Mechanochemistry of Cardiac Muscle

V. INFLUENCE OF THYROID STATE ON ENERGY UTILIZATION

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A B S T R A C T The possibility that alterations in the rate or efficiency of energy utilization could be involved in the control of cellular oxygen consumption by thyroid hormone was examined in right ventricular papillary muscles isolated from normal euthyroid cats and cats with experimentally induced hyperthyroidism and hypothyroidism. Energy production in the muscles was inhibited and isolated from the process of energy utilization by exposure to iodoacetic acid and nitrogen. After resting or performing variable amounts of contractile element work under isometric conditions, muscles were frozen, and the total amount of chemical energy ($\sim$ P = creatine phosphate + ATP) used was determined. The resting rate of energy utilization in muscles from euthyroid animals was 0.78 ± 0.07 μmoles/g per min of $\sim$ P. This rate was elevated in muscles from hyperthyroid cats to 1.00 ± 0.09 μmoles/g per min and decreased in muscles from hypothyroid cats to 0.23 ± 0.14 μmoles/g per min. Isometrically contracting muscles from cats with hypothyroidism utilized only 64% as much energy as muscles from euthyroid cats while performing 81% as much contractile element work at a moderately decreased level of contractile state. Muscles from hyperthyroid cats utilized an average of 41% more energy than did muscles from euthyroid cats while contracting an identical number of times and performing an equal amount of contractile element work at a slightly increased level of contractile state. These results suggest that thyroid hormone directly influences the rate of cellular energy utilization. Furthermore, the increase in energy utilization in muscles from hyperthyroid cats could not be attributed entirely to observed alterations in contractile behavior, which indicates that excess thyroid hormone may decrease the efficiency of the conversion of cellular energy to work. However, the opposite effect, an increased efficiency of energy utilization, was not observed in muscles from hypothyroid cats. Thus, it is concluded that the calorigenic effects of thyroid hormone may be explained, at least in part, by alterations in the process of energy utilization.

INTRODUCTION

Thyroid hormone has many effects on cellular function, the most striking of which is its influence on oxygen consumption or energy metabolism. The stimulation of oxygen consumption found in most tissues after the administration of thyroid hormone has been attributed to changes in the rate of energy generation, the efficiency of energy generation, or a combination of these (1). The chief features of clinical and experimental hyperthyroidism and hypothyroidism have been postulated to result from changes in the process of energy generation (2), shown schematically in Fig. 1 as Coupling A. However, it is also possible that a change in the rate or efficiency of energy utilization, Coupling B, could be an important determinant of the alterations in energy metabolism found in hyperthyroidism and hypothyroidism. In order to determine whether alterations in energy utilization are involved in the stimulation of cellular oxygen consumption by thyroid hormone, it is
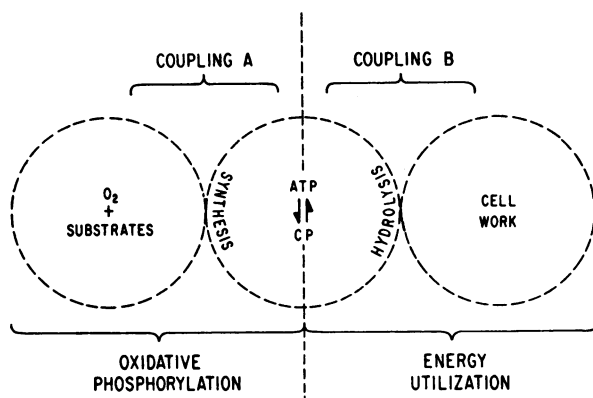
FIGURE 1 Schematic representation of those processes involved in the synthesis and degradation of high-energy phosphates (ATP + creatine phosphate) in the cell. Coupling A = processes of energy generation; Coupling B = processes of energy utilization.

necessary to measure energy transformation under conditions where energy generation has been eliminated. Cardiac muscle has been found to be a convenient tissue for this purpose (3, 4).

Adenosine triphosphate (ATP) is thought to be the immediate energy source for muscle contraction (5). Creatine phosphate is in equilibrium with ATP, and together they provide the readily available energy supply for the heart. When cardiac muscle is exposed to an appropriate concentration of iodoacetic acid, anaerobic glycolysis is inhibited. In addition, if cardiac muscle is deprived of oxygen by placing it in an atmosphere of nitrogen, oxidative phosphorylation is also inhibited, and the result of the combination of these procedures is to inhibit completely the process of energy generation. Measurement of myocardial high-energy phosphate concentrations (ATP and creatine phosphate) under conditions in which no net synthesis of ATP or creatine phosphate can occur provides a means of directly assessing the rate of energy utilization in cardiac muscle under conditions of rest or activity.

Measurement of the basal rate of energy utilization in resting cardiac muscle is analogous to measurements made in tissues where the effects of thyroid hormone have been most thoroughly studied, e.g., liver. In liver and resting cardiac muscle, there is no readily available measure of the quantity of cellular work performed, information that is essential for determining the efficiency of cellular energy utilization. On the other hand, the contraction of cardiac muscle provides a form of cellular work where the extent of the process may be determined directly and correlated with energy utilization to provide an estimate of the efficiency of cellular energy utilization (3, 4).

The purpose of the present study was to determine the influence of alterations of thyroid state on the process of energy utilization. We used a cardiac muscle preparation, the cat right ventricular papillary muscle, because both the rate and efficiency of energy utilization can be readily determined in this preparation while permitting the direct demonstration of the effects of excess thyroid hormone or its deprivation on myocardial mechanical performance.

METHODS

Experimental preparation. Studies were performed using normal adult cats, cats made hyperthyroid by the intraperitoneal injection of l-thyroxine (0.75 mg/kg per day) for 10–17 days, and cats in which hypothyroidism had been induced by a single intraperitoneal injection of 1 mCi/kg [131]I 116–160 days before study. The animals were anesthetized with intraperitoneal pentobarbital (25 mg/kg), and blood was obtained for determination of serum protein-bound iodine and cholesterol. The hearts were then removed, and right ventricular papillary muscles were rapidly excised and transferred to a myograph containing a Krebs-bicarbonate solution (pH 7.4) aerated with a mixture of 95% O_2–5% CO_2 at a constant temperature of 26°C (3). The base of each muscle was held in a spring-loaded plastic clip while the upper tendinous end was fastened to an isometric force transducer [1] by a short length of braided silk suture. Muscles were stimulated [2] directly by stainless steel wires on the inner surface of the attachment clip. Square wave impulses of 5 msec duration and a voltage of 10% above threshold were employed.

Tension and the stimulus artifact were recorded at a paper speed of 100 mm/sec on a multichannel oscillographic recorder.[3] Resting and active tension, rate of tension development, and time-to-peak tension were determined from these recordings. The maximal rate of tension development (dT/dt) measured at the peak of the length-active tension curve, L_{max}, was used as an index of myocardial contractile state for comparing muscle function in the three muscle groups. This parameter has been shown to have a direct relation to the intensity of muscle active state as determined by the quick release method (6). In order to compare the mechanical function of papillary muscles of different sizes, tension was expressed in grams per square millimeter. The cross-sectional area of each muscle was calculated by dividing its wet weight (mg) by its length (mm) assuming the muscle to be cylindrical with a density of 1.0. Mean cross-sectional area was similar in muscles obtained from euthyroid (1.39 ±0.04 mm²) and hyperthyroid cats (1.41 ±0.04 mm²) but was somewhat smaller in muscles from the hypothyroid animals (1.19 ±0.06 mm², $P < 0.05$).

Glycolysis was inhibited in all muscles by exposure to 5×10^{-4} M iodoacetic acid for 30 min. The adequacy of this blockade has been demonstrated previously (3). Inhibition of aerobic metabolism was achieved by bathing the muscles in Krebs solution previously equilibrated with 95% N_2–5% CO_2. After each experiment the muscle was rapidly frozen

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[1] Model FTA 100; Sanborn Div. of Hewlett-Packard, Waltham, Mass.
[2] Model 104A; American Electronic Laboratories, Inc., Lansdale, Pa.
[3] Model 150; Sanborn Div. of Hewlett-Packard.

by replacing the muscle bath with a beaker of 2-methyl-butane (isopentane) previously cooled in liquid nitrogen to from -150 to $-160°C$. This procedure froze the muscle without further contraction.

Experimental design. The initial portion of the experiment was similar for all muscles studied. Each muscle was stimulated to contract isometrically at a frequency of 12/min for 30 min with a resting tension of approximately 0.1 g. Its length-active tension curve was then determined, and the muscle was stimulated to contract at the apex of this curve for an additional 30 min. Stimulation was then terminated and iodoacetic acid added to the bath. After the muscle had remained in this solution for 30 min without resting tension, the bath was abruptly drained, flushed with 95% N_2–5% CO_2, and refilled with Krebs solution previously equilibrated with this gas mixture.

To determine the energy utilization of resting muscle, 86 muscles from euthyroid, 45 muscles from hyperthyroid, and 17 muscles from hypothyroid cats, left unstimulated at zero resting tension, were frozen either immediately after the change to the nitrogenated solution or after 3-, 7-, or 10-min periods of nitrogenation. Data on resting energy utilization of 76 muscles from the euthyroid cats included in this study have been reported previously (4).

The energy cost of isometric contraction was determined in 16 muscles from hypothyroid cats, 28 muscles from euthyroid animals, and 35 muscles from hyperthyroid cats. After 3 min of rest in the nitrogenated Krebs solution, muscles were stretched to that resting tension, which had been found at the apex of their previously determined length-active tension curves, and were stimulated to contract isometrically 25 times at a frequency of 30/min before freezing.

Chemical analysis. Frozen muscles were stored in liquid nitrogen. At the time of analysis each muscle was reduced to powder at $-50°C$ (7), and after addition of 0.3 M perchloric acid, was allowed to thaw and extract for 10 min at 0°C. Creatine phosphate, inorganic phosphate, ATP, and creatine contents were determined on aliquots of the supernatant following centrifugation at 25,000 g for 10 min at 0°C. Total creatine was determined by the α-naphthol-di-acetyl method (8, 9). Creatine phosphate and inorganic phosphate contents were determined by the Furchgott and de-Gubareff (10) modification of the Fiske and SubbaRow (11) technique. ATP was assayed by a modification of the firefly luminescence technique of Strehler and McElroy (12) using firefly lantern extract.[4]

To avoid possible errors from weighing small samples in the frozen state, muscle weights were calculated from the creatine content of the muscle sample using creatine concentrations measured in two separately obtained right ventricular samples from the same heart. The validity of this method was confirmed in a previous study (3) in which it was shown that the creatine concentration of papillary muscles was similar to that of adjacent right ventricular muscle. Muscles weighing less than 5 mg or more than 15 mg were discarded.

Calculations. Calculations of contractile element work (CEW), i.e. the work performed by the contractile element in stretching the series elastic component, were made from the formula CEW = f/k where f equals the summated total active tension in grams per square centimeter generated by each muscle during the experimental period, and k is the series elastic constant previously derived (13) from the relation of the modulus of elasticity of the series elastic (df/dl) to load, f: df/dl = k·f. It has been demonstrated

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

previously that the constant of the series elastic component is not altered by changes in thyroid state (14).

The basal rate of energy utilization in the resting muscles was calculated by a linear regression on time with total high-energy phosphate concentration ($\sim P = ATP +$ creatine phosphate) as the dependent variable. In addition total energy utilization ($\Delta \sim P$) was calculated for each muscle which had been contracted 25 times. Muscle content of ATP (ATPc), creatine phosphate (CPc), and creatine (creatine$_c$) was directly assessed in each muscle. It was not possible to measure directly the high-energy phosphates present in any given muscle both before and after stimulation, since one muscle could not be sampled twice. Thus, the initial high-energy phosphate contents in each active muscle were estimated from the average values obtained in muscles from the same treatment group after 3 min of rest during the period of metabolic blockade. Both initial and final high-energy phosphate contents were expressed as the quotient of

$$\frac{ATPc + CPc}{creatine_c}$$

with creatine$_c$ serving as an index of tissue extraction. The result obtained by subtracting the final quotient from the estimated initial quotient represents the utilization of ATP and creatine phosphate in each muscle in terms of directly assessed contents. This value was converted into the concentration of high-energy phosphate used ($\Delta \sim P$) by multiplying it by the creatine concentration of the adjacent right ventricle which has been shown to be similar to that found in the papillary muscle (3).

All values are expressed as the mean $\pm SE$. Statistical tests of significance were performed by the t test of the difference between group means (15). Differences between the groups were considered significant when $P < 0.05$.

RESULTS

Characterization of thyroid state. Induction of the hyperthyroid state caused an increase in the serum protein-bound iodine and a decrease in serum cholesterol. The hypothyroid cats had a decreased serum protein-bound iodine and an increased serum cholesterol (Table I). Each of these changes was statistically significant when compared with the euthyroid group. The hyperthyroid cats lost 750 ± 30 g body weight during the period of 1-thyroxine administration, whereas the hypothyroid cats gained 100 ± 50 g body weight after ^{131}I treatment. The rapid weight loss observed in the hyper-

TABLE I
Characterization of Thyroid State

	Hypothyroid	Euthyroid	Hyperthyroid
Serum PBI ($\mu g/100$ ml)	2.0 $\pm 0.3*$	5.8 ± 0.1	$>20*$
Serum cholesterol ($mg/100$ ml)	86 $\pm 7*$	68 ± 3	54 $\pm 3*$
Initial body weight (kg)	3.11 ± 0.25	2.65 ± 0.09	3.14 ± 0.20
Ventricular weight (g)	6.44 ± 0.36	6.68 ± 0.33	7.63 ± 0.30
$\dfrac{\text{Ventricular weight }(g)}{\text{Initial body weight }(kg)}$	2.07 $\pm 0.12*$	2.52 ± 0.06	2.43 ± 0.04

* $P < 0.05$ when compared with euthyroid.

thyroid animals suggests that a marked degree of hyperthyroidism was induced in these cats. A major portion of this weight loss was accounted for by loss of body fat since little subcutaneous or visceral fat was present in the hyperthyroid animals at postmortem examination. The hearts and papillary muscles from the hyperthyroid animals did not appear to be hypertrophied on gross examination. Ventricular weights were similar in the three groups. Due to the rapid loss of body weight in the hyperthyroid cats, ventricular weight was compared with initial body weight to obtain an estimate of relative ventricular size for each experimental group. When compared in this manner, the hearts from the hypothyroid cats were found to be relatively smaller than those from the other two groups (Table I) while hearts from hyperthyroid cats did not differ from normal.

Myocardial mechanics. During metabolic blockade, muscles from each group were stimulated to contract 25 times at a frequency of 30/min before freezing. There was no evidence of contracture in any of the muscles during the contraction period. Resting tension at L_{max} was similar in the three muscle groups averaging 1.2 ± 0.2, 1.1 ± 0.2, and 1.1 ± 0.1 g/mm^2 in the hypothyroid, euthyroid, and hyperthyroid groups respectively. Peak active tension and rate of tension development were attained after 15 ± 0.8 contractions in the muscles from the hyperthyroid cats, whereas the euthyroid group required 20 ± 1.0 contractions and the hypothyroid 23 ± 1.0 contractions. Once attained, peak tension and rate of tension development remained essentially unchanged in each muscle until the 25 contractions were concluded, and the muscle was frozen. Peak isometric contractile function in the three muscle groups is compared in Fig. 2. The rate of tension development (dT/dt) was

greater in muscles from hyperthyroid than euthyroid cats although the differences were not statistically significant. Time-to-peak tension and developed tension were significantly less in the hyperthyroid group. Developed tension and dT/dt were significantly less, and time-to-peak tension significantly greater in muscles from the hypothyroid group than in muscles from euthyroid animals.

Total contractile element work performed during the 25 isometric contractions in muscles from euthyroid cats (371 ± 20 g-cm/g) was similar to that in muscles from hyperthyroid cats (370 ± 25 g-cm/g) while muscles in the hypothyroid group performed significantly less work (301 ± 23 g-cm/g, $P < 0.05$).

$\Sigma dT/dt$ for the 25 contractions was averaged in each muscle group to provide an estimate of contractile state during the contraction period. $\Sigma dT/dt$ was somewhat greater in the hyperthyroid group (590 ± 35 g/mm^2 per sec, $0.10 > P > 0.05$) and significantly less in the hypothyroid cats (331 ± 41 g/mm^2 per sec, $P < 0.05$) than in the euthyroid animals (515 ± 31 g/mm^2 per sec).

Resting energy utilization. Initial high-energy phosphate stores were similar in papillary muscles from euthyroid and hyperthyroid cats (16.6 ± 0.7 vs. 16.4 ± 0.6 μmoles/g), whereas stores in muscles from hypothyroid cats were somewhat lower (14.2 ± 0.6 μmoles/g, $0.10 > P > 0.05$). A regression analysis of total energy stores with time (Fig. 3) indicated that the resting rate of energy utilization tended to be elevated in muscles from hyperthyroid animals (1.00 ± 0.09 μmoles/g per min) compared with the rate in muscles from euthyroid animals (0.78 ± 0.07 μmoles/g per min, $0.10 > P > 0.05$), whereas the rate was significantly and
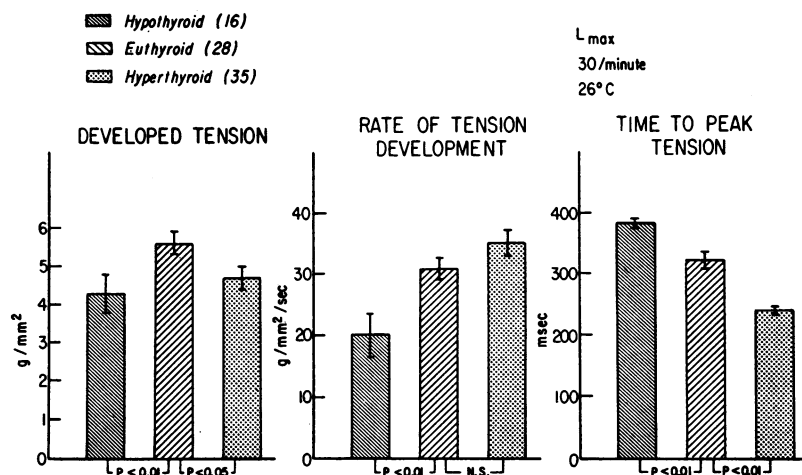

FIGURE 2 Isometric contractile function analyzed as a function of level of thyroid state. Measurements made at contraction frequency of 30/min at apex of length-active tension curve, L_{max}, during period of metabolic blockade. NS = $P > 0.05$.

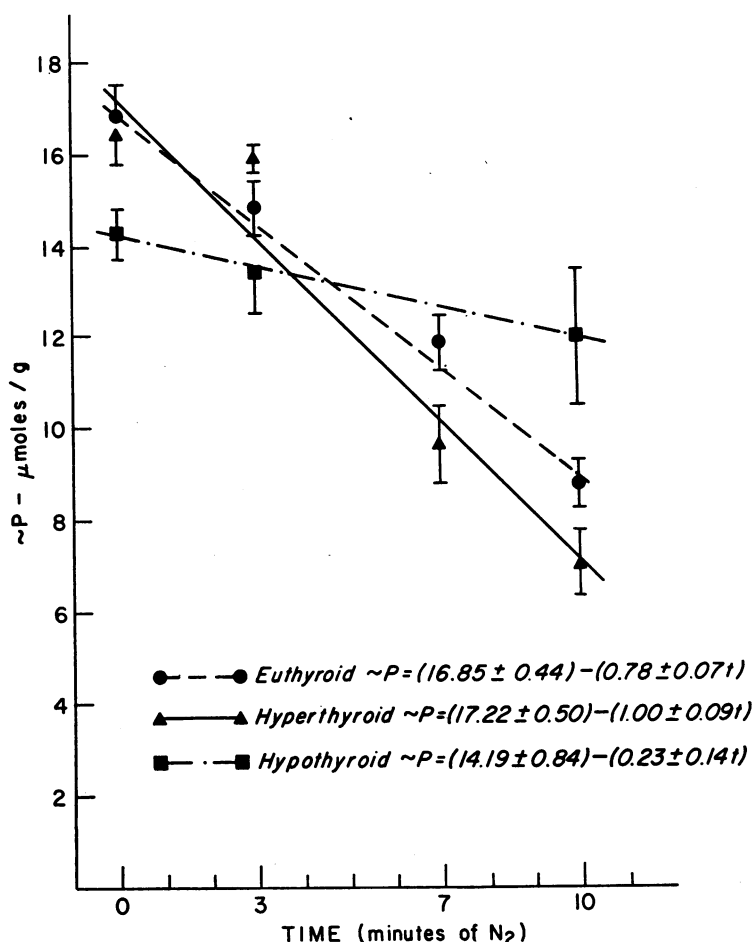*C. L. Skelton, P. E. Pool, S. C. Seagren, and E. Braunwald*

FIGURE 3 Resting energy utilization in papillary muscles from euthyroid, hyperthyroid, and hypothyroid cats as determined by regression analysis. $\sim$ P = sum of creatine phosphate and ATP. t = time in minutes of nitrogenation. Mean $\sim$ P $\pm$ SE is plotted at 0, 3, 7, and 10 min of nitrogenation.

markedly depressed in muscles from hypothyroid cats (0.23 ±0.14 μmoles/g per min, $P < 0.01$).

Energy utilization of isometric contractions. The measured content of ATP, creatine phosphate, and creatine in each muscle frozen after 25 contractions is shown in Table II. The initial high-energy phosphate content in each active muscle was estimated from the average value found in muscles from the same experimental group frozen after 3 min of rest. The total energy utilized in the active muscles was then determined by subtracting the final high-energy phosphate stores from the estimated initial stores. This average total energy utilization was 2.36 ±0.56, 4.07 ±0.33, and 5.64 ±0.34 μmoles/g, respectively, in muscles from hypothyroid, euthyroid, and hyperthyroid animals. These values for the hypothyroid and hyperthyroid groups differed significantly from that of the euthyroid cats ($P < 0.01$).

The average amount of energy used per muscle for activation and the performance of work was obtained by subtracting resting energy utilization during the 50 sec contraction period from the total energy used (Fig. 4). Average active energy utilization was greater in the hyperthyroid group (4.81 μmoles/g) and less in the hypothyroid muscles (2.18 μmoles/g) as compared with the euthyroid group (3.42 μmoles/g).

Comparative average values for muscle mechanics and active energy utilization are depicted in Table III. Muscles from hypothyroid animals utilized an average of only 64% as much energy as muscles from euthyroid cats while performing 81% as much work at a moderately decreased level of contractile state. Muscles from hyperthyroid cats utilized an average of 41% more energy than did muscles from euthyroid cats while per-

TABLE II

Summary of Biochemical Data

Experiment no.	ATP content	CP content	Creatine content	$\dfrac{ATPc + CPc}{Creatine_0}$	$\dfrac{\Delta \sim Pc}{Creatine_0}$	Creatine concentration	Total $\Delta \sim P$
	μmoles	μmoles	μmoles			μmoles/g	μmoles/g
Hypothyroid							
1	0.073	0.127	0.219	0.913	0.091	16.0	1.46
2	0.034	0.076	0.152	0.724	0.280	20.9	5.85
3	0.025	0.035	0.090	0.667	0.337	16.9	4.99
4	0.062	0.065	0.142	0.894	0.110	12.3	1.35
5	0.051	0.068	0.144	0.826	0.178	16.9	3.01
6	0.075	0.126	0.260	0.773	0.231	16.8	3.88
7	0.037	0.066	0.127	0.811	0.193	15.9	3.07
8	0.045	0.083	0.174	0.736	0.268	15.7	4.21
9	0.027	0.078	0.139	0.755	0.249	16.0	3.98
10	0.025	0.052	0.099	0.778	0.226	15.4	3.48
11	0.033	0.051	0.092	1.022	−0.018	13.2	−0.24
12	0.044	0.063	0.110	0.973	0.031	14.8	0.46
13	0.040	0.048	0.106	0.830	0.174	16.3	2.84
14	0.030	0.064	0.089	1.056	−0.052	15.2	−0.79
15	0.039	0.070	0.100	1.090	−0.086	9.5	−0.82
16	0.028	0.038	0.068	0.971	0.033	12.0	0.40
Mean	0.042*	0.069	0.132	0.864	0.140‡	15.2	2.36‡
SE	0.004	0.007	0.013	0.265	0.033	0.7	0.56
Euthyroid							
1	0.051	0.067	0.162	0.728	0.300	17.0	5.10
2	0.040	0.027	0.085	0.788	0.240	15.2	3.65
3	0.039	0.036	0.099	0.758	0.270	17.3	4.67
4	0.053	0.070	0.147	0.837	0.191	16.0	3.06
5	0.041	0.059	0.103	0.971	0.057	14.4	0.82
6	0.048	0.067	0.149	0.772	0.256	19.9	5.09
7	0.078	0.112	0.242	0.785	0.243	18.8	4.57
8	0.041	0.041	0.100	0.820	0.208	19.4	4.04
9	0.039	0.043	0.101	0.812	0.216	12.0	2.59
10	0.033	0.036	0.088	0.784	0.244	16.7	4.07
11	0.050	0.055	0.130	0.808	0.220	15.4	3.39
12	0.054	0.090	0.166	0.867	0.161	13.7	2.21
13	0.041	0.033	0.090	0.822	0.206	12.8	2.64
14	0.063	0.060	0.150	0.820	0.208	11.2	2.33
15	0.034	0.040	0.099	0.747	0.281	16.5	4.64
16	0.045	0.054	0.138	0.717	0.311	16.4	5.10
17	0.050	0.073	0.176	0.699	0.329	19.8	6.51
18	0.047	0.047	0.110	0.855	0.173	18.0	3.11
19	0.068	0.065	0.158	0.842	0.186	17.2	3.20
20	0.084	0.107	0.278	0.687	0.341	20.0	6.82
21	0.058	0.083	0.188	0.750	0.278	17.9	4.98
22	0.044	0.062	0.118	0.898	0.130	14.2	1.85
23	0.074	0.141	0.309	0.696	0.332	22.1	7.34
24	0.033	0.051	0.108	0.778	0.250	15.4	3.85
25	0.066	0.067	0.162	0.821	0.207	15.8	3.27
26	0.041	0.052	0.155	0.600	0.428	19.8	8.47
27	0.048	0.065	0.141	0.801	0.227	15.8	3.59
28	0.059	0.058	0.142	0.824	0.204	14.6	2.98
Mean	0.051	0.063	0.146	0.789	0.239	16.5	4.07
SE	0.002	0.005	0.011	0.014	0.014	0.5	0.33

TABLE II—(*Continued*)

Experiment no.	ATP content	CP content	Creatine content	$\dfrac{\text{ATPc} + \text{CPc}}{\text{Creatine}_0}$	$\dfrac{\Delta \sim \text{Pc}}{\text{Creatine}_0}$	Creatine concentration	Total $\Delta \sim \text{P}$
	μmoles	*μmoles*	*μmoles*			*μmoles/g*	*μmoles/g*
Hyperthyroid							
1	0.058	0.062	0.140	0.857	0.290	14.2	4.12
2	0.071	0.080	0.195	0.774	0.373	16.5	6.15
3	0.055	0.030	0.112	0.759	0.388	13.8	5.35
4	0.055	0.055	0.151	0.728	0.419	13.4	5.61
5	0.046	0.049	0.104	0.913	0.234	13.0	3.04
6	0.058	0.062	0.160	0.750	0.397	14.4	5.72
7	0.051	0.097	0.163	0.908	0.239	14.2	3.39
8	0.045	0.031	0.098	0.776	0.371	15.1	5.60
9	0.077	0.075	0.187	0.813	0.334	12.8	4.28
10	0.045	0.029	0.124	0.597	0.550	15.8	8.69
11	0.047	0.042	0.109	0.817	0.330	13.2	4.36
12	0.059	0.063	0.146	0.836	0.311	12.2	3.79
13	0.052	0.070	0.153	0.797	0.350	14.4	5.04
14	0.046	0.027	0.091	0.802	0.345	11.4	3.93
15	0.038	0.033	0.077	0.922	0.225	11.8	2.66
16	0.042	0.042	0.114	0.737	0.410	14.5	5.95
17	0.030	0.008	0.095	0.400	0.747	14.8	11.06
18	0.065	0.063	0.168	0.762	0.385	12.9	4.97
19	0.040	0.017	0.110	0.518	0.629	15.0	9.44
20	0.052	0.030	0.113	0.726	0.421	13.4	5.64
21	0.094	0.152	0.330	0.745	0.402	23.9	9.61
22	0.033	0.025	0.079	0.734	0.413	14.6	6.03
23	0.050	0.028	0.113	0.690	0.457	14.6	6.67
24	0.082	0.091	0.190	0.911	0.236	16.4	3.87
25	0.054	0.068	0.150	0.813	0.334	17.4	5.81
26	0.068	0.068	0.171	0.795	0.352	16.4	5.77
27	0.069	0.060	0.183	0.705	0.442	12.4	5.48
28	0.056	0.048	0.171	0.608	0.539	12.5	6.74
29	0.049	0.043	0.106	0.868	0.279	14.3	3.99
30	0.033	0.040	0.096	0.760	0.387	14.3	5.53
31	0.051	0.041	0.154	0.597	0.550	15.1	8.31
32	0.035	0.030	0.077	0.844	0.303	13.9	4.21
33	0.032	0.037	0.075	0.920	0.227	13.7	3.11
34	0.047	0.038	0.134	0.634	0.513	14.9	7.64
35	0.040	0.020	0.088	0.682	0.465	12.8	5.95
Mean	0.052	0.050*	0.135	0.757	0.390‡	14.4‡	5.64‡
SE	0.003	0.005	0.008	0.020	0.020	0.4	0.34

The change in the ratio of total high-energy phosphate content to creatine content ($\Delta \sim \text{Pc/creatine}_0$) which occurred during the series of isometric contractions was determined for each muscle by subtracting the quotient of the assessed contents $\dfrac{\text{ATPc} + \text{CPc}}{\text{creatine}_0}$ found at the end of the experiment from the average assessed value of this quotient for each group at the beginning of the isometric contraction phase of the experiment (1.004, 1.028, and 1.147 for the hypothyroid, euthyroid, and hyperthyroid groups, respectively). High-energy phosphate utilization ($\Delta \sim \text{P}$) was then determined by multiplying $\Delta \sim \text{Pc/creatine}_0$ by the creatine concentration of the adjacent right ventricle. CP = creatine phosphate.

* $P < 0.05$ when compared with euthyroid.
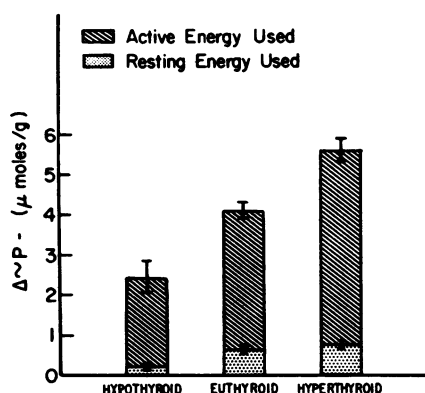‡ $P < 0.01$ when compared with euthyroid.

FIGURE 4 Total energy utilization in papillary muscles from euthyroid, hypothyroid, and hyperthyroid cats during isometric contraction. Total energy utilization = sum of active energy used and resting energy used. $\Delta \sim P$ = utilization of creatine phosphate and ATP.

forming an equal amount of contractile element work at a slightly increased level of contractile state.

DISCUSSION

The purpose of the present study was to determine whether or not the rate or efficiency of energy utilization is changed in cardiac muscle by alterations in thyroid state. It was observed that the resting rate of energy utilization was increased in muscles from hyperthyroid cats and decreased in those from hypothyroid cats. Under conditions where the frequency and number of isometric contractions were identical, muscles from hyperthyroid cats utilized an average of 41% more chemical energy than did muscles from euthyroid animals, whereas muscles from hypothyroid cats used 36% less. These results were obtained under conditions which measured only energy utilization. Therefore, changes in intermediary metabolism affecting energy generation and conservation, such as uncoupling of oxidative phosphorylation, could not be responsible for the demonstrated changes in energy utilization.

Myocardial energy requirements during isometric contraction are largely determined by the mechanical behavior of the muscle. Tension development and contractile state have been identified as the most important determinants of myocardial energy needs (16, 17). Contractile element work, a linear function of active tension, was nearly identical in the muscles from the hyperthyroid and euthyroid cats, whereas dT/dt, an estimate of myocardial contractile state, was 14% greater in the muscles from the hyperthyroid group. This increase in dT/dt in hyperthyroidism probably contributed to the increased energy requirements of muscles in the hyperthyroid group. In a previous study attempting to define the relative role of contractile state in determining myocardial oxygen consumption, it was found that a 43% increase in contractile state, measured as V_{max}, was associated with a 50% increase in oxygen consumption (18). Thus, it seems unlikely that the small increase in myocardial contractile state noted in hyperthyroidism in the present study could entirely account for the large increase in energy utilization measured in this condition. Rather, our results suggest that the increased energy utilization of contracting cardiac muscle from hyperthyroid cats is attributable to alterations in the conversion of chemical energy to mechanical work, i.e., an inefficient process of energy utilization.

It is possible that alterations of cellular energy utilizing processes in hyperthyroidism not directly related to muscle contraction could account for a portion of the increase in high-energy phosphates used during contraction. Thus, it is possible that an increased amount of energy is required in the hyperthyroid hearts for the process of activating muscle contraction. The design of the present study, however, precluded any attempt to estimate activation-related energy utilization in these muscles. Wang and Benmiloud recently reported that hearts from hyperthyroid rats had an increased adenosine triphosphatase activity in cellular fractions containing mitochondria (19). Similar results have been reported from human skeletal muscle (20). These find-

TABLE III

Comparative Average Values for Muscles from Euthyroid Cats and Cats with Experimental Hypothyroidism and Hyperthyroidism

	Hypothyroid	Euthyroid	Hyperthyroid	$\dfrac{\text{Hypothyroid}}{\text{Euthyroid}} \times 100$	$\dfrac{\text{Hyperthyroid}}{\text{Euthyroid}} \times 100$
No. of contractions	25	25	25	100%	100%
Contractile element work (*g-cm/g*)	310	371	370	81%	100%
$\Sigma dT/dt$ (*g/mm² per sec*)	331	515	590	64%	114%
Active $\sim P$ used (*μmoles/g*)	2.18	3.42	4.81	64%	141%

ings are consistent with the alterations in resting rate of energy utilization found in the muscles from hyperthyroid animals in the present study. However, the increase in the resting rate of energy utilization in hyperthyroid muscles accounts for only 11% of the increase in total energy utilization observed in these muscles during contraction. Thus, while a portion of the increase in myocardial energy utilization found in hyperthyroidism may be attributed to nonspecific processes occurring continually, the major portion of this increase in energy utilization probably occurs in association with the contractile process.

In contrast to the findings in hyperthyroidism, contracting muscles from hypothyroid cats utilized only 64% as much chemical energy as those from euthyroid cats while performing 81% as much contractile element work at a contractile state level reduced by 36% as estimated by dT/dt. Although quantification of their individual contributions is not possible, the magnitude of these decreases in contractile element work and contractile state appears sufficient to explain the decreased energy utilization observed in muscles from hypothyroid cats.

Previous attempts to define myocardial efficiency in hyperthyroidism have given conflicting results (21-23). In some instances myocardial efficiency has been thought to be increased (22, 23). However, alterations of myocardial efficiency calculated on the basis of minute work output of the heart may be difficult to interpret, especially in states in which the level of thyroid state is altered causing marked changes in cardiac output. Evans and Matsuoka (24) have demonstrated that an increase in cardiac work resulting primarily from an increase in cardiac output was associated with a much smaller increase in oxygen consumption than an increase in cardiac work resulting from increasing aortic pressure. These findings have been repeatedly confirmed and were recently extended to the isolated process of energy utilization (4). Thus, increases in myocardial oxygen consumption in hyperthyroidism which are proportional to the increase in minute work may actually reflect an inefficient process of energy utilization, since an increase in cardiac output in the absence of hyperthyroidism should result in a less than proportional increase in oxygen consumption. More recently, the oxygen consumption of papillary muscles from hyperthyroid cats was found to be greater than that of muscles from euthyroid cats (25). Although consistent with the hypothesis that thyroid hormone reduces the efficiency of energy utilization, these results were obtained under conditions which did not exclude possible alterations in the process of energy generation.

Attempts to characterize myocardial energetics in hypothyroidism have also given conflicting results. Goh

and Dallam (26) reported that nonworking ventricular preparations from hypothyroid rats had a decreased oxygen consumption. Myocardial oxygen consumption was found to be decreased in hypothyroid dogs (27, 28) but myocardial efficiency was also reported to be decreased (28). In one patient with hypothyroidism secondary to thyroiditis, a normal myocardial oxygen consumption was found (29). The interpretation of these results is limited, however, because none of these studies were performed under conditions allowing adequate control or measurement of the important mechanical determinants of myocardial energy requirements.

In a previous study Buccino, Spann, Pool, Sonnenblick, and Braunwald (30) demonstrated that hyperthyroidism was associated with a shift in the force-velocity curve upward and to the right and an increase in the maximal rate of isometric tension development (dT/dt) in feline papillary muscles stimulated to contract 12 times per minute, whereas opposite changes were associated with hypothyroidism. From these data it was concluded that myocardial contractile state was increased by hyperthyroidism and decreased by hypothyroidism. However, as with most inotropic influences (31), the augmentation of contractile state in hyperthyroidism was rate dependent. At stimulation frequencies of 30 and 48 per min, no significant differences in dT/dt were observed in the muscles from the euthyroid and hyperthyroid groups. The rate dependence of the increased contractility in hyperthyroidism was confirmed by Murayama and Goodkind using atria isolated from hyperthyroid guinea pigs (32). The explanation for the rate dependency of inotropic influences is not known but may be related to an upper mechanical limit on the contractile response (31, 33). In the present study a stimulation frequency of 30/min was purposely selected because this frequency reduced the differences in contractile state between muscles from hypothyroid, euthyroid, and hyperthyroid animals. Under these conditions a direct effect of thyroid hormone on energy utilization could be more readily identified.

The calorigenic action of thyroid hormone could be the result of a fundamental action at several levels of metabolism. *First,* it could involve the process of energy production and result from an uncoupling of oxidative phosphorylation. Many investigations have been devoted to this aspect of energy metabolism and have yielded varying results. At the present time it appears that uncoupling of oxidative phosphorylation is not produced in vivo with physiologic doses of thyroid hormone (34). In the present investigation in order to focus on energy utilization, the process of energy production was specifically inhibited, and therefore no information relative to energy production is available. *Second,* abnormally increased energy utilization could be responsible

for the calorigenic action of thyroid hormone. This increase could involve accelerated basal processes, perhaps not directly related to processes of cellular work, e.g. increased activity of mitochondrial ATPase (19, 20). Some support for this concept was found in the present study in the changes in basal energy utilization of resting heart muscle produced by alterations of thyroid state. *Finally,* it is also possible that the calorigenic action of thyroid hormone results from a decrease in the efficiency of energy utilization as the level of thyroid state is increased so that the amount of energy used in hyperthyroidism is in excess of that required of euthyroid tissues for performing identical levels of cellular work. The results obtained in contracting muscles from hyperthyroid animals in the present study, support this latter hypothesis. Whereas its calorigenic effect is one of the most characteristic actions of thyroid hormone, it also influences growth and maturation, water and salt metabolism, protein and lipid synthesis and breakdown, bone metabolism, and skeletal muscle function (2). Whether or not the calorigenic effect of thyroid hormone is a fundamental one on which all of the other actions depend is not known. Thus, the possible contribution of inefficient energy utilization to these other actions of thyroid hormone cannot be directly assessed.

The fundamental mechanism by which thyroid hormone alters the utilization of energy by the heart remains to be elucidated. Recent studies of the basic mechanism of action of thyroid hormone have stressed the importance of its effect on protein synthesis (35, 36), and it has been postulated that thyroid hormone exerts its characteristic effects by a selective control of the synthesis of enzymes or structural proteins. Applying this concept to heart muscle, it seems possible that thyroid hormone could directly alter the actin-myosin linkages or myosin adenosine triphosphatase in such a way as to control the efficiency of energy utilization. It is also possible that these effects could result from other changes in the contractile proteins or the sarcoplasmic reticulum.

In summary, this investigation has provided evidence that thyroid hormone exerts a profound direct influence on the process of myocardial energy utilization. Both the basal rate of cardiac energy utilization and energy utilization during isometric contraction are increased in hyperthyroidism and decreased in hypothyroidism. The decreased energy utilization in contracting muscles from hypothyroid animals may be largely attributed to decreases in tension generation and contractile state. However, alterations in mechanical behavior of muscles from hyperthyroid cats do not adequately account for the observed increase in cardiac energy utilization, which suggests that the conversion of energy to work

is inefficient in these muscles. Thus, alterations in both the rate and efficiency of energy utilization may be important in the control of cellular energy metabolism by thyroid hormone.

ACKNOWLEDGMENTS

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