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The Effect of Thyroxine on Lipid and Carbohydrate Metabolism in the Heart *

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The administration of thyroxine to a number of experimental animals has resulted in marked structural and metabolic changes in the myocardium. Thyroxine has been shown to produce myocardial hypertrophy (1, 2) and to increase oxygen consumption and fatty acid utilization by the heart (3). In the dog made hyperthyroid by the administration of large doses of thyroxine, increased free fatty acid utilization by the heart was accompanied by a decreased uptake of glucose (3).

In this communication data are presented which show that the administration of *l*-thyroxine to guinea pigs resulted in an increased rate of long chain fatty acid oxidation and a decreased rate of glucose oxidation by myocardial homogenates. The stimulatory effect on fatty acid oxidation was associated with elevated concentrations of free carnitine and acylcarnitines and increased long chain acyl coenzyme A (CoA)-carnitine acyltransferase (CAT) activity. The decreased glucose oxidation was associated with elevated levels of myocardial hexosemonophosphates and citrate. Citrate, the tissue concentration of which is increased by enhanced fatty acid oxidation, has been identified as one of several compounds that exert rate-controlling influences on glycolysis by inhibiting the activity of phosphofructokinase (PFK) (4, 5).

Methods

White male guinea pigs weighing 200 to 325 g were used. All animals were offered a standard diet consisting of 50% Purina rabbit chow and 50% oats supplemented with fresh cabbage. Guinea pigs treated with thyroxine received 50 μg of *l*-thyroxine intraperitoneally in 0.2 ml of 0.01 M phosphate buffer, pH 7.8, daily for 10 days. Body weights were obtained before and after the thyroxine treatment was completed. The animals were then killed by a blow on the head. The entire heart was removed immediately, the great vessels were trimmed off at their origin, and the cardiac cavities were opened and freed of blood. The opened hearts were blotted on filter paper and weighed before further analyses were carried out.

Hearts from treated animals and their controls used to assess the effect of thyroxine on total heart weight and on the concentrations of protein, ribonucleic acid, and deoxyribonucleic acid were homogenized in 8.0 ml of calcium-free Krebs-Ringer phosphate buffer, pH 7.4. The hearts used for enzymatic assays were placed in a volume of homogenizing medium such that their final protein concentrations were approximately equal.

Protein concentration was measured by the biuret method (6). After completion of the biuret reaction the solution was filtered through a layer of Celite in a Büchner type funnel. This served to remove lipids, which produce turbidity of the solution. The results obtained by this procedure agreed closely with those obtained by Kjeldahl nitrogen determination (7).

Analyses of myocardial DNA and RNA were done on trichloroacetic acid extracts of whole hearts. The *p*-nitrophenylhydrazine procedure of Webb and Levy (8) was used for DNA, and the orcinol method of Ceriotti (9) was used for RNA determination.

The concentration of FFA in the myocardium was determined by the method of Dole as modified by Trout, Estes, and Friedberg (10). Quantification of triglycerides in the heart was done by the procedure of Van Handel and Zilversmit (11).

Assays of palmitate, pyruvate, and glucose oxidations were carried out as previously described (12). Since the myocardial concentrations of lipid in the treated and control animals were not the same, the endogenous pools of FFA in the hearts were ascertained before the addition of radioactive palmitate. By this means the specific activities of the substrate pools were determined at the beginning of the incubation period. In the treated group the FFA concentration was significantly higher (mean 4.2 μmoles per g, SE 0.5) than in the control groups (mean 2.4, SE 0.3) ($p < 0.01$). These differences in endogenous FFA and the subsequent differences in specific activity of the substrate resulting from the addition of 0.1 μmole of labeled palmitate to both the treated and

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control incubations were taken into account in the calculation of the rates of long chain fatty acid oxidation. In these calculations full mixing of the endogenous FFA and the added radioactive palmitate was assumed. In time course experiments the rates of fatty acid oxidation in both treated and control preparations were linear with a fixed ratio of $^{14}CO_2$ evolved being maintained between them. This was considered to indicate that significant lipolysis with dilution of the FFA pools did not occur during the course of the incubation.

In glucose and pyruvate oxidation studies the amount of radioactive substrate added was over a hundredfold greater than the endogenous levels, and no corrections were made for differences in specific activities.

Tissue levels of free and bound carnitine were determined by the method of Tubbs, Pearson, and Chase (13). The short chain acyl CAT used in the carnitine assay was prepared from pigeon breast muscle by the procedure of Chase, Pearson, and Tubbs (14) and had a specific activity of 6.5 (micromoles coenzyme A released from acetyl CoA per minute per milligram protein at 25°).

Assays of palmityl CoA-carnitine acyltransferase were carried out by a modification of the isotope exchange method of Norum (15). In our assay procedure the rate of palmitate-1-^{14}C incorporation into palmitylcarnitine-^{14}C was followed. Palmitylcarnitine-^{14}C was extracted from the reaction mixture with chloroform-methanol and separated by thin layer chromatography on basic silica gel plates in a two-dimensional system (16). The palmitylcarnitine spots were scraped off the plates and counted in a liquid scintillation spectrometer. The assays were carried out for 10 minutes according to the procedure of Norum (15) and stopped by the addition of trichloroacetic acid.

To assess the incorporation of glucose into cardiac glycogen, guinea pigs were injected intraperitoneally with glucose-U-^{14}C. One hour later, the animals were anesthetized with sodium pentobarbital (5 mg per 100 g ip) and the hearts removed and frozen in a mixture of solid carbon dioxide and acetone. The hearts were then weighed, and slices of known weight were placed in 1.8 ml of 30% KOH and heated in a boiling water bath for 30 minutes. Glycogen was precipitated by the addition of 3.7 ml of absolute ethanol at 4° overnight. The precipitate was dissolved in 1.5 ml of water, and 0.2-ml samples were taken for glycogen quantification by the anthrone method of Seifter, Dayton, Novic, and Muntwyler (17).

TABLE I

*Cardiac weight and protein concentration in animals given l-thyroxine**

	Heart, wet weight			Myocardial protein		
	Control	Thyroxine	Difference	Control	Thyroxine	Difference
	g			*mg/ml*		
Mean	0.83	1.10	0.27	9.3	11.7	2.4
SE	0.01	0.16	0.06	0.3	0.5	0.5
p			<0.01			<0.01

* Each group contained ten animals.

TABLE II

*Myocardial concentrations of RNA and DNA and ratio of RNA to DNA in control and l-thyroxine-treated guinea pigs**

	Control	Thyroxine	Difference
	μg/mg protein		
	RNA		
Mean	2.39	2.31	0.08
SE	0.05	0.05	0.06
p			<0.20 > 0.10
	DNA		
Mean	1.34	0.81	0.53
SE	0.06	0.03	0.08
p			<0.01
	RNA/DNA		
Mean	1.80	2.84	1.04
SE	0.08	0.06	0.12
p			<0.01

* Each group contained ten animals.

Samples of 0.5 ml were placed in 15 ml of a phosphor solution consisting of 10 ml toluene containing 2,5-diphenyloxazole (4 g per L) and 1,4-bis-2-(5-phenyloxazolyl) benzene (100 mg per L), and 5 ml of Triton X-100. Radioactivity was determined with a Packard Tri-Carb liquid scintillation spectrometer.

The analyses for glucose 6-phosphate and fructose 6-phosphate were done enzymatically by the method of Hohorst (18). In this procedure fructose 6-phosphate was converted to glucose 6-phosphate by phosphohexoseisomerase, and the glucose 6-phosphate was quantified by means of glucose 6-phosphate dehydrogenase and TPN.

Myocardial pyruvate analyses were done by the enzymatic method described by Bucher, Czok, Lamprecht, and Latzku (19). This method is based on the conversion of pyruvate to lactate in the presence of DPNH and lactic dehydrogenase. Lactate concentrations were determined enzymatically by the method of Horn and Brun (20). Myocardial citrate assays were done by the procedure of Ettinger, Goldbaum, and Smith (21).

Carboxyl-labeled palmitate, glucose-U-^{14}C,[1] pyruvate-3-^{14}C,[1] carnitine,[2] purified RNA and DNA,[2] glucose 6-phosphate dehydrogenase,[3] phosphohexoseisomerase,[3] and lactic dehydrogenase [3] were obtained commercially.

The statistical significance of the data was evaluated by applying the *t* test to the mean difference between paired control and experimental animals (22).

Results

Heart weight. Administration of thyroxine resulted in cardiac hypertrophy (Tables I, II).

[1] New England Nuclear Corp., Boston, Mass.

[2] Calbiochem, Los Angeles, Calif.

[3] Sigma Chemical Co., St. Louis, Mo.

TABLE III

*Body weight changes in control and l-thyroxine-treated guinea pigs**

	Control			Thyroxine		
	Before	After	Change	Before	After	Change
			g			
Mean	258	299	+41	268	216	−53
SE	8	6	5	10	11	6
p			<0.01			<0.01

* Each group contained ten animals.

When compared with the controls, the hearts of the treated guinea pigs showed significantly greater weights, higher protein concentrations, lower concentrations of DNA, unchanged concentrations of RNA, and higher RNA to DNA ratios. These features are characteristic of myocardial hypertrophy in that cellular content of both protein and RNA are increased, whereas DNA remains constant (23–25). In contradistinction to the heart, total body weight in the treated animals decreased (Table III).

Myocardial lipids. Guinea pigs receiving thyroxine had myocardial concentrations of FFA and triglyceride that were significantly higher than those of the controls (Table IV). The mechanism whereby thyroxine may induce this effect is probably related to the accelerated release of FFA from adipose tissue and the consequent rise in plasma levels of FFA brought about by this hormone (26–29).

Long chain fatty acid oxidation. Myocardial homogenates from thyroxine-treated guinea pigs showed higher rates of fatty acid oxidation than the controls (Table V). Although both the treated and control groups oxidized about the same per cent of the fatty acid substrate in 30 minutes (treated 26.6, control 25.6), the treated group had a greater FFA pool (treated 0.64, control 0.36, μmole per 15 mg protein) and therefore oxidized a greater total amount of FFA during the course of the incubations.

TABLE IV

*Myocardial concentrations of free fatty acid and triglyceride in l-thyroxine-treated animals**

	Free fatty acid			Triglyceride		
	Control	Thyroxine	Difference	Control	Thyroxine	Difference
	μmoles/g wet wt			*mg/g wet wt*		
Mean	3.4	4.2	1.8	3.6	8.3	4.6
SE	0.2	0.2	0.1	0.3	0.6	0.7
p			<0.01			<0.01

* Each group contained six animals.

Myocardial carnitine and palmityl CoA-carnitine acyltransferase. The hearts of the thyroxine-treated guinea pigs contained higher levels of both free and acylcarnitines (Table VI), and myocardial homogenates of the treated animals had increased levels of palmityl CoA-carnitine acyltransferase activity (Table VII).

TABLE V

*Myocardial palmitate-1-^{14}C oxidation by animals given l-thyroxine**

	$^{14}CO_2$		
	Control	Thyroxine	Difference
	μmoles/g protein/30 minutes		
	8.4	9.6	1.2
	6.3	14.2	7.9
	7.8	10.8	3.0
	6.0	15.7	9.7
	8.3	11.6	3.3
	8.8	17.2	8.4
	7.6	12.3	4.7
	10.2	13.8	3.6
Mean	7.9	13.2	5.2
SE	0.5	0.9	1.1
p			<0.01

* Each reaction mixture contained from 12 to 16 mg of guinea pig heart homogenate protein in 1 ml of calcium-free Krebs-Ringer phosphate buffer, pH 7.4, and palmitate-1-^{14}C, 100 mμmoles (100,000 cpm). Final reaction volume was 1.2 ml. Incubations were at 35° for 30 minutes.

Glucose metabolism. The rate of oxidation of glucose-U-^{14}C by the myocardium of the treated animals was significantly lower than that of the controls (Table VIII). This occurred in spite of lower concentrations of myocardial glycogen in the thyroxine-treated animals, which might have raised the specific activity of the oxidizable pool of glucose in the cardiac homogenates. To minimize this effect, the amount of radioactive glucose added was a hundredfold greater than the endogenous levels.

To investigate the basis of the lower rate of glucose-U-^{14}C conversion to $^{14}CO_2$ in the treated animals, the pathways of cardiac glucose metabolism were studied. As shown in Table IX, cardiac homogenates from the treated animals formed significantly more glucose 6-phosphate plus fructose 6-phosphate, and less lactate from glucose than did

TABLE VI

Effect of l-thyroxine on concentration of myocardial carnitine

Animal	Free carnitine			Bound carnitine			Total carnitine		
	Control	Thyroxine	Difference	Control	Thyroxine	Difference	Control	Thyroxine	Difference
				μmoles/g wet wt					
1	2.03	2.26	0.23	0.69	0.93	0.24	2.72	3.19	0.47
2	2.07	2.09	0.02	0.61	0.85	0.24	2.68	2.94	0.26
3	1.86	2.18	0.32	0.51	1.20	0.69	2.31	3.28	0.97
4	1.76	2.74	0.98	0.44	1.16	0.72	2.20	3.90	1.70
5	2.10	2.88	0.78	0.48	1.23	0.75	2.58	4.11	1.53
Mean	1.96	2.43	0.46	0.55	1.07	0.52	2.50	4.38	0.98
SE	0.07	0.16	0.18	0.04	0.08	0.12	0.10	0.22	0.28
p			$<0.05 > 0.025$			<0.01			$<0.025 > 0.01$

those from control animals. In addition, thyroxine-treated animals incorporated greater amounts of radioactive glucose into cardiac glycogen than did control animals (Table X). These magnitudes of differences in incorporation of radioactive glucose into cardiac glycogen were found at 30 minutes and 1 hour. Although the glycogen concentration in the treated animals was less than that of the controls (Table X), the total glycogen content of the heart was the same in both treated and control groups because of the greater cardiac mass in the treated group (Table I).

Pyruvate oxidation. The rate of myocardial oxidation of pyruvate-3-^{14}C was significantly higher in the treated animals than in the controls (Table XI). The endogenous pools of pyruvate were determined before addition of radioactive substrate to the cardiac homogenate. The amount of radioactive pyruvate used in the incubations (10 μmoles) was over a hundredfold that of the levels of endogenous pyruvate. Therefore, no corrections were made for differences in specific activities.

Myocardial citrate. The concentration of citrate was higher in hearts of treated animals, as shown in Table XII.

TABLE VII

*Myocardial palmityl coenzyme A-carnitine acyltransferase activity**

	Palmitylcarnitine-^{14}C		
	Control	Thyroxine	Difference
	μmoles/g protein		
	0.17	0.42	0.25
	0.22	0.51	0.29
	0.25	0.60	0.35
	0.19	0.49	0.30
	0.23	0.58	0.35
Mean	0.21	0.52	0.31
SE	0.01	0.03	0.01
p			<0.01

* Each reaction mixture contained from 5 to 7 mg of guinea pig heart homogenate protein; 25 μmoles Tris·HCl buffer, pH 7.4; 5 μmoles reduced glutathione; 5 μmoles ATP; 0.2 μmole CoA; 0.2 μmole (−)-carnitine; and 0.5 μmole palmitate-1-^{14}C (200,000 cpm). Final reaction volume was 1.2 ml. Incubations were at 25° for 10 minutes.

Discussion

The metabolism of the guinea pig heart hypertrophied by thyroxine treatment was characterized by alterations in lipid and carbohydrate metabolism. The lipid changes included higher concentrations of FFA and triglyceride, augmented rates of long chain fatty acid oxidation, elevated levels of bound and free carnitine, and increased palmityl CoA-carnitine acyltransferase activity. The carbohydrate changes found were decreased rates of

TABLE VIII

*Myocardial glucose-U-^{14}C oxidation by animals given l-thyroxine**

	$^{14}CO_2$		
	Control	Thyroxine	Difference
	μmoles/g protein/30 minutes		
	7.7	3.3	4.4
	11.8	2.7	9.1
	8.6	5.4	3.2
	17.8	5.1	12.7
	16.4	4.7	11.7
	12.9	2.7	10.2
	15.2	3.5	11.7
	10.8	8.8	2.0
Mean	12.7	4.6	8.1
SE	1.3	0.7	1.5
p			<0.01

* Each reaction mixture contained from 12 to 16 mg of guinea pig heart homogenate protein in 1 ml of calcium-free Krebs-Ringer phosphate buffer, pH 7.4, and glucose-U-^{14}C, 10 μmoles (190,000 cpm). Final reaction volume was 1.2 ml. Incubations were at 35° for 30 minutes.

TABLE IX

*Effect of l-thyroxine on myocardial concentrations of hexosemonophosphates and lactate**

	Hexosemonophosphates			Lactate		
	Control	Thyroxine	Difference	Control	Thyroxine	Difference
			μmoles/g protein/30 minutes			
	1.54	2.36	0.82	6.6	4.8	1.8
	1.68	2.73	1.05	5.8	3.7	2.1
	1.44	2.94	1.50	7.7	5.2	2.5
	1.76	2.54	0.78	5.7	3.7	2.0
				8.3	3.9	4.4
Mean	1.61	2.46	1.03	6.8	4.3	2.6
SE	0.07	0.13	0.17	0.5	0.3	0.5
p			<0.01			<0.01

* Each reaction mixture contained from 10 to 12 mg of guinea pig heart homogenate protein in 1 ml of calcium-free Krebs-Ringer phosphate buffer, pH 7.4, and 50 μmoles of glucose. Final reaction volumes were 1.2 ml. Incubations were at 35° for 30 minutes. Hexosemonophosphate and lactate concentrations were determined as indicated in Methods.

glucose conversion to both lactate and CO_2, increased rates of conversion of pyruvate to CO_2 and of glucose to glycogen, and elevated rates of hexosemonophosphate formation from glucose.

Lipid metabolism. The stimulatory effect of carnitine on long chain fatty acid oxidation has been attributed to the formation of long chain fatty acylcarnitines, which, in contrast to the acyl CoA, can permeate to the mitochondrial sites of fatty acid oxidation (30). Acylcarnitine formation from acyl CoA is catalyzed by the long chain fatty acyl CoA-carnitine acyltransferase, most of which is localized in the mitochondrial fraction (31). Shepherd, Yates, and Garland have shown that this is the rate-limiting enzyme in long chain fatty acid oxidation (32). Norum has suggested that increased levels of plasma FFA and enhanced rates of FFA uptake by tissues may play a key role in the level of palmityl CoA-carnitine acyltransferase activity (31). Increased activity of this rate-limiting enzyme has been found in mitochondria from the livers of fat-fed, starved, and alloxan diabetic rats (31). These states of increased palmityl CoA-carnitine acyltransferase activity are known to be associated with elevated plasma FFA levels, elevated levels of tissue lipids, and increased concentrations of long chain fatty acyl CoA in tissues (31, 33). In addition, increased levels of both free and acylcarnitines have been found in the tissues of cold-acclimated rats, which have increased rates of fatty acid oxidation (34). The thyroxine-treated guinea pig heart exhibits the same changes in fatty acid metabolism as the aforementioned states of augmented lipid utilization. The increased availability of FFA in the thyroxine-treated animals, which results from the increased mobilization from adipose tissue, may be the means by which thyroxine exerts its stimulatory influence on long chain fatty acid oxidation in the heart. This is consonant with the thesis that

TABLE X

*Glycogen metabolism in hearts of animals treated with l-thyroxine**

	Glycogen			Glycogen-^{14}C		
	Control	Thyroxine	Difference	Control	Thyroxine	Difference
	mg/g wet wt			*cpm/mg glycogen*		
	7.0	5.4	1.6	933	2,100	1,167
	6.4	4.7	1.7	744	3,650	2,906
	7.6	5.7	1.9	638	2,470	1,832
	5.8	4.6	1.2	858	3,350	2,492
	7.8	4.9	2.9	842	2,950	2,108
Mean	6.9	5.1	1.8	803	2,904	2,101
SE	0.4	0.2	0.4	51	282	294
p			<0.01			<0.01

* Fifty μmoles of glucose-U-^{14}C (5×10^6 cpm) was injected intraperitoneally, and the animals were sacrificed 1 hour later. Glycogen was isolated and the radioactivity determined as described in Methods.

TABLE XI

*Myocardial pyruvate-3-^{14}C oxidation by animals given l-thyroxine**

	$^{14}CO_2$		
	Control	Thyroxine	Difference
	μmoles/g protein/30 minutes		
	20.9	42.9	22.0
	33.6	47.1	13.5
	36.5	43.2	6.7
	23.7	29.2	5.5
	19.3	48.7	29.4
	24.2	41.2	17.1
Mean	26.4	42.1	15.7
SE	2.9	2.8	3.7
p			<0.01

* Each reaction mixture contained from 12 to 16 mg of guinea pig heart homogenate protein in 1 ml calcium-free Krebs-Ringer phosphate buffer, pH 7.4, and pyruvate-3-^{14}C, 10 μmoles (200,000 cpm). Final reaction volume was 1.2 ml. Incubations were at 35° for 30 minutes.

TABLE XII

Myocardial concentration of citrate in animals treated with l-thyroxine

	Citrate		
	Control	Thyroxine	Difference
	μg/g wet wt		
	29	69	40
	36	63	27
	18	76	58
	20	58	38
	33	48	15
Mean	27	63	36
SE	4	5	7
p			<0.01

the rate of long chain fatty acid oxidation in tissues is regulated by the availability of fatty acids (35).

Carbohydrate metabolism. In 1942 Cori presented evidence that gave first indication of a regulatory role for phosphofructokinase in glycolysis (36). Lardy and Parks later ascertained that ATP inhibited a PFK preparation from rabbit muscle, and they postulated a regulatory feedback between the energy state of the cell and glycolysis (37). Studies on glycolysis in liver flukes, heart, and diaphragm showed that glycolysis was stimulated when ATP levels decreased or when inorganic phosphate, AMP, or ADP levels increased (38–40). These stimulated states of glycolysis were associated with increased levels of PFK activity.

The possibility that other factors were involved in the regulation was investigated by Newsholme, Randle, and Manchester, who found that fatty acids depressed PFK in the isolated diaphragm and the perfused rat heart (41), and by Parmeggiani and Bowman, who ascertained that the fatty acid inhibition of PFK activity and glycolysis was due to increased levels of tissue citrate, which were derived from the metabolism of fatty acid (4). It was found that in states of augmented lipid utilization such as fasting or diabetes, elevated plasma FFA were associated with increased concentrations of myocardial citrate and decreased PFK activity (4, 5, 41, 42). The extensive studies of Randle and co-workers have suggested that the uptake of glucose and its conversion to lactate and CO_2 by the heart are under the regulatory influence of fatty acids, which act via conversion to citrate, to inhibit PFK (5, 41–43).

The decreased conversion of glucose to CO_2 and lactate and the elevated levels of tissue citrate in the thyroxine-treated guinea pig heart are consistent with a decrease in PFK activity. Inhibition of PFK could contribute to the increased concentrations of hexosemonophosphates found in the treated hearts, and an elevation of glucose 6-phosphate would help to explain the *in vivo* increase in conversion of glucose to glycogen (Table X). Glucose 6-phosphate can promote glycogen synthesis by acting as both a cofactor and a precursor (44). Glucose 6-phosphate has been shown to inhibit mammalian hexokinases, and the elevated levels in the thyroxine-treated hearts may play a role in the decreased glucose uptake (45). That a defect beyond glycolysis could not be implicated in the decreased rate of glucose oxidation was indicated by the nondepressed rate of pyruvate oxidation in the treated guinea pig. Thus, in contrast to the enhanced rate of long chain fatty acid oxidation, the rate of glucose oxidation in the animals treated with thyroxine was depressed, and the basis of the depression could be an inhibition of PFK activity produced by the elevated concentrations of myocardial citrate.

Summary

Myocardial homogenates from guinea pigs treated with thyroxine for 10 days were found to have increased rates of palmitate oxidation and decreased rates of glucose oxidation. There were increased levels of myocardial lipids, of free and

bound carnitine, and of palmityl coenzyme A-carnitine acyltransferase activity associated with the augmentation of palmitate oxidation. The depressed glucose oxidation was associated with increased rates of glycogen and hexosemonophosphate formation and elevated tissue citrate levels. The possible regulatory role of the increased rate of fatty acid oxidation in the control of glucose metabolism is discussed.

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