Fatty acid desaturation and microsomal lipid fatty acid composition in experimental hypothyroidism

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We have studied the influence of experimental hypothyroidism in the rat on the synthesis of unsaturated fatty acids and on liver microsomal lipid fatty acid composition. Hypothyroid rats demonstrated an 80% decrease in Δ^9 (stearate) desaturation and a 43% decrease in Δ^6 (linoleate) desaturation. Liver microsomal fatty acid composition was altered in the hypothyroid animals with a significantly decreased proportion of arachidonate and increased proportions of linoleate, eicosa-8,11,14-trienoate, eicosapentaenoate and docosahexaenoate. The bulk of these changes occurred in both of the two major phospholipid components, phosphatidylcholine and phosphatidylethanolamine. All of the changes were corrected by treatment of the hypothyroid rat with 25 μ g of tri-iodothyronine/100g body wt. twice daily. The diminished Δ^9 desaturation did not lead to any changes in fatty acid composition. The increased linoleate and decreased arachidonate levels may be due to the diminished Δ^6 desaturase activity, the rate-controlling step in the conversion of linoleate into arachidonate. The increases in the proportions of the other polyunsaturated fatty acid components cannot be explained by changes in the synthesis of unsaturated fatty acids, but are probably due to diminished utilization of these fatty acids.

Altered liver lipid fatty acid composition in hypothyroidism with reduced amounts of arachidonate was first described about 20 years ago (Michel & Truchot, 1962). Ellefson & Mason (1964) suggested that this might be due to impaired arachidonate synthesis. They found increased amounts of linoleate and diminished arachidonate in hepatic phospholipid of hypothyroid rats. A number of investigators have since described a variety of alterations in plasma, liver and other tissue lipid fatty acid composition in hypothyroid animals and man (Jensen & Privett, 1969; Jurand & Oliver, 1970; Patton & Platner, 1970, 1971; Kirkeby, 1972; Shaw & Hoch, 1976, 1977; Chen & Hoch, 1976, 1977; Felt & Husek, 1977; Hoch, 1980; Hoch *et al.*, 1981). The most frequent abnormalities reported, regardless of tissue or subcellular fraction, have been increased proportions of palmitoleate and linoleate and decreased proportions of arachidonate. Changes in oleate and several long-chain (C_{18} , C_{20} and C_{22}) polyunsaturated fatty acids normally present in small amounts have been variable, depending on

which organ, cell subfraction or lipid class was studied.

It has only been in the past decade that the enzymes involved in the synthesis of unsaturated fatty acids have been well understood (Brenner, 1974). The Δ^9 desaturase enzyme converts saturated fatty acids into monounsaturated fatty acids. The Δ^6 desaturase enzyme converts linoleate into γ -linolenate, the first and rate-limiting step in the conversion of linoleate into arachidonate, whereas the Δ^5 desaturase is involved in the last step of arachidonate synthesis, converting eicosa-8,11,14-trienoate into arachidonate. The Δ^6 and Δ^5 desaturase enzymes are also involved in the conversion of linolenate into eicosapentaenoate and docosahexaenoate. These enzyme activities have only recently been studied to a limited extent in hypothyroidism.

Rats treated with propylthiouracil had no change in either liver microsomal Δ^9 or Δ^6 desaturase activity (deGomez Dumm *et al.*, 1977). More recently, Hoch *et al.* (1980), with thyroidectomized male rats maintained on a low-iodine diet, reported unchanged Δ^9 desaturase activity in animals fed *ad*

Abbreviation used: T_3 , (L)-tri-iodothyronine.

lib., but noted diminished induction of Δ^9 desaturase activity after starvation and sucrose refeeding compared with controls. Although Hoch (1980) has reported preliminary data showing decreased Δ^6 desaturase activity in hypothyroid animals and a trend toward a decrease in Δ^5 desaturase activity, the changes he found in mitochondrial fatty acid patterns were felt to be most consistent with diminished Δ^5 desaturase activity (Chen & Hoch, 1977).

Thyroid hormones are generally thought to exert their effects by an intracellular mechanism. Although the way in which changes in lipid membrane fatty acid composition might relate to the molecular basis of the effects of hypothyroidism is unknown, there is information to suggest that membrane lipid composition may relate to thyroid hormone action (Faas & Carter, 1981). Since an alteration in fatty acid desaturation is one mechanism for regulating membrane lipid composition, the present study was carried out to determine if altered fatty acid desaturation could account for the changes in liver microsomal lipid fatty acid composition in experimental hypothyroidism.

Materials and methods

Materials

Radiochemicals were purchased from New England Nuclear; stearoyl-CoA was purchased from P-L Biochemicals. All lipid standards were purchased from Applied Science Labs or Sigma Chemical Co. Silica gel 60 F254 t.l.c. plates were purchased from MCB Manufacturing, Cincinnati, OH, U.S.A.

Animals and their treatment

Normal and thyroidectomized white male Sprague-Dawley rats from Charles River Laboratories (Wilmington, MA, U.S.A.) were maintained on Purina Laboratory Chow 5001 diet (Ralston Purina Co., St. Louis, MO, U.S.A.). Fatty acid composition of the diet has been described previously (Faas & Carter, 1981). The thyroidectomized rats were observed and weighed periodically for at least 3 weeks before use. Only those animals that demonstrated the marked decline in growth rate (Table 1) were considered hypothyroid. In the treated hypothyroid animals, T_3 (0.1–0.2 ml) dissolved in 5 mM-NaOH in 0.9% NaCl ('saline') was injected intraperitoneally (25 μ g/100 g body wt.) twice daily. Control animals were injected with 5 mM-NaOH in saline.

Preparation of microsomes

In each experiment, control, hypothyroid and T_3 -treated rats were killed on the same day. Liver microsomes were prepared for desaturase enzyme

assays and for lipid analyses as described previously (Faas & Carter, 1981).

Enzyme assays

The Δ^9 ($C_{18:0} \rightarrow C_{18:1}$) and Δ^6 ($C_{18:2} \rightarrow C_{18:3}$) desaturase enzyme assays were carried out by measuring the rate of incorporation of the ^{14}C -labelled fatty acyl-CoA substrate into the corresponding ^{14}C -labelled fatty acid product (Faas & Carter, 1981). Stearoyl-CoA desaturation reaction mixtures containing 36 μ M-[1- ^{14}C]stearoyl-CoA (200000 d.p.m.), 1 mM-NADH, 50 mM-potassium phosphate buffer (pH 7.4) and approx. 1 mg of microsomal protein in a total volume of 1 ml were incubated at 37°C for 20 min. Linoleate desaturation reaction mixtures containing 40 μ M-[1- ^{14}C]linoleate, 5 mM-MgCl₂, 2 mM-ATP, 50 μ M-CoA, 1 mM-NADH, 50 mM-potassium phosphate buffer (pH 7.4) and approx. 2.5 mg of microsomal protein in a total volume of 1 ml were incubated at 37°C for 15 min. Rates of desaturation are nearly linear with time under these conditions. The reactions were terminated by the addition of 2 ml of ethanolic KOH (25% 10M-KOH and 75% ethanol). The reaction mixtures were hydrolysed at 70°C for 1 h and titrated to pH 3–4 using Bromophenol Blue as indicator. The fatty acids were extracted with light petroleum (b.p. 37.7–54.4°C) and methylated using BF₃ in methanol. The fatty acid methyl esters were separated by argentation t.l.c. with a hexane/benzene (1:1, v/v) solvent system for the stearoyl-CoA desaturase assays and a toluene/acetone (19:1, v/v) solvent system for the linoleate desaturase assays.

Lipid analyses

Total microsomal lipid was extracted and analysis of the fatty acid composition carried out by g.l.c. as described previously (Faas & Carter, 1981). By these methods, microsomal lipid is approx. 83% phospholipid on a molar basis with very little triacylglycerol. Peaks were identified by comparison of retention times with authentic standards, except for eicosapentaenoate, whose retention time was determined from a fatty acid methyl ester analysis of hydrolysed Menhaden oil (kindly given by Anthony P. Bimbo, Zapata Haynie Corp., Reedville, VA, U.S.A.) of known fatty acid composition.

In those experiments in which the individual phospholipids were separated and analysed for fatty acid composition, a portion of the total lipid extract from each sample was taken for total phosphorus determination. Another portion was taken for phospholipid separation by t.l.c. on silica gel H by the method of Skipski *et al.* (1967) as modified by Lee & Morris (1974). 2',7'-Dichlorofluorescein was used to locate the lipid spots. Phosphatidylserine and phosphatidylinositol travelled together in this

system. The spots were identified by comparison with authentic standards. The spots were scraped and eluted with chloroform/methanol/aq. 7M-NH₃ (13:7:1, by vol.). One portion of the eluate was used for lipid phosphorus determination to calculate the proportion of each major phospholipid. Mean recovery of lipid phosphorus was 87% of the amount applied to the chromatogram. The remainder of the eluate was used for preparation of fatty acid methyl esters, which were separated by g.l.c.

Analytical procedures

Phospholipid phosphorus (Fiske & Subbarow, 1926), cholesterol (Rudel & Morris, 1973) and protein (Hartree, 1972) determinations were carried out as described previously.

Statistical analysis

The results of replicate experiments were pooled for statistical analysis. Significant differences between groups were determined by using the one-way analysis of variance.

Results

Weight gain and microsomal lipid composition in hypothyroid animals

Table 1 shows the markedly retarded weight gain in the hypothyroid animals. In a large group of hypothyroid animals, growth as measured by weight gain over a 9-day period before use was markedly retarded, being only 42% that of controls. In a smaller group of animals weighed periodically over a 21-day period, the hypothyroid animals gained only 28% as much as controls.

Table 1 also shows the amounts of cholesterol and phospholipid phosphorus in the liver microsomal

Table 1. *Weight gain and microsomal lipid composition in hypothyroidism*

The number of animals in each group is shown in parentheses. Results are means $\pm$ S.E.M.

	Control	Hypothyroid
Initial weight (g)	94 (22)	111 (29)
Weight gain (g)		
During 21 days	162 $\pm$ 7 (5)	46 $\pm$ 10 (5)*
During 9 days	76 $\pm$ 3 (22)	32 $\pm$ 2 (29)*
Microsomal lipid (nmol/mg of protein)		
Cholesterol	98 $\pm$ 5 (5)	89 $\pm$ 6 (5)
Phospholipid (total)	406 $\pm$ 13 (6)	403 $\pm$ 13 (5)
Phosphatidylcholine	232 $\pm$ 6 (6)	230 $\pm$ 7 (5)
Phosphatidylethanolamine	104 $\pm$ 6 (6)	109 $\pm$ 4 (5)
Phosphatidylserine + phosphatidylinositol	37 $\pm$ 2 (6)	35 $\pm$ 1 (5)

* $P < 0.01$ (hypothyroid versus control).

lipid extract from control and hypothyroid rats. There were no significant alterations in the amounts of either of these lipids in the hypothyroid animals. Although the total amount of phospholipid was not altered in hypothyroidism, the phospholipids were separated into their major components, phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine plus phosphatidylinositol (the latter two were not readily separated by the t.l.c. techniques used) to determine if the proportion of the individual phospholipids might be altered. These three components make up greater than 90% of the total phospholipid phosphorus. Table 1 shows the quantity of each phospholipid present in control and hypothyroid animals. Again, no changes in the quantity of any of these fractions were seen in the hypothyroid animals.

Fatty acid desaturase activity in hypothyroidism

Fig. 1 shows the results of stearate (Δ^9) and linoleate (Δ^6) desaturase activity in control, hypothyroid and treated hypothyroid rats (25 μ g of T₃/100g body wt. daily for 2 days). The hypothyroid rats had an 80% decrease in stearate desaturation ($C_{18:0} \rightarrow C_{18:1}$) and a 43% decrease in

Fig. 1. *Microsomal fatty acid desaturase activity in hypothyroidism*

Desaturase enzyme assays and animal treatments were carried out as described in the Materials and methods section. Mean ($\pm$ S.E.M.) control stearate ($C_{18:0} \rightarrow C_{18:1}$) and linoleate ($C_{18:2} \rightarrow C_{18:3}$) desaturase activities were 3.79 ± 0.44 and 1.70 ± 0.16 nmol/mg of protein per 15 and 20 min incubation period respectively. The numbers of animals in each group are shown within the columns. The stearate and linoleate desaturase activities in the hypothyroid group were significantly lower ($P < 0.01$) than both the controls and the treated hypothyroids. The bars represent S.E.M.

linoleate desaturation ($C_{18:2} \rightarrow C_{18:3}$). Both the diminished Δ^9 and Δ^6 desaturase activities in the hypothyroid rats were corrected by the administration of T_3 ($25 \mu\text{g}/100 \text{g body wt.}$) to hypothyroid rats for 2 days (Fig. 1). Although administration of $50 \mu\text{g}$ of $T_3/100 \text{g body wt.}$ for 3 days likewise corrected the Δ^9 desaturase activity to $109 \pm 22\%$ ($n=6$) of control activity, this regimen failed to correct the Δ^6 desaturase activity ($44 \pm 5\%$ of control; $n=6$). This is consistent with the observation that large doses of thyroid hormone depress Δ^6 desaturase activity (de Gomez Dumm *et al.*, 1977; Faas & Carter, 1981).

Microsomal fatty acid composition in hypothyroidism

To determine if the diminished desaturase activities in the hypothyroid animals resulted in corresponding changes in microsomal membrane lipid composition, the fatty acid composition of the lipid membrane was analysed in normal, hypothyroid and treated hypothyroid rats. The results from two combined experiments are shown in Fig. 2. No changes in the proportion of any of the saturated or monounsaturated fatty acids were seen. However, the proportions of all polyunsaturated fatty acids were altered in the hypothyroid animals. The major changes occurred in the linoleate ($C_{18:2}$) and arachidonate ($C_{20:4}$) fractions. The hypothyroid animals had a 20% increase in the proportion of $C_{18:2}$ fatty acid ($19.2 \pm 0.4\%$) compared with normal animals ($16.0 \pm 0.4\%$) and a reciprocal 27% decrease in the proportion of $C_{20:4}$ fatty acid ($15.3 \pm 0.4\%$) compared with controls ($20.9 \pm 0.5\%$).

These changes are consistent with the observed decrease in Δ^6 (linoleate) desaturase activity, the first step in the conversion of linoleate into arachidonate. After T_3 treatment, these changes reverted to normal. Two quantitatively minor components, $C_{20:5}$ and $C_{20:3(8,11,14)}$ fatty acids, which are both prostaglandin precursors, were increased in the hypothyroid animals. The proportion of $C_{20:5}$ fatty acid was $2.6 \pm 0.3\%$ in hypothyroid compared with $1.1 \pm 0.1\%$ in control animals and the proportion of $C_{20:3(8,11,14)}$ fatty acid was $2.0 \pm 0.1\%$ in the hypothyroid compared with $1.4 \pm 0.1\%$ in control animals. Docosahexaenoate ($C_{22:6}$) levels were also slightly increased ($P < 0.05$) in hypothyroid animals ($10.0 \pm 0.5\%$) compared with controls ($8.6 \pm 0.3\%$). The increased proportions of $C_{20:3}$, $C_{20:5}$ and $C_{22:6}$ fatty acids in hypothyroidism all reverted to normal after 2 days of T_3 therapy.

We then wondered if the changes in microsomal total lipid fatty acid composition in hypothyroidism were due to a larger change in fatty acid composition of one of the phospholipid molecular species or if the change was generalized among the various phospholipid components. Therefore, in one experiment the microsomal phospholipids from control, hypothyroid and treated hypothyroid rats were separated into their major fractions phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine plus phosphatidylinositol. Each of these three fractions was isolated and analysis of the fatty acid composition was carried out. The results of these studies are shown in Fig. 3. The major increase in $C_{18:2}$ fatty acid with the reciprocal decrease in $C_{20:4}$ fatty acid in the total lipid extract from the hypothyroid rats was seen in the two major phospholipid fractions,

Fig. 2. Liver microsomal fatty acid composition in hypothyroidism

Fatty acid composition was determined by g.l.c. Animal treatments are described in the Materials and methods section. The numbers of animals in each group are shown within the first set of columns. Results are shown as means $\pm$ S.E.M. (represented by the bars). Only those statistical differences significant at the 1% level are shown.

Fig. 3. Fatty acid composition of individual phospholipids in hypothyroidism

Animal treatments are given in the Materials and methods section. The individual phospholipids of the liver microsomal extract were separated and fatty acid analysis of each fraction was carried out as described in the Materials and methods section. The numbers of animals in each group are shown within the first set of columns. Results are shown as means $\pm$ S.E.M. (represented by the bars). Statistically significant differences are shown above the columns.

phosphatidylcholine and phosphatidylethanolamine. A very small increase in $C_{18:2}$ fatty acid without a significant change in $C_{20:4}$ fatty acid occurred in the phosphatidylserine plus phosphatidylinositol fraction. The increases in $C_{20:5}$ and $C_{22:6}$ fatty acids also occurred in the two major fractions phosphatidylcholine and phosphatidylethanolamine, but the increased $C_{22:6}$ fatty acid in phosphatidylcholine was not statistically significant. No measurable amounts of $C_{20:5}$ and $C_{22:6}$ fatty acids appeared in the phosphatidylserine plus phosphatidylinositol fraction. No change occurred in the $C_{20:3}$ fatty acid in phosphatidylcholine, the only phospholipid fraction containing measurable amounts of this fatty acid. Treatment with thyroid hormone reversed all the changes seen except for the increased $C_{22:6}$ fatty acid in phosphatidylethanolamine. It thus appears that the changes in fatty acid composition in the lipid membrane are due mainly to changes in both of the major phospholipids, phosphatidylcholine and phosphatidylethanolamine.

Discussion

Although it has been postulated for many years that linoleate desaturation may be decreased in hypothyroidism because of the decreased arachidonate (and frequently increased linoleate) levels found in cellular lipids, only recently have the fatty acid desaturation reactions been studied in hypothyroidism. In our studies, a 43% decrease in linoleate desaturation was found in thyroidectomized animals supporting the thesis that linoleate desaturation is decreased in hypothyroidism. This agrees with the preliminary report of Hoch (1980) but differs from the studies of deGomez Dumm *et al.* (1977), who found unchanged desaturase activity in propylthiouracil-treated rats. Treatment with 25 μ g of T_3 /100g body wt. for 2 days corrected the linoleate desaturase defect. Thus, the Δ^6 desaturase enzyme is sensitive to thyroid hormone requiring normal hormone levels for normal activity in spite of the fact that hyperthyroid animals have decreased Δ^6 desaturase activity (Faas & Carter, 1981; deGomez Dumm *et al.*, 1977).

In the present study, thyroidectomized animals fed Purina rat chow diet *ad lib.* had an 80% decrease in stearate (Δ^9) desaturation, which was promptly repaired after 2 days of T_3 therapy. This differs from the studies of Hoch *et al.* (1980), who found that thyroidectomized animals fed a low-iodine diet had unchanged stearate desaturation in animals fed *ad lib.*, but failed to normally induce stearate desaturase activity after a starvation/refeeding regimen. The more easily demonstrable defect in Δ^9 desaturase activity in the present study may be due either to

dietary differences (Brenner, 1974) or to the different method of determining desaturase enzyme activity (Hoch *et al.*, 1980). Large doses of thyroid hormone are necessary to increase Δ^9 desaturase activity in normal rats (Faas & Carter, 1981). Thus, both Δ^6 and Δ^9 desaturase activities are more sensitive to thyroid hormone deprivation than to thyroid hormone excess in the rat.

The major changes we found in microsomal lipid fatty acid composition were increased proportions of linoleate and decreased arachidonate consistent with diminished Δ^6 desaturase activity. The decreased arachidonate and increased linoleate levels are consistent with virtually all reports, regardless of organ or subcellular component studied, including the one previous report on microsomal lipid (Hoch *et al.*, 1981). Although liver triacylglycerol linoleate levels decrease in hypothyroidism (Ellefson & Mason, 1964), phospholipid linoleate invariably increases. The liver microsomal linoleate and arachidonate changes occurred in the two major phospholipid fractions, phosphatidylcholine and phosphatidylethanolamine, with minimal or no changes in the phosphatidylserine plus phosphatidylinositol fraction. This is consistent with the findings in liver mitochondria (Hoch *et al.*, 1981) and in total liver lipids of hypophysectomized rats (Jensen & Privett, 1969). Correction of the fatty acid composition and Δ^6 desaturase activity changes with 2 days of thyroid hormone therapy supports the notion that the fatty acid composition may be due to the altered desaturase activity.

No changes in amounts of monounsaturated fatty acids were found in the hypothyroid animals in spite of the fact that the Δ^9 desaturase activity was suppressed to only 20% of normal. Since oleate is so plentiful in dietary sources (32% of the total fatty acids in Purina rat chow), oleate levels are not likely to be greatly affected by changes in Δ^9 desaturation. However, in diabetic animals with impaired Δ^9 desaturase activity (Faas & Carter, 1980), palmitoleate levels are significantly decreased. The lesser decrease in Δ^9 desaturase activity combined with the hypometabolic state of the hypothyroid animals may allow the animal to maintain normal monounsaturated fatty acid levels. Other factors may be playing a role here, since, somewhat paradoxically, other investigators have noted increased palmitoleate levels in hypothyroidism (Ellefson & Mason, 1964; Chen & Hoch, 1977).

The other changes in fatty acid composition we found in the hypothyroid animals include a small increase in eicosa-8,11,14-trienoate, a substantial increase in eicosapentaenoate ($C_{20:5}$) and a small increase in docosahexaenoate ($C_{22:6}$). Shaw & Hoch (1977) and Chen & Hoch (1977) have noted increased eicosa-8,11,14-trienoate levels and have suggested that this may be due to diminished Δ^5

desaturase activity, but were unable to confirm this (Hoch, 1980). Shaw & Hoch (1976, 1977) found increased C_{22:6} fatty acid levels in myocardial mitochondria but decreased levels in liver nuclei of hypothyroid animals. The increase in microsomal C_{20:5} and C_{22:6} fatty acid levels, products of linolenate desaturation and elongation, in hypothyroidism probably result from decreased utilization of these fatty acids, since there is no evidence of increased desaturation or chain elongation in hypothyroidism (Landriscina *et al.*, 1976; deGomez Dumm *et al.*, 1977).

Although the changes in linoleate, arachidonate and eicosa-8,11,14-trienoate levels in hypothyroidism may be due to diminished desaturase activity, other evidence suggests that this may not be true. In hyperthyroid rats converse changes in these fatty acids occur; yet there is no evidence of increased Δ^6 desaturase activity in the hyperthyroid animals (deGomez Dumm *et al.*, 1977; Hoch, 1980; Faas & Carter, 1981). The decreased food intake of the hypothyroid animals cannot explain the observed changes in fatty acid composition since such changes have not been observed in food-restricted control animals (Faas & Carter, 1980, 1981). Diet and fatty acid desaturation are probably the major factors that might alter the pool of fatty acids available for incorporation into phosphoglycerides. Other factors, such as alterations in glycerophosphate acyltransferase activity, the phosphoglyceride deacylation reacylation cycle (lysophosphoglyceride acyltransferase and phospholipase A₂ activity), fatty acid oxidation or transport of fatty acids from one site to another must be considered as possible factors that may influence membrane fatty acid composition in hypothyroid animals.

The major implications of altered fatty acid desaturation would be that this might alter lipid membrane composition and hence function, and/or influence prostaglandin production. Horrobin (1981) has recently postulated that loss of Δ^6 desaturase activity may be a key factor in aging through such changes.

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