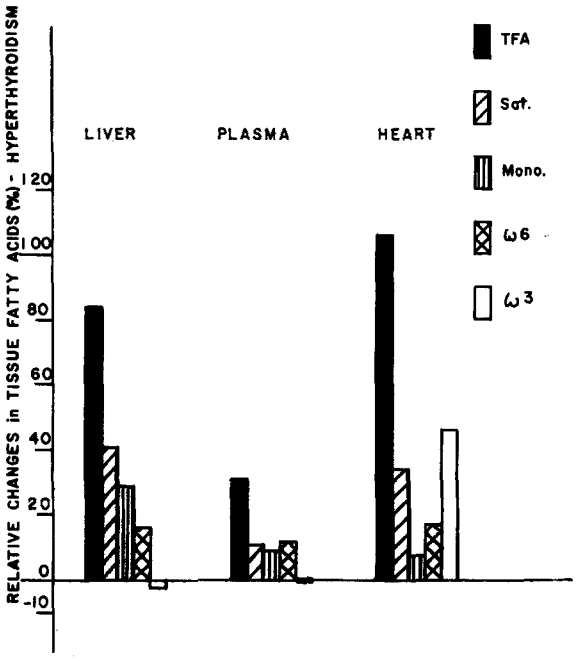

FIG. 2. Relative contributions of saturated (Sat.) and monounsaturated (Mono.) acids and the linoleate (ω6) and linolenate (ω3) families of acids to the total increases of fatty acids (TFA) in the tissues of hyperthyroid rats.

18:1, 20:4, and 22:6 in the liver, plasma, and heart, respectively, were the more obvious shifts in the distribution of available fatty acids in hyperthyroid rats. Hyperthyroid animals have an increased requirement for PUFA (18, 19) and it may be that this need is partially compensated for by the elevated tissue levels of 20:4 and 22:6.

The hyperthyroid condition is known to be accompanied by an accelerated biosynthesis of saturated fatty acids (4–7), and Gompertz and Greenbaum (20) noted an enhanced activity of stearoyl CoA dehydrogenase in the liver microsomes of hyperthyroid animals. These effects would account for the higher levels of 16:0, 18:0, and 18:1 in the tissues of hyperthyroid rats used in the present study (Table 3) and by Ellefson and Mason (2). Hyperthyroidism has also been reported to accelerate the conversion 18:2 → 20:4 in the liver (20). The results of the present study suggest that the biosynthesis of 22:6, as well as of 20:4, is accelerated in hyperthyroid animals receiving significant amounts of both 18:3 and 18:2. When miniature piglets received 18:3 as their major source of exogenous PUFA, the onset of a hyperthyroidism was accompanied by an accelerated biosynthesis of 22:6 in the liver (Peifer and Guzman, data to be published).

At the present time, it is not apparent how a thyroid imbalance might affect the availability of malonyl CoA and the microsomal enzyme systems (31, 32) required for the conversions 18:2 → 20:4 and 18:3 → 22:6. Brown, McLean, and Greenbaum (33) have found some evidence that hyperthyroidism enhances the activities of a series of enzymes involved in the biosynthesis of fatty acids and other lipids in adipose tissue. A failure to demonstrate the in vitro effects of thyroid hormone by some investigators (34) suggests that other hormonal factors, possibly the catecholamines (35), may be more directly responsible for some of the observed effects on fatty acid metabolism.

The magnitude and nature of the changes in the tissue levels of 18:1, 20:4, and 22:6 did not appear to be related to the differences in food intakes between the two groups. Although some of the hyperthyroid rats received as much as 25% more 18:2 and 18:3 because of their increased intakes of food (Methods and Materials), these differences were not reflected in the tissue levels of these exogenous PUFA. When euthyroid rats receive increasing amounts of 18:2 and 18:3, they accumulate proportionately greater amounts of these same acids in their tissues (36).

The beneficial effect of treating hypercholesterolemic individual with thyroid hormone has often been correlated with its stimulatory effect on the biliary excretion of cholesterol and bile acids (37, 38). The results of the present study suggest that such individuals would also

have greater amounts of the longer-chain PUFA available for transport of cholesterol out of the vascular system. Previous investigations have demonstrated that very small amounts of 20:4 or a concentrate of 20:5 and 22:6 is sufficient to elicit significant hypocholesterolemic responses in man and experimental animals (16–19).

At the turn of the century, Henriques and Hansen (39) noted that animals living in colder environments tended to accumulate increased amounts of unsaturated acids in their tissues. Since then, other investigators have reported that homeotherms, such as the pig and chicken (40, 41), and poikilotherms, including fish (42–44), have elevated tissue levels of the longer-chain PUFA after they have been exposed to subnormal environmental temperatures. A colder environment also stimulates a greater release of thyroid hormone in man (45) and other homeotherms, including the rat (22, 46). Fish, amphibia, and probably other poikilotherms, have some ability to adjust their body temperatures to above that of their colder surroundings (47, 48); this thermogenic response also appears to be related to the effects of the thyroid hormone (48).

However, until now there has been little evidence of a physiological explanation for this relationship between tissue levels of PUFA and changes in the environmental temperatures of homotherms and poikilotherms. In view of the present results, it seems likely that the increased release of thyroid hormone is largely responsible for the greater biosynthesis and accumulation of longer-chain PUFA in cold-stressed animals. Sometimes there is a rapid adaptation to the changes in environmental temperatures and only a transient hyperthyroid activity is experienced in these cases (46); similar transient changes have also been noted in the tissue levels of PUFA in cold-adapted fish (44).

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