THYROID HORMONE CONTROL OVER RAT LIVER MEMBRANE LIPIDS

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A relationship between essential fatty acid metabolism and the thyroid state of animals was suggested in 1931 by Wesson and Burr's observations[21] of an increased basal metabolic rate in fat-deficient animals, correctible by feeding small amounts of methyl linoleate;[14] by Morris and his co-workers'[15] demonstration that thyroid hypersecretion does not occur in EFA-deficiency; by Klein and Johnson's[12] comments that fat deficiency and hyperthyroidism have in common a deleterious effect on oxidative phosphorylation that might involve the mitochondrial unsaturated fatty acids; and by Ershoff's[5] summary of evidence showing that dietary linoleic acid protects against thyrotoxicity. Recent findings begin to provide mechanisms for this relationship.

Both hyperthyroidism and hypothyroidism modify the function of energy-linked mitochondrial electron- and metabolite-transporters involved in oxidative phosphorylation by changing their membrane-dependency, as measured by Arrhenius plot profiles and Hill coefficients.[3,9,10] Membrane-dependency reflects lipid–protein interactions and is influenced by lipid–protein ratios, proportions of different phospholipid classes, and relative contents of unsaturated fatty acyl groups and of cholesterol. We find that the liver mitochondria of control (euthyroid) and hypothyroid rats contain similar amounts, per mg protein, of extractable total lipids, phospholipids, neutral lipids, and lipid P. The relative contents of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and PS, measured from total P after TLC resolution, are also similar in both groups but the hypothyroid preparations contain 72% more cardiolipin (CL) than do controls.[11] In hypothyroid rats, the mitochondrial phospholipid contains excess 18:2 and less than the normal proportion of 20:4 acyls.[3] These abnormalities appear in PC and PE, less in phosphatidylserine (PS), and not at all in CL fractions. The increased amount of CL with its normally high (55%) 18:2 and low 20:4 (3.5%) contents contributes somewhat to the overall redistribution of phospholipid fatty acyls but because PC and PE comprise 75% of the total phospholipids, their abnormal unsaturated fatty acid (UFA) contents contribute more. Hypothyroidism produces this pattern of high 18:2 and low 20:4 acyl contents not only in liver mitochondria but also in liver microsomes[11] and nuclei.[18] The generality of these membrane changes speaks for a defect in hepatic metabolism of essential fatty acids (EFA) rather than of specific phospholipids or organelles.

Injection with thyroid hormones stimulates, and hypothyroidism depresses, several processes in rat liver fatty acid and cholesterol metabolism that would be expected to affect membrane fluidity. A selection of the specific process that is accelerated seems to depend on whether a hypothyroid animal is pretreated with one dose or a control animal is injected several times over a period of days to make it thyrotoxic. Among the many observations on hepatic lipid metabolism, the hormone activates or is necessary for maintaining normal activities of the following rate-controlling steps (Fig. 1): (A) In the synthesis of saturated fatty acids from acetyl CoA, the acetyl CoA carboxylase is activated[6] and the activity and amount of the fatty acid synthetase are increased;[17] the saturated fatty acids (SFA) decrease fluidity when incorporated into membranes. (B) In the biosynthesis of monounsaturated fatty acids, the Δ^9-desaturase is accelerated in thyrotoxicosis,[7] but the 16:1 and 18:1 produced contribute little (<20%) toward increasing membrane fluidity because of their relatively minor incorporation into membranes and their single unsaturated bond. (C) In the conversion of dietary 18:2 to 20:4 fatty

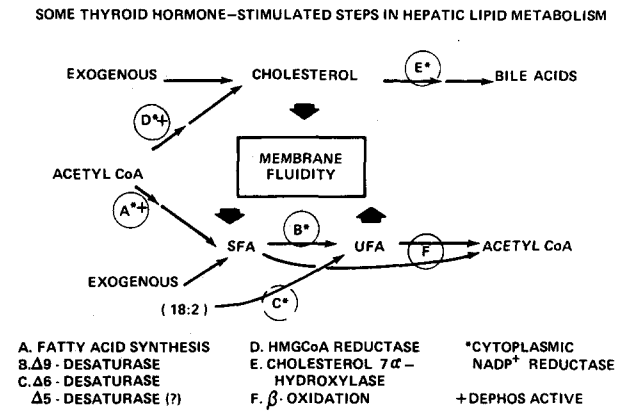

FIG. 1.

acyls, the Δ^6- and Δ^5-desaturations seem to be hormone-sensitive,[10] and the 20:4 is the major contributor (>50%) to membrane fatty acyl unsaturation. (D) In the biosynthesis of cholesterol from acetyl CoA, the HMG CoA reductase is accelerated;[8] cholesterol incorporation into membranes decreases fluidity. (E) The conversion of cholesterol to bile acids at the 7α-hydroxylation reaction is even more sensitive to the hormone than is (D).[19] (F) The rate of mitochondrial β-oxidation of fatty acids depends on thyroid hormone levels;[1] although essential fatty acid metabolites are normally protected against β-oxidation, no information is on hand concerning their oxidation rates in abnormal thyroid states. Increased production of cytoplasmic reducing equivalents to drive processes A–E is provided by thyroid-induced $NADP^+$-reducing enzymes of the pentose shunt,[20] and by a cytoplasmic transhydrogenase system whereby NADH can reduce $NADP^+$.[22] It may be important that steps A and D are activated by protein dephosphorylation.[13]

We are examining processes (B) and (C) at present. The Δ^9-desaturase rate constant, k, was measured spectrophotometrically from the reoxidation of NADH-reduced cytochrome b_5.[16] The rate constants are similar in routinely fed control and hypothyroid rats, and the cytochrome b_5 content is elevated 54% in microsomes of hypothyroid animals (Table 1). The specific Δ^9-desaturase protein is known to be induced and cytochrome b_5 content to decrease when normal animals are fasted 48 hr and then fed carbohydrates.[4] On such a regimen (we offered 20% sucrose solution), our control animals increase k 7-fold and decrease cytochrome b_5 levels but hypothyroid rats fail to do so although they consume as much sucrose.

The microsomal Δ^6-desaturase activity was measured spectrophotometrically; variation of the concentration of substrate, 18:2ω6 CoA, allowed the extrapolation of the rate constants to infinite substrate concentration (k_{max}), and the determination of Km. The Δ^9-desaturase kinetics were measured the same way (Table 2). Hypothyroidism does not affect the Δ^9-desaturase k_{max} of these fed animals (cf. Table 1); thyrotoxicosis raises the k_{max} almost 4-fold and depresses the microsomal cytochrome b_5 content. The Δ^6-desaturase k_{max} is significantly below normal in hypothyroidism but is not changed in

TABLE 1. Rat Liver Microsomes: Dietary Induction of Δ^9-Desaturase (Means ± SEM)

	Fed		Fasted–refed sucrose	
	Controls (11)	Hypo (5–9)	Controls (7)	Hypo (5)
Cytochrome b_5 (nmol/mg)	0.13 ± 0.01	0.20 ± 0.01*	0.10 ± 0.01*	0.17 ± 0.02
Δ^9-Desaturase k (min^{-1})	1.17 ± 0.33	0.75 ± 0.24	7.47 ± 1.9*	1.06 ± 0.28
Sucrose consumed (mg/g BW)			17.6	20.5
$p < {}^*0.001$				

TABLE 2. Desaturase Activities in Rat Liver Microsomes (Means ± SEM)

Desaturase	Controls (6–8)	Hypothyroids (6–10)	Thyrotoxics (8)
Δ^9: k_{max} (min^{-1})	2.7 ± 0.6	3.1 ± 0.3	10.2 ± 1.3[c]
Km (μm)	2.0 ± 0.5	1.6 ± 0.6	5.3 ± 2.4
Δ^6: k_{max} (min^{-1})	4.5 ± 1.0	2.2 ± 0.3[a]	2.8 ± 0.5
Km (μm)	17.9 ± 7.0	3.6 ± 0.9	2.6 ± 0.9
Δ^5: V_{max} (pmol min^{-1} mg^{-1})	12.8 ± 4.0	7.0 ± 0.7	26.0 ± 9.8
Km (μm)	0.15 ± 0.08	0.06 ± 0.02	0.14 ± 0.03
Cyto b_5 (nmol mg^{-1})	0.13 ± 0.01	0.20 ± 0.01[c]	0.09 ± 0.007[b]

$p <$ [a]0.05; [b]0.01; [c]0.001

hyperthyroidism. Assays for Δ^5-desaturase activity are shown as V_{max}, extrapolated from activities measured by thin layer chromatography resolution of the substrate, the ^{14}C-labeled 20:3ω6 acid, and the product, labeled 20:4.[2] The mean V_{max} values are half normal in hypothyroidism and twice normal in hyperthyroidism, but the great variance of these activities makes the differences not significant statistically. Adequate thyroid levels appear to be necessary for normal activities of one or both of these microsomal desaturations in 20:4 acyl synthesis. Thyroid state affects only their maximal velocities, not the Km values, and so seems to regulate either the specific activity of existing desaturase or the amount of enzyme but not the enzyme–substrate interactions.

Liver microsomal lipid composition and metabolism in hypothyroid rats respond rapidly to a hormone injection (Table 3).

TABLE 3. Liver microsomal Total Lipids: Triiodothyronine-LT_3-Injected Hypothyroid Rats (Means ± SEM)

	Controls (6–12)	Hypothyroids: 0 (8–10)	Hours after LT_3 injection (N) 0.5 (2)	1.0 (6)	2.5 (5)	4.0 (6)
20:4 acyl (%)	26.4 ± 0.9[c]	18.5 ± 0.5	19.2	16.4 ± 1.0	14.9 ± 0.7[b]	13.0 ± 0.9[c]
Cholesterol (g/g FAME)	0.11 ± 0.02[c]	0.23 ± 0.02	0.22	0.16 ± 0.03	0.23 ± 0.05	0.18 ± 0.02
Δ^9-Desaturase, k_{max} (min^{-1})	2.7 ± 0.6	3.1 ± 0.3	3.7	4.9 ± 1.0	5.6 ± 1.4	8.9 ± 0.8[c]
Δ^6-Desaturase, k_{max} (min^{-1})	4.5 ± 1.0[a]	2.2 ± 0.3		4.4 ± 0.7[b]	6.0 ± 0.7[c]	3.5 ± 0.7
Cytochrome b_5 (nmol/mg)	0.13 ± 0.01[c]	0.20 ± 0.01	0.20	0.17 ± 0.02	0.14 ± 0.01[b]	0.18 ± 0.01

$p <$ [a]0.05; [b]0.005; [c]0.001.

With a latent period of 1–4 hr, the depressed microsomal 20:4 acyl content and unsaturation index decrease even further, the Δ^9-desaturase k_{max} almost triples, and there is an unsustained increase in Δ^6-desaturase activity and decrease in cytochrome b_5 content, while the high levels of cholesterol persist. Microsomal lipid metabolism thus seems worth examining further as a site of hormonal action or indirect activation, in view of the close coupling between organelle membrane fatty acyl composition and hormone availability.

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