Effects of Thyroxine and Cold-Acclimation on Hepatic Fatty Acid Metabolism in the Hamster[1] (35543)

JOHN F. PATTON[2] AND WESLEY S. PLATNER

Department of Physiology, University of Missouri Medical School, Columbia, Missouri 65201

A previous report from this laboratory (1) showed that during the process of cold-acclimation, a relatively higher degree of fatty acid unsaturation occurred in hamster whole liver. In the rat, however, the fatty acid desaturating mechanism does not appear to be operating as cold-acclimation fails to increase the proportion of unsaturated hepatic fatty acids (1, 2).

The mitochondria represent the site of oxidative metabolism in the liver cell and are known to undergo structural and functional changes during cold-acclimation (3, 4). Indeed, cold-acclimation results in a decrease in total fatty acid unsaturation in rat liver mitochondria (2) and induces changes in the proportion of individual fatty acids of both rat (2) and hamster (5) liver mitochondria. Furthermore, the thyroid gland is known to participate in an animal's response to a cold environment (3), effect a number of parameters of fatty acid metabolism (6–9), and produce structural and functional changes in liver mitochondria (10).

The present experiments were performed, therefore, to evaluate in the hamster the role of the thyroid gland and cold-acclimation on fatty acid unsaturation as well as changes in individual fatty acids in both whole liver and liver mitochondria.

Methods and Materials. Adult male hamsters were caged singly and cold-acclimated at 5 ± 2° for 6–7 weeks. Warm-acclimated hamsters were kept at room temperature (23 ± 2°) for 4–5 weeks. Both the warm- and cold-acclimated animals were divided into three groups: controls, prophylthiouracil (PTU)-treated animals and thyroxine-treated animals. PTU and thyroxine were administered for 10 days prior to sacrifice by ip injection. L-Thyroxine was administered as the sodium salt at a dose of 2 mg/100 g of body weight while the dosage of PTU was 5 mg/100 g of body weight (11).

Animals were maintained on synthetic diets and were given distilled water *ad libitum*. The diet had the following composition(%): promine R (25), corn starch (58.8), corn oil (65), Briggs salt mixture (4), alphacel (4), vitamin mixture (2), and methionine (0.2). The fatty acid analysis of the diet expressed in percentage composition was as follows: palmitic (12.40); stearic (2.65); oleic (26.00); linoleic (57.00); and linolenic (1.95).

Animals were killed by decapitation, the livers were removed and two samples were taken: one for whole liver total fatty acid extraction and one for mitochondrial preparation. The methods used for mitochondrial isolation and fatty acid extraction have previously been described (2).

A Barber-Colman gas chromatograph was used to separate and quantitate the fatty acids. The operating conditions of this instrument have been outlined previously (2). Peak area of the fatty acids was obtained with the model 205 Disc Chart Integrator. The fatty acids from 14 to 22 carbons in chain length are included in this study. The fatty acid designations used give the number of carbon atoms and double bonds present: thus myristic is 14:0, palmitic 16:0, palmitoleic 16:1, stearic 18:0, oleic 18:1, linoleic

[1] This study was supported by a grant to W. S. Platner from the National Institute of Arthritis and Metabolic Diseases Research Grant AM-12437-02 and the University of Missouri Space Sciences Research Center.

[2] Present address: Arctic Medical Research Laboratory, Alaska, APO Seattle, Wash. 98731.

TABLE I. Hamster Whole Liver Percentage Unsaturated Fatty Acid Composition; A Comparison Between Treatments.[a]

Comparison percentage unsaturation	DBM	Significance (.05 level)	CI
Warm C vs warm PTU (65.52 ± 0.56) (63.70 ± 0.45)[b]	1.82	NS	(—0.29, 3.93)
Warm C vs warm T_4 (65.02 ± 0.56) (64.05 ± 0.45)	1.47	NS	(—0.64, 3.58)
Warm C vs cold C (65.52 ± 0.56) (65.84 ± 0.36)	1.32	NS	(—0.79, 3.43)
Cold C vs cold PTU (66.84 ± 0.36) (65.48 ± 0.48)	1.36	NS	(—0.75, 3.47)
Cold C vs cold T_4 (66.84 ± 0.36) (66.81 ± 0.71)	0.03 D = 2.11	NS	(—2.08, 2.14)

[a] Abbrev.: DBM = difference between means; CI = 95% confidence interval; C = control; PTU = propylthiouracil; T_4 = thyroxine; NS = not significant; D = mean square times Q value.

[b] Mean ± SEM; 16 animals/treatment.

18:2, linolenic 18:3, arachidonic 20:4, and docosahexanoic 22:6. Three fatty acids, designated as X_1, X_2, and X_3, were not positively identified as standards could not be obtained. However, from previously reported fatty acid compositions of rat liver (12), the authors feel that X_1 may represent 5,8, 11-eicosatrienoic; X_2, 8,11,14-eicosatrienoic;

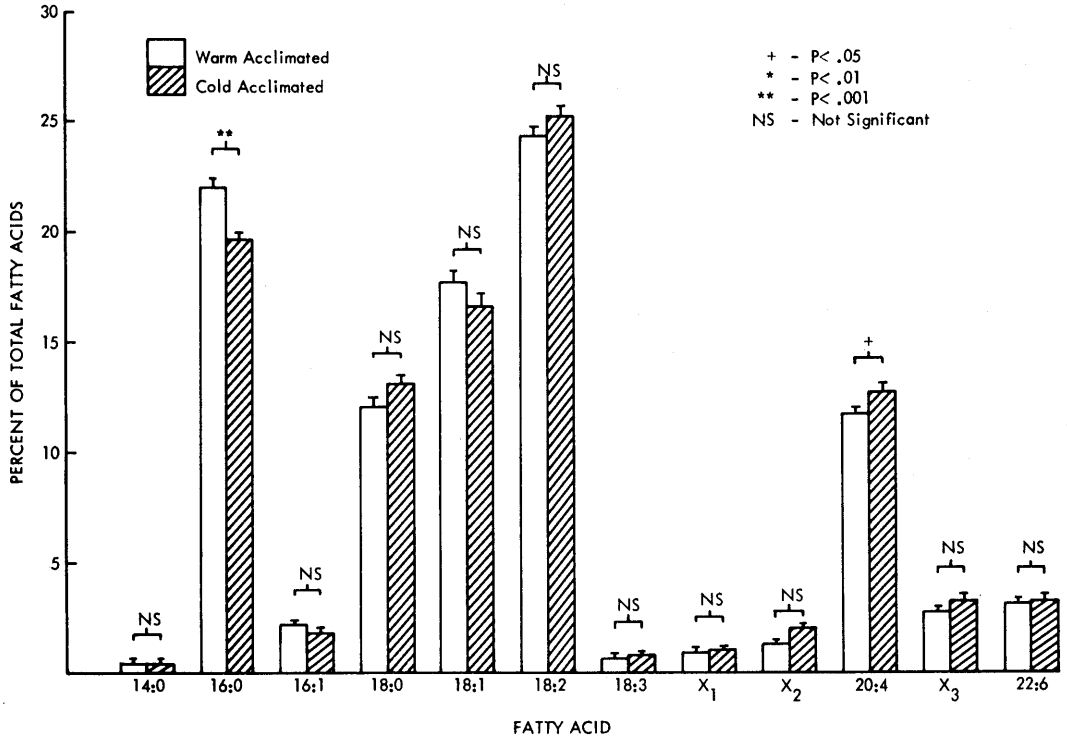

FIG. 1. Hamster: warm-acclimated controls compared to cold-acclimated controls; percentage fatty acid composition of whole liver.

and X_3, docosapentanoic acids.

The Studentized Range Test (13) was used for testing comparisons among treatment means for total fatty acid unsaturation of whole liver and liver mitochondria. The difference between means of individual fatty acids was statistically analyzed in accordance with the Student's *t* test.

Results and Discussion. Table I shows that neither cold-acclimation nor PTU or thyroxine treatments produce any significant changes in the degree of total unsaturation of whole liver fatty acids. The data suggest, therefore, that during cold-acclimation the fatty acid desaturating mechanism is not utilized to any significant extent which is consistent with the data that have been reported for the cold-acclimated rat (2).

Although total fatty acid unsaturation is not effected by cold-acclimation, changes in individual hepatic fatty acids occur as seen by a relative decrease in palmitate and an increase in arachidonate (Fig. 1). These findings may reflect changes in the triglyceride to phospholipid ratio for Therriault and Poe (14) showed a decrease in triglycerides with no change in total phospholipids of hepatic lipid from chronically cold-exposed rats.

PTU and thyroxine treatments also produce marked changes in individual fatty acids of liver from warm-acclimated hamsters (Fig. 2). The large increases in palmitate and oleate as a result of thyroxine most likely represent mobilization from adipose tissue as it is known that these two fatty acids comprise 70% of the total fatty acids of this tissue (1). Similar findings have been reported in the thyroxine-treated rat by Ellefson and Mason (7). The decrease in stearate may represent increased desaturation of this particular fatty acid forming oleate since this conversion is known to be stimulated by thyroxine in the rat (8). The decrease in the polyunsaturated fatty acids linoleate, X_2 and arachidonate, following thyroxine injection are striking and may represent increased utilization. However, this is contrary to what has been reported in the rat (7), but since we are dealing with percentage composition,

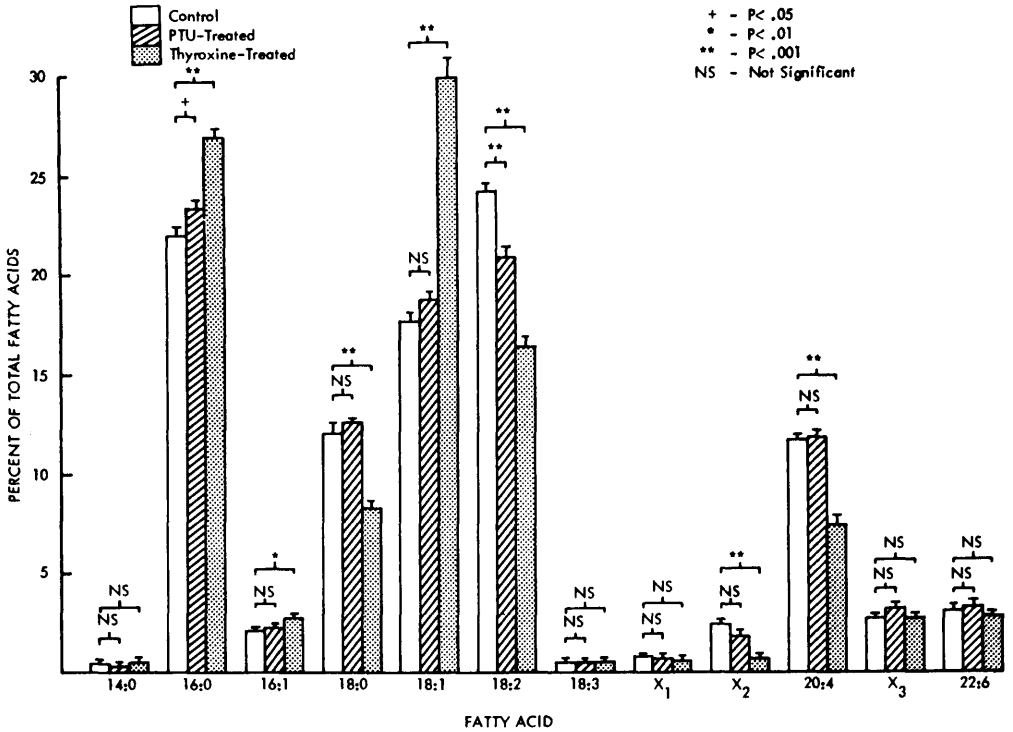

FIG. 2. Warm-acclimated hamster: percentage fatty acid composition of whole liver.

TABLE II. Hamster Liver Mitochondria Percentage Unsaturated Fatty Acid Composition; A Comparison Between Treatments.[a]

Comparison percentage unsaturation	DBM	Significance (.05 level)	CI
Warm C vs warm PTU (68.99 ± 0.53) (67.96 ± 0.41)[b]	1.03	NS	(−0.56, 2.62)
Warm C vs warm T_4 (68.99 ± 0.53) (62.53 ± 0.37)	6.46	Significant	(4.87, 8.05)
Warm C vs cold C (68.99 ± 0.53) (68.85 ± 0.23)	0.14	NS	(−1.45, 1.73)
Cold C vs cold PTU (68.85 ± 0.23) (69.50 ± 0.32)	0.64	NS	(−0.89, 2.24)
Cold C vs cold T_4 (68.85 ± 0.23) (65.26 ± 0.40)	3.59 D = 1.59	Significant	(2.00, 5.18)

[a] Abbrev.: DBM = difference between means; CI = 95% confidence interval; C = control; PTU = propylthiouracil; T_4 = thyroxine; NS = not significant; D = mean square times Q value.

[b] Mean ± SEM; 16 animals/treatment.

these changes may be an inverse resultant of higher percentages of other fatty acids. Regardless of the mechanism(s) which may be involved, it is readily apparent that thyroxine can drastically alter the distribution and metabolism of individual hepatic fatty acids in the hamster.

With regard to liver mitochondria, cold-acclimation does not affect the degree of total fatty acid unsaturation (Table II). In the

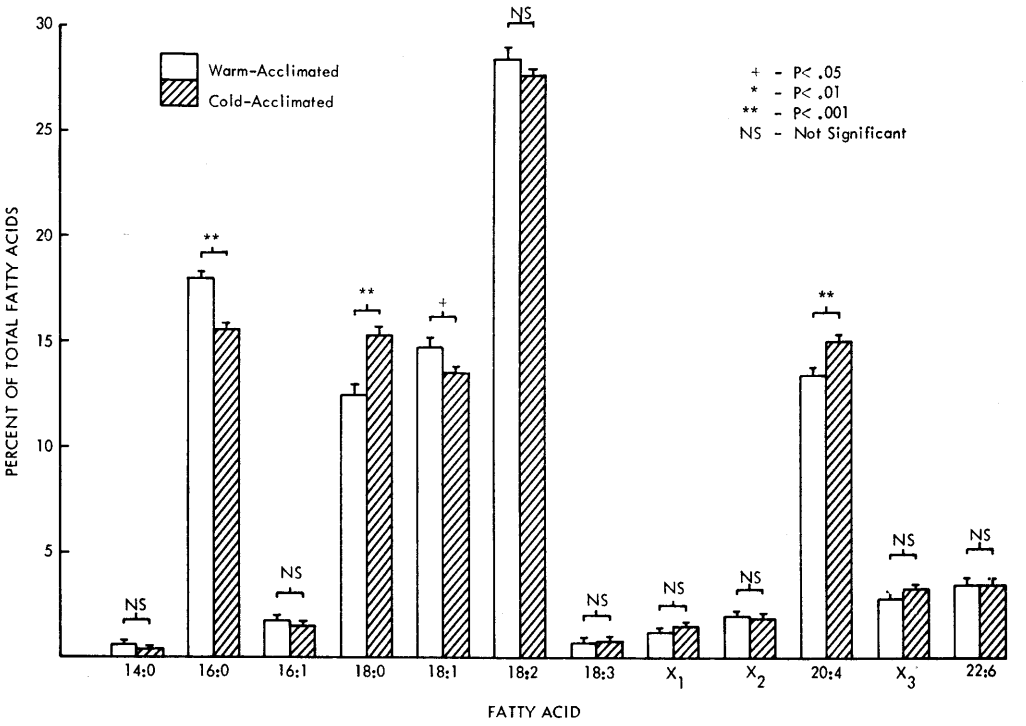

FIG. 3. Hamster: warm-acclimated controls compared to cold-acclimated controls; percentage fatty acid composition of liver mitochondria.

rat, however, the cold-acclimated state did induce a significant decrease in total unsaturation (2). This finding suggests that differences exist between these two species concerning mitochondrial function in the cold.

Cold-acclimation does produce a number of changes in individual mitochondrial fatty acids as shown by decreases in palmitate and oleate and increases in stearate and arachidonate (Fig. 3). These changes may represent structural alterations in the lipoprotein components of the mitochondrial membrane. Indeed, studies on essential fatty acid deficiency (15, 16) have shown that the fatty acid composition of mitochondria is altered which is associated with changes in both their structure and function. Furthermore, Green and Fleischer (17) have suggested that the stability of the phospholipid component of the oxidative phosphorylating apparatus of mitochondria depends, in part, on the nature of the fatty acids of the phospholipids. The data suggest, therefore, that changing the environmental temperature can modify the biochemical components of mitochondria. Such modifications may play a role in the increased heat production characteristic of the cold-acclimated state.

Table II and Fig. 4 show the effects of PTU and thyroxine on total mitochondrial unsaturation and individual fatty acids from warm-acclimated animals. Thyroxine treatment results in a decrease in total unsaturation and produces marked changes in individual fatty acids as evidenced by increases in palmitate, stearate, arachidonate and X_3 and by a decrease in linoleate and X_2. These findings appear unrelated to those demonstrated in whole liver. Apparently thyroxine can alter the fatty acid composition of liver mitochondria independent of its effect on whole liver.

From the present experiments it is not possible to assess the significance of the data in relation to the action of thyroxine on mitochondria. However, it is proposed that by bringing about changes in the fatty acid composition, thyroxine can sufficiently modify the structure of the mitochondrial membrane to cause altered enzymatic activity and permeability. The effects of thyroxine are dramatic and may represent an important site of

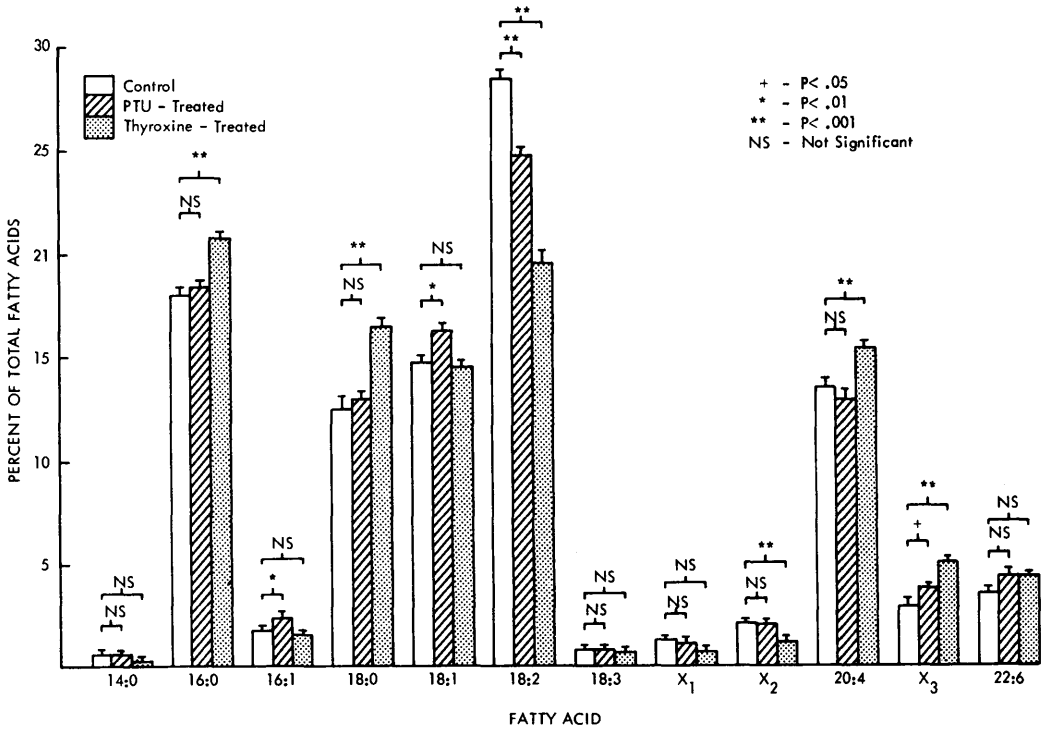

FIG. 4. Warm-acclimated hamster: percentage fatty acid composition of liver mitochondria.

hormone action for it is well documented that the thyroid hormones can affect both the structure and function of mitochondria (10, 18).

The present experiments fail to show analogous effects between cold-acclimation and thyroxine treated, warm-acclimated animals regarding the fatty acid composition of mitochondria. In the rat, however, Patton and Platner (2) have reported similarities between cold-induced changes in mitochondrial fatty acids and those occurring as a result of thyroxine treatment.

Summary. Relative concentrations of fatty acids from whole liver and liver mitochondria of cold-acclimated hamsters were analyzed by gas chromatography. Involvement of the thyroid gland was studied by the administration of PTU and thyroxine to both warm- and cold-acclimated animals. No change was observed in total unsaturation of either whole liver or mitochondrial fatty acids as a result of cold-acclimation but changes did occur in the composition of individual fatty acids. These were a decrease in palmitate and an increase in arachidonate in whole liver while mitochondria showed increases in stearate and arachidonate and decreases in palmitate and oleate. Injection of warm-acclimated animals with thyroxine also resulted in no change in whole liver unsaturation but did produce a decrease in the total unsaturation of mitochondria. Marked effects were also observed in individual fatty acids following thyroxine treatment as evidenced by increases in palmitate and stearate and a decrease in linoleate in mitochondria while in whole liver there were increases in palmitate and oleate and decreases in stearate, linoleate, and arachidonate. Analogous effects on fatty acid metabolism between thyroxine-treated, warm-acclimated hamsters and cold-acclimation were not observed.

1. Williams, D., and Platner, W. S., Amer. J. Physiol. **212**, 167 (1967).

2. Patton, J. F., and Platner, W. S., Amer. J. Physiol. **218**, 1417 (1970).

3. Smith, R. Em., and Hoijer, D. J., Physiol. Rev. **42**, 60 (1962).

4. Lusena, C. V., and Depocas, F., Can. J. Physiol. Pharmacol. **45**, 683 (1967).

5. Chaffee, R. R. J., Platner, W. S., Patton, J., and Jenny, C., Proc. Soc. Exp. Biol. Med. **127**, 102 (1968).

6. Dayton, S., Dayton, J., Drimmer, F., and Kendall, R. E., Amer. J. Physiol. **199**, 71 (1960).

7. Ellefson, R. D., and Mason, H. L., Endocrinology **75**, 179 (1964).

8. Gompertz, D., and Greenbaum, A. L., Biochim. Biophys. Acta **116**, 441 (1966).

9. Deykin, D., and Vaughan, M. J., J. Lipid Res. **4**, 200 (1963).

10. Hoch, F. L., N. Engl. J. Med. **266**, 446 (1962).

11. Nelson, D. R., and Cornatzer, W. E., Endocrinology **77**, 37 (1965).

12. Getz, G. S., Bartley, W., Stripe, F., Notton, B. M., and Renshaw, A., Biochem. J. **83**, 181 (1963).

13. Snedecor, G. W., "Statistical Methods," 534 pp. Iowa State Univ. Press, Ames, (1966).

14. Therriault, D. G., and Poe, R. H., Can. J. Biochem. **43**, 1427 (1965).

15. Johnson, R. M., and Ito, J., J. Lipid Res. **6**, 75 (1965).

16. Smith, J. A., and DeLuca, H. F., J. Cell Biol. **21**, 15 (1964).

17. Green, D. E., and Fleischer, S., Biochim. Biophys. Acta **70**, 554 (1963).

18. Piatnek-Luenissen, D. A., and Luenissen, R. L. A., Endocrinology **84**, 456 (1969).

Received Oct. 5, 1970. P.S.E.B.M., 1971, Vol. 137.