Effect of graded doses of tri-iodothyronine on ventricular myosin ATPase activity and isomyosin profile in young and old rats

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Ventricular myosin ATPase activity, V_1 isomyosin content and serum T_3 (tri-iodothyronine) values decrease with age in male Fischer 344 rats. To determine if the age decrement in ATPase activity and V_1 isomyosin content are caused by decreased T_3 levels or an age-related decrease in V_1 isomyosin induction by T_3 , 3-, 12- and 24-month-old male Fischer 344 rats were given constant T_3 infusions by osmotic minipump. Rats at all ages were given 0.75, 5 and 15 $\mu\text{g}/(100\text{ g per } 24\text{ h})$ doses of T_3 , whereas 12- and 24-month-old rats were given an additional 0.4 μg dose. In control rats, T_3 levels decreased from 97 ± 2.7 at 3 months to 75 ± 4.7 ng/100 ml at 24 months. Likewise, Ca^{2+} -activated myosin ATPase activity decreased from 1.04 ± 0.05 to 0.68 ± 0.05 $\mu\text{mol of } P_i/\text{min per mg of protein}$, and the relative proportion of V_1 of isomyosin decreased from 90 ± 4.0 to $26 \pm 2.0\%$. The lowest (0.4 μg) T_3 dose, which was sufficient to restore T_3 levels in 24-month-old animals to 3-month control values, abolished the age decrement in myosin ATPase activity and markedly increased the proportion of V_1 isomyosin present in the ventricle. These findings indicate that the senescent ventricle responds readily to small doses of T_3 and strongly suggest that the age decrement in serum T_3 levels is sufficient to contribute to the age-related decrease in myosin ATPase activity and V_1 isomyosin content. Since these parameters correlate with ventricular contractility, the age decrement in T_3 levels may also contribute to the decreased ventricular contractility and cardiac output observed in senescent rats.

INTRODUCTION

The present study explores the relationship between declining T_3 levels and altered ventricular isomyosin distribution in senescent Fischer 344 rats. In animals and man, thyroid hormone increases the rate of myocardial fibre shortening and tension development in the ventricle (Buccino *et al.*, 1967; Merillon *et al.*, 1981). There is a corresponding thyroid-hormone stimulation of Ca^{2+} -activated myosin ATPase activity in the cardiac ventricle (Rovetto *et al.*, 1972; Yazaki & Raben, 1975). The rate of myosin-mediated ATP hydrolysis appears to limit the velocity of muscle shortening by determining the rate of actin-myosin cross-bridge formation (Alpert *et al.*, 1979). Thyroid hormone causes these changes in ventricular function by inducing the synthesis of an isomyosin with high ATPase activity (Morkin *et al.*, 1983). Intact myosin from the rat ventricle can be separated by native gel electrophoresis into three distinct components, V_1 , V_2 and V_3 , in order of decreasing Ca^{2+} -activated ATPase activity and mobility (Hoh *et al.*, 1977). These isomyosins contain two heavy-chain species, α and β , that differ in amino acid sequence (Hoh *et al.*, 1979). V_1 isomyosin contains two α -chains, V_2 contains one α - and one β -chain and V_3 contains two β -chains. The β heavy chain predominates in the hypothyroid state, and thyroid

hormone induces the synthesis of the α heavy chain (Dillmann *et al.*, 1983a).

Senescent male Fischer 344 rats show a fall in circulating T_3 levels that is accompanied by decreased cardiac output, decreased shortening velocity of isolated ventricular muscle fibres and decreased Ca^{2+} -activated myosin ATPase activity (Rothbaum *et al.*, 1973; Chesky & Rockstein, 1977; Klug & Adelman, 1979; Capasso *et al.*, 1986). In addition, senescent rats have a decreased proportion of V_1 isomyosin in the cardiac ventricle, indicating an increased β - and a decreased α -heavy-chain content (Capasso *et al.*, 1986). Since the thyroid-hormone level can influence ventricular isomyosin composition and ventricular contractility, is the decline in V_1 isomyosin and α -heavy-chain content in old rats caused by decreased circulating T_3 levels in these animals? Alternatively, is the genetic mechanism for α -heavy-chain induction less responsive to T_3 in old animals? To answer these questions, various T_3 doses have been infused in 3-, 12- and 24-month-old male Fischer 344 rats, with measurement of Ca^{2+} -activated myosin ATPase activity and isomyosin distribution in the cardiac ventricle. This study has shown that the senescent ventricular myocardium is very sensitive to T_3 . A small dose of T_3 , just sufficient to raise T_3 levels in 24-month-old animals to that of 3-month-old animals, abolished

Abbreviation used: T_3 , tri-iodothyronine.

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the age decrement in myosin ATPase activity and greatly increased the percentage of V_1 isomyosin in the ventricle.

EXPERIMENTAL

Materials

Male Fischer 344 rats aged 3, 12 and 24 months were purchased from the National Institute on Aging, Bethesda, MD, U.S.A. Alzet Osmotic Minipumps (model 2ML2) were purchased from the Alza Corp., Palo Alto, CA, U.S.A. Acrylamide, NN' -methylenebisacrylamide, $NNN'N'$ -tetramethylethylenediamine and ammonium persulphate were obtained from Bio-Rad Laboratories, Richmond, CA, U.S.A., and T_3 was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. All other chemicals used were of reagent grade.

Treatment of experimental animals

Alzet Osmotic Minipumps were loaded with T_3 dissolved in poly(ethylene glycol) 300/water (3:1, v/v) at the concentrations required to deliver doses of 0.75, 5.0 and 15.0 $\mu\text{g}/24\text{ h}$ per 100 g in all age groups and a 0.4 μg dose in the 12- and 24-month-old groups. The pumps were implanted subcutaneously in the interscapular area by using anaesthesia induced by intramuscular injection of 5.0 mg of ketamine and 0.1 mg of acepromazine/100 g body weight. The anaesthetic dose was decreased to half in 24-month-old rats because such a dose produced adequate anaesthesia and allowed rapid recovery. Control animals received sham implantations. Young and old rats recovered rapidly from the pump implantation with no mortality. During the 12-day T_3 infusions, rats were housed individually, weighed daily and fed Purina Rat Chow (Ralston Purina, St. Louis, MO, U.S.A.) *ad lib*. At the end of the infusion, rats were killed by decapitation and blood collected for determination of total T_3 , free T_3 and T_3 binding by serum proteins. In addition, the heart and kidneys were rapidly removed, the cardiac chambers opened and both organs rinsed in ice-cold 0.9% NaCl, blotted and weighed. The hearts were stored at -80°C until processed.

Methods

Total and free T_3 levels were measured by using radioimmunoassay kits [T_3 RIA Bead Kit (Abbott Laboratories, North Chicago, IL, U.S.A.) and Magic Free T_3 Kit (Corning Medical and Scientific Division, Walpole, MA, U.S.A.)]. The capacity of serum proteins

to bind T_3 was estimated by using an '*in vitro*' T_3 resin uptake Kit (Travenol-Genetech, Cambridge, MA, U.S.A.). Electrophoretically homogeneous myosin was extracted from the cardiac ventricles by the method of Shiverick *et al.* (1975). Myosin purity was checked by electrophoresis on SDS/polyacrylamide slab gels (Weber & Osborn, 1969). Myosin ATPase activity was measured by incubating purified myosin in 10 mM- CaCl_2 /5 mM-dithiothreitol/5 mM-ATP, pH 7.6, as described by Bhan & Malhotra (1976). Isomyosins were separated by electrophoresis in 6 cm tube gels containing 4% (w/v) total acrylamide and 3% (w/w) NN' -methylenebisacrylamide cross-linker, the latter value being expressed as a percentage of total acrylamide (Hoh *et al.*, 1977). The gel buffer contained 10% (v/v) glycerol, 20 mM-sodium pyrophosphate, pH 8.8, and the running buffer contained these components plus 1 mM-EDTA and 2 mM-L-cysteine. The gels were run in a Pharmacia GE-4 apparatus at 90 V for 20–24 h with recirculation of the running buffer, which was cooled to 0–2 $^\circ\text{C}$. The gels were stained with Coomassie Blue R, scanned in a densitometer, and the percentage of total myosin represented by each isomyosin was estimated by measuring the area under each peak (Dillmann, 1980; Capasso *et al.*, 1986). Protein concentrations were measured by a modified Lowry procedure (Hartree, 1972).

RESULTS

Total circulating T_3 levels

Table 1 shows total serum T_3 levels measured at the end of the 12-day T_3 infusion. Eight animals were studied in each treatment category. In control animals, serum T_3 levels progressively declined in the 12- and 24-month-old age groups when compared with the 3-month-old group. Although the 0.75 μg T_3 dose is a physiological replacement dose in young rats given constant T_3 infusions (Brown & Millward, 1983), it increased serum T_3 levels in 12- and 24-month-old rats above 3-month-old-control levels. In contrast, the 0.40 μg T_3 dose increased serum T_3 levels in 12- and 24-month-old rats to that of 3-month-old controls. The 5 and 15 μg T_3 doses caused progressive serum T_3 elevations in all age groups, which were greater in older animals.

Circulating free T_3 levels and the capacity of serum proteins to bind T_3

To determine whether free T_3 levels were also decreased in untreated 12- and 24-month-old animals, free T_3 levels

Table 1. Serum T_3 levels at the end of the 12-day treatment period

As described under 'Methods', 3-month-old animals were given 0.75, 5.0 and 15.0 $\mu\text{g}/24\text{ h}$ per 100 g doses of T_3 , whereas 12- and 24-month-old animals were given an additional 0.4 μg dose. Serum T_3 measurements were made after a continuous 12-day T_3 infusion. Values presented below are means $\pm$ S.E.M., with eight animals in each treatment category. Statistical significance: * $P < 0.01$, ** $P < 0.001$, when compared with control animals of corresponding age by Student's *t* test.

Age group (months)	Dose of T_3 (μg) ...	Serum T_3 level (ng/100 ml)				
		0 (Control)	0.4	0.75	5.0	15.0
3		97 $\pm$ 2.7	—	101 $\pm$ 4.7	476 $\pm$ 20**	1643 $\pm$ 71**
12		89 $\pm$ 2.2	100 $\pm$ 5.4	113 $\pm$ 4.2**	562 $\pm$ 59**	1896 $\pm$ 107**
24		75 $\pm$ 4.7	95 $\pm$ 4.7*	140 $\pm$ 12.0**	664 $\pm$ 59**	3082 $\pm$ 446**

and the capacity of serum proteins to bind T_3 were estimated in control rats of each age group. Free T_3 levels (mean $\pm$ S.E.M., $n = 8$) were as follows: 3-month-old animals, 3.36 ± 0.29 pg/ml; 12-month-old animals, 2.96 ± 0.29 pg/ml; and 24-month-old animals, 1.92 ± 0.17 pg/ml. Serum-protein-binding capacities as estimated by T_3 resin uptakes *in vitro* were as follows: 3-month-old animals, $56 \pm 0.5\%$; 12-month-old animals, $54 \pm 0.6\%$; and 24-month-old animals, $54 \pm 0.5\%$. There was a downward trend in free T_3 levels in the 12-month-old rats, and a clear decrease in the 24-month-old rats. Furthermore, the slight decrease in T_3 resin uptake in the 12- and 24-month-old rats did not support decreased protein binding as the cause of the decreased total T_3 levels in these animals. Therefore the senescent rats appeared to have decreased free and total T_3 levels.

Changes in body, heart and kidney weight resulting from the T_3 infusions

As shown in Table 2, the 3-month-old controls had a 7% gain in body weight during the 12-day treatment interval, whereas the 12-month-old controls had no change and the 24-month-old controls had a 2% loss. In addition, the 12- and 24-month-old rats lost more weight in response to higher (5 and 15 μ g) T_3 doses than the 3-month-old rats. Since hyperthyroidism causes cardiac and renal hypertrophy in rats (Beznak, 1962; Goulding

et al., 1976), heart and kidney weights are presented in Table 2 as an index of clinical hyperthyroidism. The 0.4 μ g T_3 dose did not cause organ hypertrophy at any age, the 0.75 μ g dose caused hypertrophy only in the 24-month-old rats, and the 5 and 15 μ g doses caused hypertrophy at all ages. These data suggest that the 0.4 μ g T_3 dose did not cause hyperthyroidism at any age, whereas the 0.75 μ g dose caused hyperthyroidism in 24-month-old rats and the 5 and 15 μ g doses caused hyperthyroidism at all ages.

Ventricular myosin ATPase activity

Table 3 shows Ca^{2+} -activated ventricular myosin ATPase activity at all ages and T_3 dose levels. In control animals, ATPase activity progressively declined in 12- and 24-month-old age groups when compared with 3-month-old-rat values. In the 3-month-old rats, the 0.75 and 5 μ g T_3 doses did not increase ATPase activity above control levels, whereas the 15 μ g dose caused a modest increase. In the 12- and 24-month-old rats, the lowest (0.4 μ g) T_3 dose restored ATPase activity to the 3-month-old-rat control level, whereas the 0.75 and 5 μ g doses caused no further increase. The highest (15 μ g) T_3 dose caused a further increase in ATPase activity in the 12-month-old group, but not in the 24-month-old group. Therefore the lowest (0.4 μ g) T_3 dose, which was just sufficient to restore T_3 levels in 24-month-old rats to 3-

Table 2. Changes in body weight (A, in g) during, and heart (B) and combined kidney (C) weights (g) at the end of, the 12-day treatment period

Values presented below are means $\pm$ S.E.M. with eight animals in each treatment category. Values in parentheses refer to the percentage of initial body weight gained or lost during the treatment interval. 'NS' indicates that the weight change was not significant. Before weighing, cardiac chambers were opened and the hearts were rinsed in ice-cold 0.9% NaCl and blotted. The renal capsule was removed before weighing the kidneys. Statistical significance: * $P < 0.01$, ** $P < 0.001$ when compared with control animals of corresponding age by Student's *t*-test.

Parameter (see above)	Age group (months)	T_3 dose (μ g) ...	0 (Control)	0.4	0.75	5.0	15.0
A	3		$+18.0 \pm 3.0$ (7%)	—	$+19.0 \pm 3.0$ (7%)	$+2.7 \pm 3.4^*$ (NS)	$-35 \pm 4.0^{**}$ (13%)
	12		$+3.1 \pm 3.1$ (NS)	-0.6 ± 2.7 (NS)	-5.1 ± 4.0 (NS)	$-39.0 \pm 4.0^{**}$ (9%)	$-74 \pm 6.0^{**}$ (18%)
	24		-8.9 ± 2.9 (2%)	-2.1 ± 3.9 (NS)	-14.0 ± 4.9 (3%)	$-60.0 \pm 9.0^{**}$ (14%)	$-90 \pm 8.0^{**}$ (22%)
B	3		0.720 ± 0.02	—	0.774 ± 0.03	$0.941 \pm 0.02^*$	$1.02 \pm 0.03^*$
	12		0.984 ± 0.03	1.06 ± 0.02	1.08 ± 0.03	$1.30 \pm 0.03^*$	$1.25 \pm 0.03^*$
	24		1.080 ± 0.03	1.08 ± 0.04	$1.29 \pm 0.04^*$	$1.36 \pm 0.05^*$	$1.42 \pm 0.02^*$
C	3		1.95 ± 0.04	—	2.01 ± 0.07	$2.35 \pm 0.04^*$	$2.38 \pm 0.05^*$
	12		2.60 ± 0.07	2.78 ± 0.06	2.77 ± 0.10	$3.28 \pm 0.07^*$	$3.20 \pm 0.04^*$
	24		2.80 ± 0.06	2.78 ± 0.06	$3.24 \pm 0.10^*$	$3.57 \pm 0.28^*$	$3.31 \pm 0.08^*$

Table 3. Ca^{2+} -activated ventricular myosin ATPase activity at the end of the 12-day treatment period

Myosin was extracted from the cardiac ventricle, and Ca^{2+} -activated myosin ATPase activity was measured as described under 'Methods'. Values presented below are means $\pm$ S.E.M., with eight animals in each treatment category. Statistical significance: * $P < 0.01$ when compared with control animals of corresponding age by Student's *t* test; ** $P < 0.01$ when compared with the 0.4 μ g- T_3 -dose activity of corresponding age by Student's *t* test.

Age group (months)	T_3 dose (μ g) ...	Activity (μ mol of P_i /min per mg of protein)				
		0 (Control)	0.4	0.75	5.0	15.0
3		1.04 ± 0.05	—	1.04 ± 0.05	1.11 ± 0.03	1.19 ± 0.05
12		0.85 ± 0.03	$1.05 \pm 0.05^*$	$1.07 \pm 0.07^*$	$1.05 \pm 0.05^*$	$1.24 \pm 0.04^{**}$
24		0.68 ± 0.05	$1.03 \pm 0.07^*$	$0.95 \pm 0.06^*$	$1.14 \pm 0.04^*$	$1.17 \pm 0.04^*$

month-old-rat levels, abolished the age decrement in Ca^{2+} -activated myosin ATPase activity in 12- and 24-month-old rats without causing weight loss or organ hypertrophy.

Ventricular isomyosin distribution

Fig. 1 shows densitometer scans of gel preparations of ventricular isomyosins from each age group. In the 3-month-old control panel, the major peak corresponds to the fastest-migrating isomyosin, V_1 , which has the highest ATPase activity. The minor peaks correspond to V_2 and V_3 isomyosins in order of decreasing mobility and ATPase activity. In the 12- and 24-month-old control panels, the proportion of V_1 isomyosin progressively decreases and proportion of V_3 isomyosin increases. The shift in isomyosin profile corresponds to the lower Ca^{2+} -

activated myosin ATPase activity in 12- and 24-month-old controls as compared with 3-month-old controls. Since V_1 isomyosin is the greatly predominant species in 3-month-old controls and T_3 induces V_1 isomyosin, the various T_3 doses had little effect on isomyosin distribution and Ca^{2+} -activated myosin ATPase activity in 3-month-old rats. On the other hand, Fig. 1 shows the marked increase in the proportion of V_1 isomyosin that occurred when the lowest ($0.4 \mu\text{g}$) T_3 dose was given to 12- and 24-month-old rats.

Table 4 shows the percentage distribution of each isomyosin in ventricular myosin preparations at all ages and T_3 dose levels. Values presented are means $\pm$ S.E.M. for six animals in each treatment group. As revealed in Fig. 1, there was a progressive decline in V_1 isomyosin and an increase in V_3 isomyosin when 12- and 24-month-

Fig. 1. Ventricular-isomyosin distribution in rats of each age group

The Figure shows representative densitometer scans of ventricular isomyosins from the indicated age and treatment categories. Isomyosins were separated by pyrophosphate gel electrophoresis as described under 'Methods'. The vertical arrows ($\downarrow$) indicate the positions of the V_1 , V_2 and V_3 isomyosin components and the horizontal arrow ($\rightarrow$) indicates the direction of protein migration in the gel.

Table 4. Percentage distribution of each ventricular isomyosin at the end of the 12-day treatment period

Ventricular isomyosins were separated by native gel electrophoresis, and the percentage distribution of each isomyosin was estimated by densitometry as described under 'Methods'. Values presented below are means $\pm$ S.E.M., with six animals in each treatment category. In the 3-month-old age group, no V_2 or V_3 isomyosins were detected at any T_3 dose level. Statistical significance: * $P < 0.05$, ** $P < 0.01$ when compared with control animals of the corresponding age by Student's t test.

Age group (months)	Iso-myosin	T_3 dose (μg) ...	Percentage distribution				
			0 (Control)	0.4	0.75	5.0	15.0
3	V_1		90 $\pm$ 4.0	—	100*	100*	100*
	V_2		7 $\pm$ 3.0	—	0*	0*	0*
	V_3		3 $\pm$ 1.0	—	0*	0*	0*
12	V_1		50 $\pm$ 3.0	64 $\pm$ 2.0**	73 $\pm$ 2.0**	91 $\pm$ 3.0**	94 $\pm$ 2.0**
	V_2		29 $\pm$ 2.0	24 $\pm$ 1.0*	19 $\pm$ 2.0**	7 $\pm$ 2.0**	5 $\pm$ 2.0**
	V_3		21 $\pm$ 2.0	12 $\pm$ 1.0**	8 $\pm$ 0.6**	2 $\pm$ 1.0**	1 $\pm$ 0.8**
24	V_1		26 $\pm$ 2.0	66 $\pm$ 2.0**	72 $\pm$ 3.0**	80 $\pm$ 2.0**	81 $\pm$ 2.0**
	V_2		30 $\pm$ 0.8	22 $\pm$ 2.0**	20 $\pm$ 2.0**	15 $\pm$ 2.0**	15 $\pm$ 1.0**
	V_3		44 $\pm$ 3.0	12 $\pm$ 1.0**	8 $\pm$ 2.0**	5 $\pm$ 0.6**	4 $\pm$ 0.2**

old controls were compared with 3-month-old controls. The lowest ($0.4 \mu\text{g}$) T_3 dose caused a marked increase in V_1 isomyosin and a decrease in V_3 isomyosin in the 12- and 24-month-old age groups. Whereas T_3 doses of $0.75 \mu\text{g}$ and greater totally eliminated V_2 and V_3 isomyosins in 3-month-old animals, residual V_2 and V_3 isomyosins remained in 12- and 24-month-old animals at all T_3 dose levels.

DISCUSSION

A small T_3 dose that was just sufficient to raise circulating T_3 levels in 24-month-old animals to that of 3-month-old animals also abolished the age decrement in ventricular myosin ATPase activity and markedly increased the percentage of V_1 isomyosin in old animals. These findings strongly suggest that the age decrement in serum T_3 levels in male Fischer 344 rats is sufficient to contribute to the age-related decrease in Ca^{2+} -activated myosin ATPase activity and V_1 isomyosin content in these animals. Furthermore, these studies show that the senescent ventricular myocardium responds readily to small T_3 doses. Since Ca^{2+} -activated myosin ATPase activity and V_1 isomyosin content correlate with muscle contractility (Alpert *et al.*, 1979; Scheuer & Bhan, 1979; Capasso *et al.*, 1986), the age decrement in T_3 levels may also contribute to the decreased rate of ventricular muscle fibre shortening and decreased cardiac output reported in senescent Fischer 344 rats (Rothbaum *et al.*, 1973; Capasso *et al.*, 1986). Additional studies are needed to determine whether small doses of T_3 can improve the contractile performance of the senescent cardiac ventricle.

Although myosin ATPase activity and the percentage of V_1 isomyosin in the ventricles of 12- and 24-month-old animals increased readily in response to the lowest ($0.4 \mu\text{g}$) T_3 dose, residual V_2 and V_3 isomyosin remained in 1- and 24-month-old animals, even at much higher (5 and $15 \mu\text{g}$) T_3 doses. Since the half-life of myosin heavy chains is 5.5 days in young rats (Martin *et al.*, 1977) and may be greater in old rats, a large fraction of the V_2 and V_3 isomyosins remaining in old rats after the 12-day T_3 infusion may have been synthesized before T_3 treatment. Therefore the small ($0.4 \mu\text{g}$) T_3 dose may have restored V_1 isomyosin levels in the old rats to, or near, 3-month-old control levels without eliminating residual V_2 and V_3 isomyosins. The decreasing percentage of V_2 and V_3 isomyosins in old rats given large T_3 doses may be related to increased degradation of V_2 and V_3 isomyosins as well as increased synthesis of V_1 isomyosin. Since the present study estimated the percentage distribution of ventricular isomyosins rather than absolute concentrations, it is uncertain whether the $0.4 \mu\text{g}$ T_3 dose restored the absolute V_1 isomyosin content in 12- and 24-month-old-rat ventricles to that of 3-month-old-rat ventricles. Nevertheless, the $0.4 \mu\text{g}$ T_3 dose appears to have at least caused a substantial increase in the V_1 isomyosin content in 12- and 24-month-old-rat ventricles, because it eliminated the age decrement in myosin ATPase activity and markedly changed the isomyosin profile.

Since malnutrition and illness may decrease T_3 levels (Wartofsky & Burman, 1982), it may be questioned whether the decreased T_3 levels and accompanying changes observed in the present study resulted from disease, altered nutritional status or old age. All rats used

in the present study were obtained from the National Institute on Aging, where efforts are made to maintain a healthy colony of animals for aging studies. All rats were entered into experiments within 2 weeks of purchase, and animals with apparent illness were excluded. Young and old animals appeared to recover rapidly from the minor surgery and the anaesthesia required for osmotic-minipump implantation which resulted in no mortality. All 24-month-old controls had T_3 levels below the mean of the 3-month-old controls, indicating that low T_3 values were uniform in the old animals and not confined to a subgroup. Furthermore, other investigators have reported similar findings in senescent Fischer 344 rats, such as decreased ventricular myosin ATPase activity (Chesky & Rockstein, 1977; Capasso *et al.*, 1986) and decreased percentage of V_1 isomyosin in the ventricle (Capasso *et al.*, 1986). Therefore it is likely that the decreased T_3 levels, diminished ventricular myosin ATPase activity and altered isomyosin profile observed in the present study are general features of senescent male Fischer 344 rats and not restricted to the sample used in these experiments.

The age-related decrement in serum T_3 levels observed in the present study is one of several instances in which a decrease in T_3 levels short of those producing frank hypothyroidism appears to have regulatory significance. Decreased T_3 levels in uraemic rats were associated with decreased hepatic α -glycerophosphate dehydrogenase and malate dehydrogenase activities, which were restored to normal by physiological doses of T_3 (Lim *et al.*, 1980). Likewise, feeding a restricted low-carbohydrate diet to rats decreased T_3 levels and V_1 isomyosin content in the cardiac ventricle (Dillmann *et al.*, 1983b). Again, a physiological dose of T_3 restored the isomyosin profile to normal.

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