# EFFECT OF THYROXINE ON SKELETAL MUSCLES OF DYSTROPHIC MICE

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UDC 616.74-009.54-055.5/.7-092-085.
357.441:577.175.444

KEY WORDS: dystrophic mice; L-thyroxine; types of muscle fibers; contractile properties of muscles.

The principal steps in the pathogenesis of human hereditary muscular dystrophies (MD) have not yet been elucidated and no conclusive evidence has yet been obtained of the effectiveness of any form of treatment [7]. Muscular dystrophies in mice have many similar phenotypic features with MD in man, and for that reason inbred mice with MD are widely used as models [9]. The modifying "therapeutic" effect of chronic low-frequency electrical stimulation of muscles in mice with MD has been demonstrated [4]. Factors modifying the phenotype of skeletal muscles include thyroid hormones. Directly, or indirectly through the nervous system, they cause changes in the level of myosin synthesis, activity of $Ca^{++}$-adenosine triphosphatase (ATPase), and the contractile properties of muscles [14].

In the investigation described below the effect of L-thyroxine ($T_4$) on the phenotype of the muscles in mice with a dystrophic genotype was studied. An argument in support of the possible therapeutic effect of $T_4$ in muscular dystrophy is provided by data on the role of thyroid hormones in the protein metabolism of the affected muscle [8], and the presence of hypothyroidism [15] and more intensive catabolism of thyroid hormones in the muscles of mice with MD [5].

## EXPERIMENTAL METHODS

Experiments were carried out on 47 mice of the 129Rj line with normal (+/+) and dystrophic (dy/dy) genotypes. The animals were bred from pure-strain mice obtained from the Scientific-Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, and were used in the experiments at the age of 3-4 months. A solution of $T_4$ (from Reanal, Hungary) was made up and injected intraperitoneally in a dose of 80 µg/100 g body weight (10 animals). The following experimental groups were distinguished: 1) +/+ mice (n = 19), 2) +/+ mice receiving $T_4$ for 3 weeks (n = 12), 3) dy/dy mice (n = 10), and 4) dy/dy mice receiving $T_4$ for 3 weeks (n = 6). A fast muscle (tibialis anterior, TAM) and a slow muscle (soleus, SM) from both limbs, but in some experiments from only limb, were studied. Isometric contraction was studied and the mean amplitude in millivolts (mMU) and number (nMU) of functioning motor units of TAM (11 experiments) *in vivo* were calculated by the method described previously [1, 2]. Direct stimulation was applied and the integral action potential of TAM recorded by the same platinum surface electrodes, each 3 mm in diameter, with interelectrode distance of 4 mm. The following parameters were assessed: $M_w$) the weight of the muscle, $P_t$) the strength of a single contraction in response to indirect stimulation, $P'_t$) the same in response to direct stimulation, $P_t/M_w$) the force of a single contraction calculated per unit weight of muscle, $P_t/P'_t$) the coefficient of functional innervation (12 experiments); CT) contraction time; $^1/_2RT$) the half-relaxation time, $P_o$) the force of tetanic contraction in response to indirect stimulation with a frequency of 150 Hz. Immediately after investigation of the functional characteristics, activity of mitochondrial succinate dehydrogenase (SDH) and ATPase (pH 9.4) of the muscle fibers was demonstrated on frozen sections 10 µ thick cut from TAM and SM [6]. On the basis of their SDH activity the muscle fibers were divided into types (Fig. 1) white (A), intermediate (B), and red (C); on the basis of their ATPase activity they were divided into pale (I), dark (IIA), and darkest (IIB). The types of fibers were counted on photographic prints. The relative percentages of the types of muscle fibers, differentiated according to SDH and ATPase activity were calculated in 34 SM and 8 TAM of animals of group 1, 23 SM

Department of Normal Physiology and Department of Nervous Diseases, Kazan Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 5, pp. 540-543, May, 1986. Original article submitted March 4, 1985.

  

TABLE 1. Effect of $T_4$ on Contractile Properties, Number and Mean Value of Motor Units of TAM from Mice with Normal and Dystrophic Genotypes (M ± m)

| Group of animals | $A_w$, g | $M_w$, mg | $P_t$, g | $P_t'$, g | $P_t/M_w$, g/mg | CT, msec | ½RT, msec | $P_0$, g | $P_t/P_0$ | $P_t/P_t'$ | nMU | mMU |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 25,9±1,5 (16) | 44,4±2,2 (35) | 9,2±0,7 (38) | 9,5±0,7 (30) | 0,22±0,02 (36) | 27,2±1,1 (38) | 24,1±2,9 (38) | 28,5±1,8 (37) | 0,35±0,02 (37) | 0,98±0,03 (34) | 50,8±4,8 (23) | 0,23±0,05 (23) |
| 2<br>$P_{2-1}$ | 29,1±1,4 (8) | 41,5±1,4 (18) | 9,7±1,0 (18) | 9,5±1,1 (17) | 0,23±0,02 (18) | 25,8±1,6 (18) | 14,6±1,4 (18)<br><0,01 | 24,6±2,5 (18) | 0,38±0,02 (18) | 1,04±0,06 (17) | 41,5±4,2 (14) | 0,45±0,05 (14)<br><0,01 |
| 3<br>$P_{3-1}$ | 15,1±0,9 (9)<br><0,001 | 20,9±1,9 (17)<br><0,001 | 2,3±0,3 (17)<br><0,001 | 2,7±0,4 (12)<br><0,001 | 0,12±0,02 (17)<br><0,001 | 28,7±2,6 (17) | 32,1±4,0 (17) | 10,4±1,4 (17)<br><0,001 | 0,24±0,02 (16)<br><0,001 | 0,84±0,06 (16)<br>0,05 | 43,9±5,4 (12) | 0,17±0,05 (12) |
| 4<br>$P_{4-1}$<br>$P_{4-3}$ | 18,6±1,4 (4)<br><0,01 | 19,9±0,7 (8)<br><0,001 | 3,3±0,5 (8)<br><0,001 | 3,4±0,5 (8)<br><0,001 | 0,16±0,02 (8)<br><0,05 | 21,0±0,9 (8)<br><0,001<br><0,01 | 22,4±2,4 (8)<br><0,05 | 15,2±2,0 (8)<br><0,001 | 0,21±0,01 (8)<br><0,001 | 0,98±0,1 (8) | 33,9±2,0 (6)<br><0,01 | 0,34±0,08 (6) |

Note. Number of mice tested given in parenthesis. $A_w$) Weight of animal.

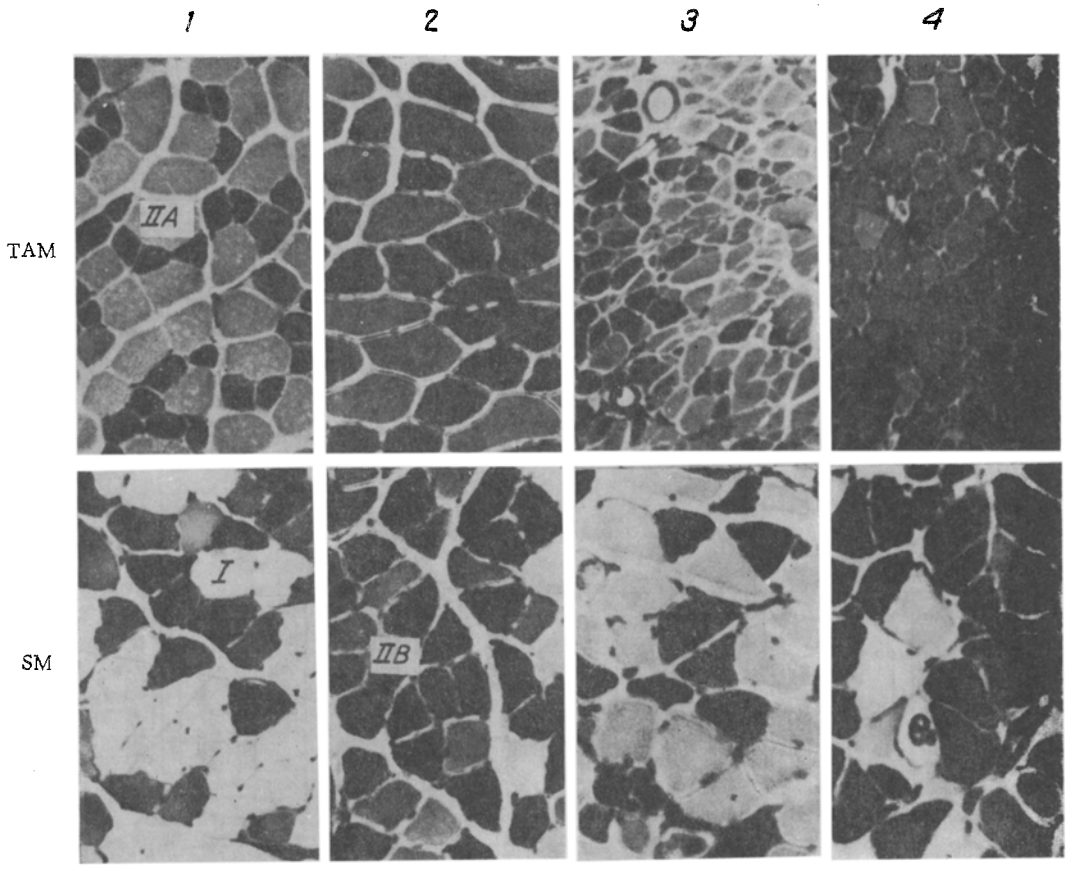


Fig. 1. Myosin ATPase activity in TAM and SM of mice with normal and dystrophic genotypes. 1-4) Nos. of corresponding experimental groups. Magnification: TAM (3 and 4) objective 3.5, ocular 7; all other sections objective 8, ocular 7.

and 13 TAM of animals of group 2, 20 SM and 7 TAM of mice of group 3, and 12 SM and 12 TAM of mice of group 4. All muscle fibers in all central regions of the sections free from artefacts were typed. The relative percentages of types of fibers in SM and TAM were calculated for each experimental group.

## RESULTS

Fibers in SM were not differentiated by SDM (Table 1, Fig. 2). The effect of $T_4$ on muscles of the animals of group 2 was manifested as an increase in the number of fast type II fibers in SM and TAM, in the absence of any significant changes in the type composition based on SDH. A decrease in the half-relaxation time of TAM was observed. A similar "quickening" of the muscles under the influence of $T_4$ was demonstrated previously [14]. In this group a decrease in the weight of TAM and an increase in the value of mMU also were observed.

The effect of $T_4$ on the type composition of the muscles in mice with MD (group 4) was manifested as an increase in the number of fast IIA and IIB fibers in SM and in the number of IIB fibers in TAM. On the basis of SDH activity the number of B fibers (intermediate type) in TAM increased. The contractile characteristics of TAM of the animals of this group showed shortening of the temporal characteristics and a tendency toward an increase in the force characteristics, so that they approached those in the control group (Fig. 2). The coefficient of functional innervation also tended to normal, mMU increased, whereas nMU decreased.

Thus the effect of $T_4$ on muscles belonging to mice of +/+ and dy/dy genotypes is characterized by an increase in the number of fibers with high ATPase activity. Under these circumstances the relative percentages of the various types in dystrophic muscles deviated even more from the normal. Meanwhile, in animals with MD treated with $T_4$, some normalization of the contractile characteristics was noted (Fig. 2).

Consequently, with respect to the contractile characteristics of their muscles, animals with MD treated with $T_4$ behave more normally than animals not receiving the hormone. The

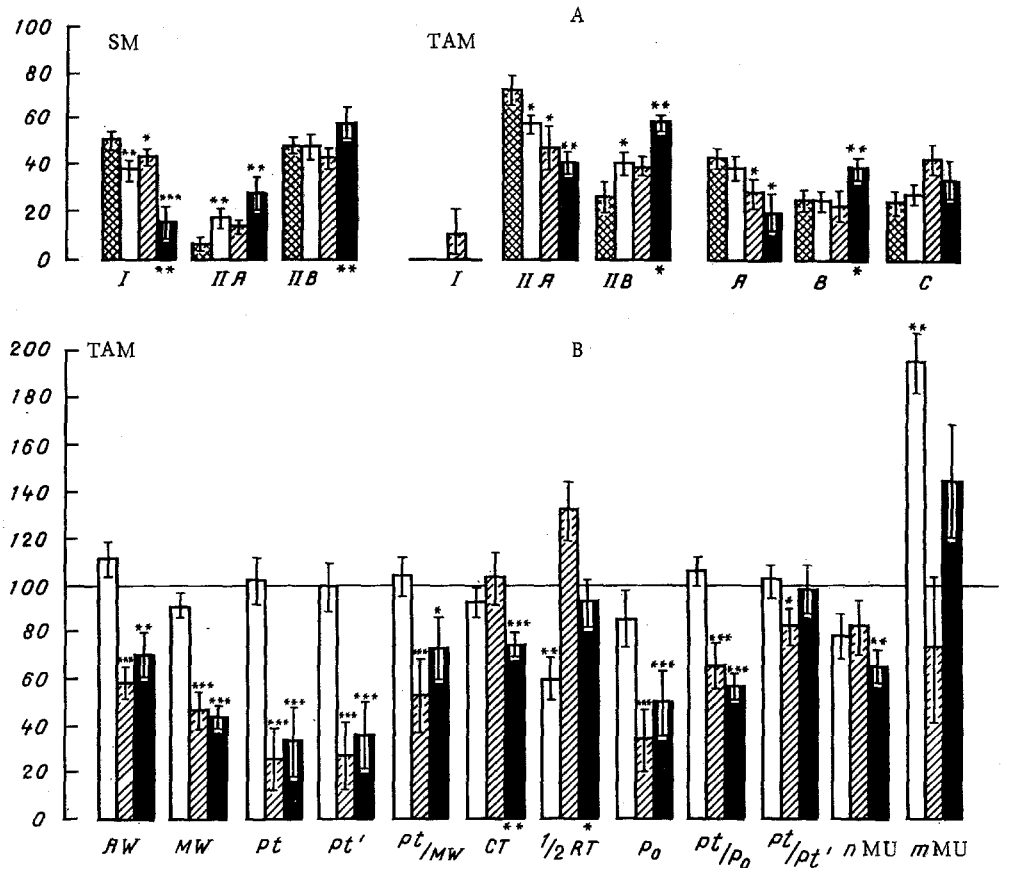


Fig. 2. Effect of $T_4$ on histochemical type composition, contractile properties of muscles, and mean value and number of motor units in mice of normal and dystrophic genotype. 1-4) Experimental groups. A) Relative percentages of types of fibers in SM and TAM of experimental groups 1-4; B) change in contractile characteristics in animals of groups 2-4 relative to control group 1 (in %). [Asterisks not explained in Russian original — Editor.]

"therapeutic" effect of $T_4$ on dystrophic muscles can probably be explained by its ability to "quicken" the muscles and thus to overcome the "slowing" of fast muscles that is characteristic of mice with MD [13]. A definite role may perhaps be played by the ability of thyroid hormones to potentiate regeneration and myelinization of axons [3]. The more marked signs of an increase in mMU observed in the muscles of the animals of group 4 is indirect confirmation of this hypothesis.

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