Plasma thyroxine levels in Duchenne muscular dystrophy

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Summary. Some young Duchenne muscular dystrophy (DMD) patients (3–7 years) had total thyroxine (T_4) levels and T_4 to thyroxine binding globulin (TBG) ratios above the normal range and significantly increased free thyroxine indices (fT_4I) which, however, remained within the normal range. Older DMD patients (7–11 years) had T_4 and TBG levels and fT_4I similar to normal. In both DMD groups the thyroxine binding index (TBI) values were in the normal range.
Key words. Duchenne muscular dystrophy; plasma thyroxine; thyroxine binding globulin; thyroxine binding index.

Duchenne muscular dystrophy is caused by deficiency of the protein dystrophin [1] which immunochemical and biochemical evidence indicates is associated with cell membranes in normal skeletal muscle [2–5]. At an early stage of the disease necrotic muscle fibres are detectable [6, 7]. It has previously been suggested that proteins released into the circulation from damaged tissue in non-thyroidal illness can inhibit binding of thyroxine to its plasma transport proteins [8]. 75% of plasma thyroxine present at physiological concentrations is bound to thyroxine binding globulin [9, 10]. It has generally been considered that the main physiological functions of TBG are to act as a buffer in the maintenance of the free hormone level and as a hormone reservoir [9, 10]. However, more recently there have been suggestions that TBG has other specific roles. Pardridge [11] has suggested that a rise in hormone binding proteins may redirect hormone delivery within the body particularly to tissues characterized by long capillary transit times where the hormone would have a longer time to equilibrate with the tissues. Ekins [12] has proposed that TBG may promote differential delivery of hormones to specific tissues. More recently Mendel et al. [13] have suggested that the major function of thyroid-binding proteins in plasma is to ensure uniform delivery of T_4 to all cells within a given tissue. In the absence of binding proteins, T_4 circulating through tissues avidly associated with the first cells it contacted. Only the specific thyroxine binding proteins TBG and thyroxine binding prealbumin ensured nonfluctuating circulating concentrations of free T_4 in vivo resulting in uniform cellular uptake of T_4.

Whichever of these suggested roles is the case, the presence of factors which inhibit binding of T_4 to TBG may result in an altered delivery of hormone to the tissues. Such an occurrence in Duchenne muscular dystrophy may compound the damaging effect of the initial lesion. Low levels of thyroxine reaching the tissues can cause myopathological changes in adults [14] and deficiency during infancy can retard intellectual development [10]. In the present investigation total free thyroxine, thyroxine binding globulin, thyroxine binding indices, T_4/TBG × 10 quotients and free thyroxine indices in younger and older DMD patients were compared with age-matched normal boys.

Materials and methods

All DMD patients were diagnosed on the basis of clinical features, elevated serum phosphokinase levels and histological examination of muscle biopsies. Blood samples were taken from DMD patients and normal non-hospitalized volunteers in southeast Scotland and Manchester and from patients undergoing minor elective surgery at the Royal Hospital for Sick Children, Edinburgh. The samples in heparinized or untreated tubes were incubated at room temperature for 2–4 h prior to centrifugation and removal of plasma or serum respectively which was aliquoted and stored at −70 °C. Neither DMD patients nor normal boys were taking drugs.

T_4, TBG and TBI were determined by immunoassay using appropriate commercial kits (Enzymum tests, Boehringer) and used to calculate T_4/TBG × 10 quotients and free fT_4I.

Results

Inspection of the data (table 1) shows that the young DMD patients tended to have higher total T_4 and fT_4I than the older patients. Therefore the DMD samples were divided into young (3–7 years) and old (7–11 years) age groups for statistical comparison with similarly grouped normal boys (table 2).

The young DMD group showed a significant 60% increase in total T_4 (table 2), patients 1, 3, 5 and 6 having values above the normal range (table 1), but TBG levels were similar to normal. Two of these patients (5 and 6) had T_4/TBG × 10 quotients above the normal range. Despite the higher T_4 values the average TBI which is a measure of exogenous T_4 binding was not different from normal. The fT_4I of the four patients with high T_4 values were also relatively high but remained within the normal range.

In a group of older DMD patients (7–11 years) T_4 and TBG levels, the T_4/TBG × 10 quotients, TBI values and fT_4I values were similar to normal. All the older DMD patients and many normal boys had T_4/TBG × 10 quotients below the normal range. This was attributed to their TBG values most of which were higher than the normal range quoted for the TBG determination kits.

Table 1. Plasma TBG and T_4 transport

	TBG (μg/ml)	T_4 (μg/dl)	T_4/TBG×10 quotient	TBI	fT_4I
Normal range	9.6–18.5	5–11.5	3.9–6.5	0.85–1.35	3.7–13.5
Normal (Age)					
1 $3^4/12$	21.5	8.0	3.72	1.20	6.67
2 $4^5/12$	17.5	6.7	3.83	1.28	5.25
3 $4^7/12$	21.3	7.2	3.45	1.29	5.66
4 $4^9/12$	20.5	7.8	3.81	1.20	6.50
5 $5^{10}/12$	25.0	7.0	2.80	1.38	5.07
6 $6^1/12$	16.5	7.8	4.73	1.28	6.09
7 $6^6/12$	17.5	6.2	3.54	1.25	4.96
8 $6^{10}/12$	16.5	7.2	4.36	1.14	6.32
9 $6^{11}/12$	17.0	7.8	4.59	1.16	6.72
10 7	23.5	7.8	3.32	1.26	6.19
11 $7^6/12$	13.5	5.6	4.15	1.07	5.23
12 $7/^{11}/12$	23.5	7.8	3.32	1.32	5.91
13 9	16.5	7.0	4.24	1.07	6.54
14 $9^7/12$	23.5	6.7	2.85	1.29	5.19
15 10	17.0	7.1	4.18	1.20	5.92
16 $10^2/12$	21.5	6.6	3.07	1.25	5.28
17 $11^3/12$	19.5	7.2	3.69	1.21	5.95
18 $11^6/12$	22.0	7.0	3.18	1.18	5.93
DMD					
1 $3^6/12$	23.0	12.4	5.39	1.18	10.51
2 $4^7/12$	23.0	8.4	3.65	1.28	6.56
3 $5^4/12$	22.0	13.6	6.18	1.30	10.46
4 $6^1/12$	20.5	10.4	5.07	1.31	7.94
5 $6^1/12$	14.0	11.8	8.43	0.98	12.04
6 $6^7/12$	16.5	13.6	8.24	1.23	11.06
7 7	21.5	7.6	3.54	1.08	7.04
8 $8^2/12$	26.0	8.2	3.15	1.30	6.31
9 $9^5/12$	18.0	6.1	3.39	1.24	4.92
10 $9^{11}/12$	21.5	8.2	3.81	1.35	6.07
11 $10^6/12$	22.5	7.2	3.20	1.22	5.90
12 11	25.0	7.2	2.88	1.16	6.21

Table 2. Plasma TBG and T_4 transport

		TBG(μg/ml)	T_4(μg/dl)	T_4/TBG×10	TBI	fT_4I
Normal range		9.6–18.5	5–11.5	3.9–6.5	0.85–1.35	3.7–13.5
Young normal	(9)	19.26 ± 0.99	7.30 ± 0.20	3.87 ± 0.20	1.24 ± 0.03	5.92 ± 0.23
Young DMD	(6)	19.83 ± 1.53^{ns}	11.70 ± 0.82***	6.16 ± 0.77*	1.21 ± 0.05^{ns}	9.76 ± 0.85***
Old normal	(9)	20.06 ± 1.22	6.98 ± 0.22	3.56 ± 0.18	1.21 ± 0.03	5.79 ± 0.16
Old MDM	(6)	22.42 ± 1.16^{ns}	7.42 ± 0.32^{ns}	3.23 ± 0.14^{ns}	1.23 ± 0.04^{ns}	6.08 ± 0.28^{ns}

Young normals, $3^4/12$–$6^{11}/12$ years; Old normals, 7–$11^6/12$ years; Young DMD, $3^6/12$–$6^7/12$ years; Old DMD, 7–11 years. Student's t-test: ns, not significant at 5% level; *, significant at 5% level; ***, significant at 0.1% level.
Normal ranges are quoted in the determination kits.

Discussion

TBI is a measure of the binding sites on TBG not occupied by thyroxine and can be an indirect measure of normal or abnormal binding capacity. TBI values did not differ between the younger and older DMD groups, remaining within the normal range, suggesting that proteins released from tissues into the circulation as they become progressively more damaged do not measurably inhibit thyroxine binding.

The significantly increased total T_4 levels in some young DMD patients correlated with an fT_4I which was relatively high in the euthyroid range suggesting a higher than normal exposure of the tissues to T_4 up to the age of 6–7 years. Occasionally TBG elevation may be the cause of hyperthyroxinemia in non-thyroid illness[15,16], but there was no significant difference between the average TBG values in normal boys and DMD patients. However in patients 5 and 6 TBG levels were lower than average resulting in a T_4/TBG × 10 quotient above the normal range. A combination of high T_4 and unchanged TBG would be expected to lead to a decreased TBI but this was not the case suggesting that the TBG in these two patients may have a higher affinity for T_4.

High levels of thyroxine reaching the tissues are associated with increases in basal metabolic rate and one consequence is muscle weakness and wasting[10,17]. However, total T_4 and free T_4 levels may have been increased in the four younger DMD patients due to a decreased extrathyroidal T_4 metabolism which is often found in non-thyroidal illness[15]. It might be expected that these levels would get even higher in older patients in a progressive disease but this was not the case. Instead the present data

indicates that T_4 was at a lower level in older patients. Thyroxine levels are regulated by thyroid stimulating hormone secreted by the pituitary anterior lobe which in turn is regulated by thyrotropin releasing hormone secreted from the hypothalamus[18]. Factors regulating the secretion of TRH are uncertain but may include influences from higher centres and a stimulatory effect of the thyroid hormones[18] presumably acting through receptors. Dystrophin is known to be associated with normal brain cells[19]. An abnormality in brain cells in DMD patients may affect functioning of the hypothalamus and hence the hypothalamus-pituitary-thyroid axis in different ways at early and later stages of the disease.

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Immunological comparison between albumins of three species of mice (genus *Mus*)

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Summary. Three closely related species of short-tailed mice (*Mus musculus musculus*, *M. spretoides* and *M. spicilegus*) were tentatively discriminated using immunological techniques based on albumin cross-reactivity. Different fractionations of crude albumin antisera allowed the recovery of antibody populations specific to the *M. m. musculus* albumin, whereas antibody population differences do not seem to exist between *M. spicilegus* and *M. spretoides*. Moreover, immunoreactivities tested with native and S-carboxymethylated albumins revealed that species-specific antibodies correspond to antigenic determinants depending on the amino acid sequence (sequential determinants). The observed immunological differences are related to species divergence and albumin sequences.
Key words. Mus musculus musculus; *Mus spicilegus*; *Mus spretoides*; albumin antiserum fractionation; native and S-carboxymethylated albumins.

The use of biochemical techniques, particularly protein electrophoresis, has made it possible to redefine the systematics of the genus *Mus*[1, 2] and to ascribe an accurate taxonomic rank to species which are very similar morphologically. Four biochemical groups of mice were thus recognized in Central and Eastern Europe[3], corresponding to *Mus musculus domesticus*, *M. m. musculus*, *M. spicilegus* and *M. spretoides* (although the nomenclature of the latter taxa was still debated, see Auffray et al.[4]). Among these, *M. m. domesticus* is the only long-tailed mouse. The three remaining taxa are all relatively short-tailed and are thus very difficult to differentiate in the field.

Albumin has often been used as an evolutionary marker, and in immunological comparisons aimed at quantifying the degree of divergence of the taxa considered[5, 6]. In the