Fatty-acid desaturation and microsomal lipid fatty-acid composition in experimental hyperthyroidism

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We have studied the influence of experimental hyperthyroidism in the rat on the synthesis of unsaturated fatty acids and on liver microsomal lipid fatty-acid composition. Tri-iodothyronine treatment (25 µg/100 g body weight) daily for 3 weeks caused no significant changes in Δ^9 (stearate) desaturation but a 24% decrease in Δ^6 (linoleate) desaturation. Much larger doses of tri-iodothyronine increased Δ^9 desaturation. Liver microsomal fatty-acid composition in hyperthyroidism is altered with significantly increased proportions of stearate and arachidonate and decreased proportions of palmitate, palmitoleate, linoleate ($C_{18:2}$) and eicosa-8,11,14-trienoate ($C_{20:3}$). These changes, other than the decreased proportion of $C_{20:3}$ fatty acid, which may be due to the diminished Δ^6 desaturase activity, cannot be attributed to changes in fatty-acid desaturation. Most of these changes were also found to be due not simply to the decreased weight gain or the increased food intake of the hyperthyroid animals. Only the decreased $C_{18:2}$ fatty-acid proportions could be mimicked by restricting food intake of control animals and none of the changes were prevented by restricting food intake of hyperthyroid animals. Thus most of the changes in microsomal lipid fatty-acid composition are likely to be due to a thyroid hormone effect on peripheral lipid mobilization or lipid degradation.

Ellefson & Mason (1964) described a variety of changes in hepatic lipid fatty-acid composition in chronically tri-iodothyronine-treated rats and suggested that these changes might be due to increased influx of certain fatty acids from adipose tissue and stimulation of the conversion of linoleate to arachidonate. A number of investigators have since described a variety of alterations in plasma, liver or other tissue lipid fatty-acid composition in hyperthyroid animals and man (Peifer, 1968; Jurand & Oliver, 1970; Kirkeby, 1972; Patton & Platner, 1970, 1971; Platner *et al.*, 1972). The most frequent abnormalities reported have been increased proportions of palmitate, stearate, oleate and arachidonate and a decreased proportion of linoleate. These changes have variably been interpreted as consistent with increased fatty-acid synthesis and desaturation, increased fatty-acid degradation with inadequate dietary supply or increased cycling of certain fatty acids between adipose tissue and liver.

Gompertz & Greenbaum (1966) reported an increased conversion of stearate into oleate in liver slices and in microsomal fraction ('microsomes') from hyperthyroid rats. Many authors have subsequently assumed that fatty-acid desaturation was

generally increased in hyperthyroidism (Peifer, 1968; Jurand & Oliver, 1970; Patton & Platner, 1970; Kirkeby, 1972). However, the enzyme responsible for the conversion of stearate into oleate (Δ^9 desaturase) is different from the enzyme responsible for the first and rate-limiting step (Hassam *et al.*, 1975) in the conversion of linoleate into arachidonate [Δ^6 desaturase, which converts linoleate ($C_{18:2}$) to γ -linolenate ($C_{18:3}$)] (Brenner, 1974). In addition, a recent report indicates that rats treated with thyroxine have impaired Δ^6 (linoleate) desaturation, but increased Δ^9 (palmitate) desaturation (de Gomez Dumm *et al.*, 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 hyperthyroidism is unknown, there is information to suggest that membrane lipid composition may relate to thyroid hormone action. Chen & Hoch (1977) found abnormalities in mitochondrial oxidative phosphorylation and in membrane fatty-acid composition in hypothyroid rats and have inferred that hormone-responsive polyenoic fatty-acid contents may participate in the control

of mitochondrial oxidative phosphorylation. Hulbert (1978) observed an increase in liver mitochondrial unsaturated fatty acids and an associated decrease in the temperature limits of the membrane phase transition in hypothyroid rats. He has proposed a thesis that thyroid hormones act by altering the extent of unsaturation of membrane fatty acids, hence affecting membrane fluidity, and that changes in membrane fluidity may be the manner by which the activities of many membrane-associated enzymes are altered by thyroid hormone. 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 hyperthyroidism. Since the synthesis of unsaturated fatty acids occurs in the microsomal fraction, microsomal fatty-acid composition might reflect early changes in fatty-acid desaturation. In addition we attempted to determine if the increased food intake and/or decreased weight gain of the hyperthyroid animal might contribute to the lipid desaturation and composition changes.

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Materials and methods

Materials

[1-¹⁴C]Stearoyl-CoA and [1-¹⁴C]linoleate were purchased from New England Nuclear. Unlabelled stearoyl-CoA was purchased from P-L Biochemicals. L-Tri-iodothyronine, linoleate, free co-enzyme A, NADH and ATP were purchased from Sigma Chemical. Authentic fatty-acid methyl ester standards were purchased from Applied Science Labs or Sigma Chemical. All other chemicals were of reagent grade.

Animals and their treatment

White male Sprague-Dawley rats from Charles River Laboratories were maintained on a Purina Laboratory Chow 5001 diet *ad libitum* unless indicated otherwise. The diet contained 5.0% total lipid with a fatty-acid composition of 1.7% myristate, 22.9% palmitate, 1.9% palmitoleate, 7.8% stearate, 32.4% oleate, 29.8% linoleate and 3.1% linolenate. Hyperthyroidism was produced by daily intraperitoneal injection of 25 µg of tri-iodothyronine/100g body weight. Tri-iodothyronine (0.1–0.2 ml) dissolved in 5 mM-NaOH in saline was injected daily for 3 weeks unless otherwise indicated.

In those experiments in which the influence of body-weight changes and food intake on fatty-acid metabolism was studied, experiments were carried out using four groups of rats monitoring daily

weights and food intake: group I, control rats fed *ad lib.*; group II, hyperthyroid rats fed *ad lib.*; group III, control rats whose food intake was restricted so that their weight gain would match the weight gain of the hyperthyroid rats; group IV, hyperthyroid rats whose food intake was restricted to match the food intake of the controls. Since hyperthyroid animals have hyperphagia but gain less weight than controls, the food-restricted controls in group III were studied to determine if changes in desaturation or lipid composition in hyperthyroidism might be due to the decreased weight gain and relatively increased fat mobilization of the hyperthyroid animals. The food-restricted hyperthyroid rats in group IV were studied to determine if changes in desaturation or lipid composition in hyperthyroidism might be due to the increased food intake of the hyperthyroid animals. Animals were killed at 3 weeks in the fed state and liver microsomes were isolated.

Preparation of microsomes

Microsomes were prepared from rats killed by a blow to the head. The liver was rinsed in 0.25 M-sucrose and was homogenized in 5 ml of 0.25 M-sucrose/g of liver. The homogenate was centrifuged for 20 min at 16 000 g, and the resulting supernatant was centrifuged for 60 min at 105 000 g. The resulting pellet's surface was washed three times with cold 0.25 M-sucrose, and the pellet was resuspended in the same solution to an appropriate protein concentration. In some instances, pellets were frozen for several days before use. All microsomal preparation was done at 0–4°C. When frozen in concentrated form (15 mg of microsomal protein/ml of 0.25 M-sucrose), enzyme activities were stable for up to 2 weeks. Washed microsomes for lipid and fatty-acid analysis were prepared by resuspending the 105 000 g pellet in 0.25 M-sucrose and centrifuging for 60 min at 105 000 g.

Enzyme assays

The desaturation reactions were carried out as described previously (Eck *et al.*, 1979). Stearoyl-CoA desaturation reaction mixtures containing 36 µM-[1-¹⁴C]stearoyl-CoA (200 000 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 µM-[1-¹⁴C]linoleate, 5 mM-MgCl₂, 2 mM-ATP, 50 µM-coenzyme A, 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% 10 M-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 by using BF_3 in methanol as described previously (Faas *et al.*, 1972). 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 (Eck *et al.*, 1979).

Lipid fatty-acid composition

Total microsomal lipid was extracted with chloroform/methanol (2:1, v/v) (Folch *et al.*, 1957). Fatty-acid methyl esters of the lipid extract were formed as above following alkaline hydrolysis. Separation of individual fatty acid methyl esters was accomplished by g.l.c. using an HP5711 gas chromatograph. Glass columns (1.83 m $\times$ 4 mm internal diam.) packed with 12% EGSS-X and 2% poly(vinylpyrrolidinone) on 100/120 mesh Chromosorb W (acid-washed) were used. The columns were operated isothermally at 185°C using a flow rate of 60 ml/min for N_2 as carrier gas. Peaks were identified by comparison of retention times with authentic standards. The percentage of each fatty acid present was determined from the peak areas determined by an HP3380 recorder-integrator. Analysis of a quantitative standard containing 20% by weight each of the methyl esters of palmitate, stearate, oleate, linoleate and linolenate revealed integrated areas between 19.88% and 20.18% for each component.

Analytical procedures

Phospholipid, cholesterol and triacylglycerol

assays were carried out on the chloroform/methanol extracts by previously described procedures (Chen *et al.*, 1956; Morris *et al.*, 1972; Rudel & Morris, 1973). Proteins were determined by a modification of the Lowry method with bovine serum albumin as standard (Hartree, 1972).

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

Food intake and body weight in hyperthyroid animals

Table 1 shows the cumulative changes in body weight and the cumulative food intake of the four groups of rats throughout the experiment as well as values for the 14–21-day interval. Mean rat weight at the beginning of the experiment was 186 g. At the end of the experiment, the hyperthyroid rats (group II) had a 23% greater food intake than the controls (group I), but a 21% lower weight gain. The food-restricted controls (group III) received 88% of the food intake of the controls to achieve a similar weight gain as the hyperthyroid rats. The food-restricted hyperthyroid rats (group IV), which were given the same food intake as the controls, had an 81% lower weight gain than control animals. Similar changes were observed during the 14–21-day interval with the hyperphagia of the hyperthyroid rats (group II) being even more pronounced during this interval (35% greater food intake than controls).

Table 1. *Weight gain, food intake and microsomal lipid composition in hyperthyroidism*
Animal treatments are described in the Materials and methods section. Results are means $\pm$ S.E.M.

	Group I (control) (n = 10)	Group II (hyperthyroid) (n = 9)	Group III (food-restricted control) (n = 9)	Group IV (food-restricted hyperthyroid) (n = 10)
Weight gain (g)				
During 21 day treatment	152 $\pm$ 7	120 $\pm$ 11*	123 $\pm$ 6	28 $\pm$ 9
14–21-day interval	48 $\pm$ 3	39 $\pm$ 4	43 $\pm$ 2	6 $\pm$ 4
Food intake (g)				
During 21 day treatment	529 $\pm$ 16	650 $\pm$ 26†	466 $\pm$ 12	529 $\pm$ 8
14–21-day interval	187 $\pm$ 7	252 $\pm$ 9†	173 $\pm$ 7	184 $\pm$ 2
Microsomal lipid (nmol/mg of protein)				
Phospholipid	533 $\pm$ 8	495 $\pm$ 16	567 $\pm$ 14‡	502 $\pm$ 27
Cholesterol	75 $\pm$ 4	70 $\pm$ 3	69 $\pm$ 3	79 $\pm$ 3
Triacylglycerol§	38 $\pm$ 1	35 $\pm$ 1	39 $\pm$ 1	40 $\pm$ 2

* $P < 0.05$ (group II versus group I).

† $P < 0.01$ (group II versus group I).

‡ $P < 0.01$ (group III versus group II and group IV).

§ $n = 7, 6, 7$ and 7 for groups I–IV respectively.

Influence of food intake and weight gain on fatty-acid desaturation in hyperthyroidism

Fig. 1 shows the results of the fatty-acid desaturase activities in the four groups of rats. The

Fig. 1. Microsomal fatty-acid desaturase activity in hyperthyroidism and with food restriction

Desaturase enzyme assays were carried out as described in the Materials and methods section in the four groups of animals described in the text. 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 8.15 ± 0.89 and 1.54 ± 0.19 nmol/mg of protein per incubation period respectively. The numbers of animals in each group are shown within the columns. The star indicates a significant difference from the controls and each of the other groups ($P < 0.05$). Animal treatments are described in the Materials and methods section.

hyperthyroid rats had a 19% decrease in liver microsomal linoleate desaturase ($C_{18:2} \rightarrow C_{18:3}$) activity compared with controls without a change in stearate desaturation ($C_{18:0} \rightarrow C_{18:1}$). Likewise, study of a larger group ($n = 12$) of hyperthyroid animals fed *ad lib.* revealed similarly unchanged stearate desaturase activity of $100 \pm 7\%$ (mean $\pm$ S.E.M.) of control and a similar $24 \pm 4\%$ inhibition ($P < 0.01$) of linoleate desaturation (Table 2). Control animals whose food intake was restricted so that their weight gain was equivalent to the hyperthyroid animals had unchanged Δ^6 desaturase but a 56% increase in Δ^9 desaturase activity. Hyperthyroid animals whose food intake was restricted to match the food intake of the controls showed a 150% increase in Δ^6 desaturase without a change in Δ^9 desaturase activity. These increases in fatty-acid desaturase activities in the food-restricted controls and hyperthyroids were unexpected since starvation decreases both Δ^9 and Δ^6 desaturase activities (Brenner, 1974) and emphasizes the need for evaluation of food intake as one factor important in the complex control of fatty-acid desaturation.

Microsomal lipid composition in hyperthyroidism

Table 1 shows the microsomal composition of the major lipid classes in the four groups of rats. There were no significant differences in phospholipid content per g wet wt. of liver. Control liver microsomal lipids contained 83% phospholipid, 12% cholesterol and 6% triacylglycerol on a molar basis. There were no substantial differences in composition in any of the groups. Therefore, changes in fatty-acid composition between the various treatment groups are likely to be due to changes in fatty-acid composition of the phospholipid fraction.

Fig. 2 shows the fatty-acid composition of microsomal lipid in the fed and food-restricted control and hyperthyroid rats. Liver microsomal

Table 2. Microsomal desaturase activity in hyperthyroidism

The rats were injected with the given dose of thyroid hormone (abbreviations used: T_3 , tri-iodothyronine; T_4 , thyroxine) daily for the time indicated as detailed in the Materials and methods section. Mean ($\pm$ S.E.M.) desaturase activities are expressed as a percentage of control values with the numbers of animals in each group given in parentheses. Mean ($\pm$ S.E.M.) control stearate and linoleate desaturase activities were 6.11 ± 0.70 ($n = 23$) nmol/mg of protein per 20 min and 2.00 ± 0.26 ($n = 22$) nmol/mg of protein per 15 min respectively.

Treatment (μ g/100 g body wt.)	Duration of treatment (days)	Stearate (Δ^9) desaturation (% of control)	Linoleate (Δ^6) desaturation (% of control)
25 (T_3)	7	104 ± 23 (5)	98 ± 5 (5)
25 (T_3)	21	100 ± 7 (13)	$76 \pm 4^*$ (12)
50 (T_3)	5	$136 \pm 5^*$ (4)	$66 \pm 4^*$ (3)
500 (T_3)	5	$167 \pm 16^*$ (4)	$56 \pm 5^*$ (3)
100 (T_4)	5	109 ± 14 (4)	
1000 (T_4)	5	$142 \pm 7^*$ (4)	
1000 (T_4)	10	$168 \pm 13^*$ (4)	

* $P < 0.01$, compared with control.

Fig. 2. Liver microsomal fatty-acid composition in hyperthyroidism and with food restriction

Fatty-acid composition was determined by g.l.c. as described in the Materials and methods section for the four groups of animals described in the text. The numbers of animals in each group are shown within the first set of columns. Results are shown as means $\pm$ S.E.M. The letters above the bars indicate significant differences at $P < 0.01$: a, group I versus group II; b, I versus III; c, I versus IV; d, II versus III; e, II versus IV; f, III versus IV. a', b', c' and d' indicate $P < 0.05$.

lipid fatty-acid composition is altered in the hyperthyroid animals with significantly decreased proportions of palmitate (C_{16:0}), palmitoleate (C_{16:1}), linoleate (C_{18:2}) and eicosatrienoate (C_{20:3(8,11,14)}) and increased proportions of stearate (C_{18:0}) and arachidonate (C_{20:4}). Of these changes, only the decreased C_{20:3} fatty acid might be related to the decreased linoleate desaturase activity. Decreased linoleate desaturation would be expected to cause the opposite changes in C_{18:2} and C_{20:4} fatty-acid proportions.

Control animals whose food intake was restricted so that their weight gain was equivalent to the hyperthyroid animals had an increased proportion of C_{16:1} fatty acid in microsomal lipid, which is consistent with the increased Δ^9 desaturase activity in these animals. A decreased proportion of linoleate occurred in these animals similar to that found in the hyperthyroid rats fed *ad lib*. No other changes in fatty-acid composition occurred, similar to those seen in the hyperthyroid animals. Thus the diminished C_{18:2} fatty acid proportions in the hyperthyroid animals may be related to the diminished weight gain with relatively enhanced lipid mobilization in these animals.

Hyperthyroid animals whose food intake was restricted to match the food intake of the controls showed microsomal lipid fatty-acid composition changes in the same direction as those seen in hyperthyroid animals fed *ad lib*. but the decreases in C_{16:0} and C_{18:2} fatty acids and the increases in C_{18:0} and C_{20:4} were significantly greater in magnitude. In addition, the proportion of oleate (C_{18:1}) was significantly increased. The further decrease in C_{18:2} fatty acid and the increase in C_{20:4} fatty-acid proportions might be at least partly related to the

associated increase in Δ^6 desaturase activity in these food-restricted hyperthyroid animals. The fact that the composition changes were in general accentuated compared with the hyperthyroid rats fed *ad lib*. rather than decreased indicates that it is unlikely that the changes in the hyperthyroid rats fed *ad lib*. are due simply to the increased food intake in the hyperthyroid animals fed *ad lib*.

Microsomal Δ^9 and Δ^6 desaturase activity in hyperthyroidism

Gompertz & Greenbaum (1966) using rats treated with 500 μ g of thyroxine daily for 4 days and de Gomez Dumm *et al.* (1977) using rats treated with 4 mg of thyroxine/100 g body wt. daily for 12 days have reported more than doubling of stearate and palmitate desaturase activities in liver microsomes. Since our studies in chronically hyperthyroid rats using a much smaller dose of thyroid hormone (25 μ g of tri-iodothyronine daily for 21 days) failed to show an increase in Δ^9 desaturase activity (see Fig. 1), experiments were undertaken to evaluate stearate desaturation at various doses of tri-iodothyronine and thyroxine. Table 2 shows that whereas 25 μ g of tri-iodothyronine daily shows no increase in stearate desaturation, 50 μ g of tri-iodothyronine/100 g body wt. causes a significant 36% increase and 500 μ g/100 g body wt. a 67% increase. Likewise, whereas 100 μ g of thyroxine/100 g body wt. daily for 5 days fails to alter desaturase activity, 1000 μ g/100 g body wt. for 5 days increases stearate desaturase activity 42% and for 10 days 68%. Thus although 25 μ g of tri-iodothyronine/100 g body wt. is clearly a hyperthyroid dose in the rat (Ingbar & Galton, 1975), resulting in diminished weight gain and increased food intake, much larger doses of

thyroid hormone in normal animals are necessary to produce increases in Δ^9 desaturation.

500 μ g of tri-iodothyronine/100g body wt. daily for 5 days caused a similar but more marked decrease in Δ^6 desaturase activity (56%) compared with the 24% decrease noted in rats treated with 25 μ g of tri-iodothyronine/100g body wt. daily for 21 days.

To determine if these large doses of thyroid hormone that produce an increase in Δ^9 fatty-acid desaturation likewise produce changes in fatty-acid composition of the microsomal membrane, fatty-acid analysis of liver microsomal lipid was carried out in the groups of rats treated with 500 μ g of tri-iodothyronine/100g body wt. and 1000 μ g of thyroxine/100g body wt. for 5 days and compared with normal controls. The tri-iodothyronine- and thyroxine-treated animals had mean weight gains of 37 ± 3 and 32 ± 2 g in 5 days compared with 56 ± 1.4 g in the controls. The results of these studies are shown in Fig. 3. There were similar decreases in $C_{18:2}$ and $C_{20:3}$ fatty acids and a similar but smaller decrease in $C_{16:1}$ fatty acid than occurred in the rats treated with 25 μ g of tri-iodothyronine/100g body wt. for 21 days. Proportions of $C_{16:0}$, $C_{18:0}$ and $C_{20:4}$ fatty acids were unchanged in contrast with the changes in the more chronically treated animals. There was a 'borderline significant' small increase in $C_{18:1}$ fatty acid in the animals treated with the large doses of thyroid hormone which could be due to the increased Δ^9 desaturase activity, compared with a slight but insignificant decrease in $C_{18:1}$ fatty

acid in those treated with the smaller doses. The other changes in fatty-acid composition, notably the decrease in $C_{16:1}$ fatty acid are not the expected result of increased Δ^9 desaturase activity, and must be due to other factors.

Discussion

Although many investigators have assumed or postulated that linoleate desaturation is increased in hyperthyroidism either because of the decreased proportion of linoleate found in cellular lipids or because of the increased stearate desaturation reported (Gompertz & Greenbaum, 1966; de Gomez Dumm *et al.*, 1977), our studies on linoleate desaturation demonstrate not only no increase but a significant decrease in linoleate desaturation compared with controls at doses of tri-iodothyronine from 25 μ g/100g to 500 μ g/100g body wt. daily. This finding agrees with the findings of de Gomez Dumm *et al.* (1977), who used only very large doses of thyroxine (4 mg/100g body wt. daily for 12 days). The lack of an increase in stearate desaturation in animals treated with 25 μ g of tri-iodothyronine/100g body wt. daily for 21 days in the present study was apparently due to the much smaller doses of thyroid hormone since larger doses of thyroid hormone verified the increased Δ^9 desaturase activity reported previously (Gompertz & Greenbaum, 1966; de Gomez Dumm *et al.*, 1977).

We chose to look at microsomal lipid fatty-acid composition, not previously investigated by others, thinking that changes in microsomal fatty-acid desaturation might be most rapidly or markedly manifest in microsomal lipid. The changes we found, consisting of decreased proportions of $C_{16:0}$, $C_{16:1}$, $C_{18:2}$ and $C_{20:3(8,11,14)}$ fatty acids and increased $C_{18:0}$ and $C_{20:4}$ fatty acids are similar in most respects to changes reported in whole rat liver or liver mitochondrial lipids (Patton & Platner, 1970; Platner *et al.*, 1972). The decreased $C_{20:3(8,11,14)}$ fatty acid may be a reflection of decreased linoleate desaturation but the other changes must be due to other factors. Specifically, decreased linoleate desaturation would be expected to result in increased proportions of $C_{18:2}$ fatty acid, whereas the tri-iodothyronine-treated animals actually had a consistent significant decrease in the proportion of $C_{18:2}$ fatty acid. Animals treated for 5 days with large doses of thyroid hormone have increased Δ^9 desaturase activity, similar fatty-acid composition changes in $C_{18:2}$ and $C_{20:3(8,11,14)}$ fatty acids and a noteworthy similar though smaller decrease in $C_{16:1}$ fatty acid than those treated with much smaller doses of tri-iodothyronine. In other situations such as in insulin-treated diabetic rats and after oestradiol treatment of hens, increased Δ^9 desaturase activity is rapidly reflected in changes in lipid fatty-acid

Fig. 3. Liver microsomal fatty-acid composition in acute hyperthyroidism

Fatty-acid composition was determined by g.l.c. as described in the Materials and methods section. The middle and right-hand columns in each group represent rats treated with 500 μ g of tri-iodothyronine/100g and 1000 μ g of thyroxine/100g body wt. daily for 5 days respectively. Means $\pm$ S.E.M. for four animals in each group are shown. a, $P < 0.05$; b, $P < 0.01$ (significantly different from controls).

composition (Benjamin & Gellhorn, 1964; Lippiello *et al.*, 1979; Faas & Carter, 1980). Thus the lack of changes in fatty-acid composition that might be expected from increased Δ^9 and decreased Δ^6 desaturase activities and the additional changes after a longer duration of treatment with smaller doses of thyroid hormone (decreased $C_{16:0}$ and increased $C_{18:0}$ and $C_{20:4}$ fatty acids) all strongly suggest that most of the changes in the hyperthyroid animals are not related to changes in fatty-acid desaturation. However, the consistent decrease in $C_{20:3(8,11,14)}$ fatty acid in the hyperthyroid animals may be related to the diminished Δ^6 desaturation.

The diminished weight gain of the hyperthyroid animals in itself does not appear to explain the fatty-acid composition changes other than possibly the decrease in $C_{18:2}$ fatty acid, since control animals with similar weight gain did not show similar changes in fatty-acid composition except for a similar decrease in $C_{18:2}$ fatty acid. The only other change in fatty-acid composition in these animals was an increased proportion of $C_{16:1}$ fatty acid. This is probably due to the increase in Δ^9 desaturase activity seen in these animals. This latter finding was somewhat surprising since starvation markedly diminishes Δ^9 desaturase activity, although starvation followed by refeeding, low-fat diet and various other dietary manoeuvres may increase Δ^9 desaturase activity (Brenner, 1974). This observation of increased desaturase activity with partial food restriction was also observed recently as an incidental finding in studies with diabetic animals (Faas & Carter, 1980) and is being studied further.

The increased food intake of the hyperthyroid animals in itself probably does not account for the lipid composition changes, since limiting food intake in hyperthyroid animals actually accentuated the fatty-acid composition changes rather than preventing such changes. This may be due to the fact that food deprivation in the thyroid hormone treated animal diminishes thyroid hormone excretion (Ingbar & Galton, 1975), resulting in a more marked hyperthyroid state, thus accentuating the changes seen. The increased proportion of $C_{18:0}$ fatty acid and the decreased proportion of $C_{16:0}$ and $C_{18:2}$ fatty acids were changed to a significantly greater extent in the same direction in these food-restricted hyperthyroid animals. The decreased proportions of $C_{16:1}$ and $C_{20:3(8,11,14)}$ fatty acids were similar in extent to that found in the hyperthyroid rats fed *ad lib*. Changes not present in the hyperthyroid animals fed *ad lib*. include significantly higher proportions of $C_{18:1}$ and $C_{20:4}$ fatty acids. Interpretation of these fatty-acid composition changes is complicated by the fact that Δ^6 desaturase activity was increased 150% in these animals rather than the 24% decrease seen in the hyperthyroid rats fed *ad lib*. Therefore, in the food-restricted hyperthyroid animals, the further

decrease in $C_{18:2}$ and increase in $C_{20:4}$ fatty acids might be due at least in part to this increase in Δ^6 desaturase activity. This change in desaturase activity exemplifies the sensitivity of these enzymes to dietary as well as hormonal factors.

Other factors such as effects on Δ^5 desaturation, fatty-acid synthesis *de novo*, fatty-acid chain elongation, membrane lipid degradation and synthesis, diet, fatty-acid oxidation and transport of fatty acids between adipose tissue and the liver must be considered as possible factors that may influence membrane fatty-acid composition in thyroid hormone treated animals. For instance increased Δ^5 desaturation might result in diminished $C_{20:3(8,11,14)}$ and increased $C_{20:4}$ fatty acid proportions. The increased proportion of $C_{18:0}$ fatty acid in the hyperthyroid animals may be due to increased chain elongation reported in hyperthyroidism (Landriscina *et al.*, 1976), although some investigators have found no change in chain elongation after thyroxine treatment (de Gomez Dumm *et al.*, 1977). It also may be due to increased fatty-acid mobilization from the periphery, since this finding was enhanced by food restriction. Since the decreased proportions of $C_{16:0}$ and $C_{18:2}$ fatty acids were magnified by food restriction in hyperthyroid animals, increased lipid degradation in response to the hyperthyroid state may be important in bringing about these changes. These changes in the hyperthyroid animals that were magnified by food restriction are probably related to a thyroid hormone effect and not simply the non-specific result of enhanced lipid breakdown, since the food-restricted control animals failed to reveal similar changes. These studies emphasize the complexity of the regulation of membrane fatty-acid composition by multiple dietary, metabolic and hormonal factors.

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