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Hyperestrogenemia in neuromuscular diseases

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SUMMARY

In order to elucidate the relationship between certain neuromuscular diseases and gonadal hormones, we measured the levels of serum estrogens and other sex-related hormones. The values were compared with those for age-matched controls. The cases, comprising bulbospinal muscular disease of the Kennedy-Alter-Sung type, Kugelberg-Welander disease, amyotrophic lateral sclerosis, and Duchenne muscular dystrophy, were all euthyroid males. The baseline levels of serum estrone were significantly higher in all of the patients than in age-matched normal subjects. Serum baseline testosterone, LH and FSH levels were all essentially normal, except low FSH levels in Duchenne muscular dystrophy. Since our patients had no overweight, liver or glandular abnormalities, we presume that the elevated serum estrone levels have resulted from increased peripheral androgen-to-estrogen conversion.

Key words: Hyperestrogenemia; Extraglandular aromatization; Kennedy-Alter-Sung type; Kugelberg-Welander disease; Amyotrophic lateral sclerosis; Duchenne muscular dystrophy

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INTRODUCTION

Weiner (1980) postulated that androgen has a trophic effect on motor nerves and that a defective androgen receptor–androgen interaction may contribute to the development of motor neuron disease. Some neuromuscular diseases exhibit signs that may be related to sex hormones. For instance, gynecomastia is often observed in male patients with X-linked bulbospinal muscular disease of the Kennedy-Alter-Sung type (KAS; Stefanis 1975), and sometimes in males with Kugelberg-Welander disease (KW). Amyotrophic lateral sclerosis (ALS) has been found to exhibit sexual dimorphism in incidence and mortality: the male-to-female patient ratio is about 2 : 1 (Kurland et al. 1967; Tsubaki 1979), and females live longer than males (Tsubaki 1979; Mukai et al. 1984).

Skeletal muscle has estrogen as well as androgen receptors (Dionne et al. 1979; Meyer and Rapp 1985). Max (1981) reported that estrogen accelerated the increase in glucose-6-phosphate dehydrogenase (G6PD) activity following denervation, mediated via estrogen receptors; G6PD is correlated with muscle regeneration (Wagner et al. 1978). These reports warrant examination of sex hormones in muscle wasting diseases.

In this study, we have evaluated the levels of serum estrogen and other sex-related hormones in men with certain motor neuron diseases including KAS, KW, ALS, and other X-linked recessive muscle wasting disease such as DMD, in order to determine their relationship to sex hormones. Women with KW or ALS were excluded from this study to avoid misinterpretation of our data.

SUBJECTS AND METHODS

Subjects

Thirty-three patients with DMD (8–19 yrs), 10 with ALS (43–62 yrs), 5 with KW (19–42 yrs), and 5 with KAS from 1 family (47–59 yrs) were investigated. The diagnosis in each case was based on clinical, electrophysiologic, laboratory and morphologic findings. All of them were rather lean men without apparent liver dysfunction. No patient had taken any anabolic hormone, and none had a history of alcohol abuse. The thyroid hormone levels in all patients were within the normal range. Three KAS patients had gynecomastia. Thirty-four normal males (7–69 yrs) without endocrine or hepatic abnormalities constituted the control group. Their age distribution is shown in Table 1.

Sample preparation

Fasting peripheral blood was sampled in the early morning, and the serum was stored at $-20\,^{\circ}C$ until used.

Two ml of serum was extracted twice with 10 ml of ether in a capped-glass tube by shaking in a Vortex mixer for 2 min. The pooled ether extracts were then dried in a water bath at $37\,^{\circ}C$ under N_2 gas. The dried residue was stored at $-20\,^{\circ}C$.

TABLE 1

AGE DISTRIBUTION OF THE SUBJECTS

All subjects were males.

	Age (yrs)						
	7–11	12–15	16–19	20–29	40–49	50–59	60–69
Normal (n = 34)	6	7	7	2	5	4	3
DMD (n = 33)	11	11	11	0	0	0	0
ALS (n = 10)	0	0	0	0	3	4	3
KW (n = 5)	0	0	0	3	2	0	0
KAS (n = 5)	0	0	0	0	1	2	2

Separation of estrogens by Sephadex LH-20 column chromatography

The dried residue was dissolved 3 times in an 0.1-ml aliquot of a diluent (benzene/methanol, 9 : 1, v/v) using the Vortex mixer, and then the extracts were applied to a minicolumn (5 × 100 mm) of Sephadex LH-20 equilibrated with a solvent system of benzene/methanol, 9 : 1 (v/v). The column was developed with the same solvent. Estrone was eluted at 1.5–3.5 ml and estradiol at 4.0–6.5 ml (Fig. 1). Each eluate was dried in a water bath at 37 °C under N_2 gas, and the dried residue was then stored at − 20 °C until assayed.

Assay procedure

The amount of estrone (E_1) or estradiol (E_2) was calculated by means of radioimmunoassay using a commercial [^{3}H]estrone or [^{3}H]estradiol RIA kit (WIEN Laboratories, Inc.), respectively. Testosterone, LH and FSH were determined in non-fractionated serum by radioimmunoassay. All samples were assayed in duplicate.

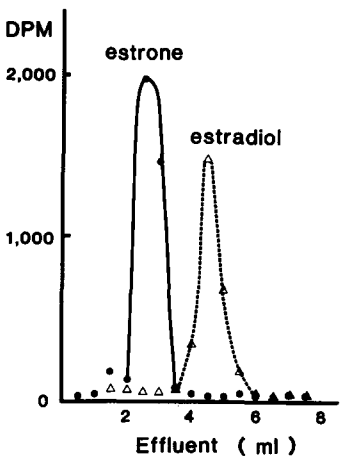

Fig. 1. Separation of estrogens on a Sephadex LH-20 column. [^{3}H]Estrone and [^{3}H]estradiol were applied on a column (5 × 100 mm) of Sephadex LH-20 equilibrated with benzene/methanol (9 : 1, v/v). 0.5-ml fractions were collected and counted in a scintillation fluid.

RESULTS

Serum estrogen levels in normal men

In boys below age 20 yrs (divided into 4-yr age groups), the baseline serum levels of estrone (E_1) and estradiol (E_2) increased with age. E_1 and E_2 levels for men over 20 yrs (divided into 10-yr age groups) remained constant (Fig. 2). We designated the latter group, aged 20–69 years, as a control group.

Serum estrogen levels in neuromuscular diseases

(a) KAS, KW and ALS

The results are shown in Fig. 3. In normal subjects, the mean serum E_1 value was 65.7 ± 13.1 (± SD) pg/ml (n = 14). The E_1 values in ALS, KW and KAS were 84.7 ± 28.4 pg/ml ($P < 0.05$), 100.6 ± 27.7 pg/ml ($P < 0.005$) and 88.1 ± 11.6 pg/ml ($P < 0.01$), respectively. On the other hand, the baseline serum E_2 levels in ALS patients (29.9 ± 10.8 pg/ml) were significantly ($P < 0.005$) lower than normal (46.8 ± 12.3 pg/ml). The mean value in KW was 60.6 ± 26.4 pg/ml (not significant), while that in KAS (72.5 ± 28.6 pg/ml) was significantly higher than normal ($P < 0.02$).

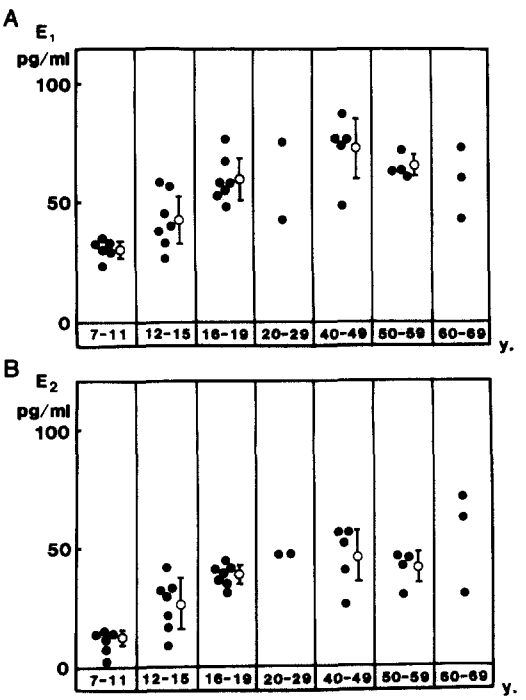

Fig. 2. Serum estrone (E_1) (A) and estradiol (E_2) (B) levels in normal men. After fractionation by Sephadex LH-20 column chromatography, the contents were calculated by radioimmunoassay using [^{3}H]estrone and [^{3}H]estradiol RIA kits. The data were analyzed as to 4–10-yr age groups.

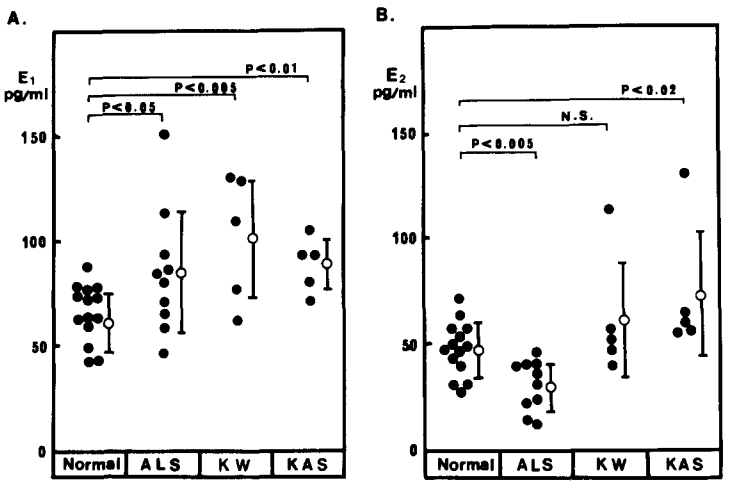

Fig. 3. Serum estrone (E_1) (A) and estradiol (E_2) (B) levels in patients with amyotrophic lateral sclerosis (ALS), Kugelberg-Welander disease (KW) and the X-linked bulbospinal muscular disease of the Kennedy-Alter-Sung type (KAS), and in the control group (20–69 yrs). Estrogens were determined as described under Methods.

The baseline serum testosterone, LH and FSH levels of these patients were not significantly different from the normal, though one FSH value and two testosterone values in ALS were higher than normal (Tables 2 and 3).

(b) Duchenne muscular dystrophy

Since the E_1 and E_2 levels for boys below 20 yrs in the control group increased with age, the data were likewise analyzed for corresponding 4-yr age groups in DMD:

TABLE 2

SERUM ESTROGENS AND OTHER SEX-RELATED HORMONES IN AMYOTROPHIC LATERAL SCLEROSIS

E_1, estrone; E_2, estradiol; T, testosterone; n.e., not examined. severe: means bedridden state; moderate: means ambulatory.

Normal men: E_1: 65.7 ± 13.1 pg/ml, E_2: 46.8 ± 12.3 pg/ml; T: 7.7 ± 1.7 ng/dl (20–49 yrs); 6.9 ± 0.8 ng/dl (50–69 yrs); LH: 18.5 ± 4.3 mIU/ml (20–49 yrs); 27.1 ± 6.3 mIU/ml (50–69 yrs); FSH: 9.3 ± 3.8 mIU/ml (20–49 yrs); 17.3 ± 3.9 mIU/ml (50–69 yrs). Values are the means ± SD.

Case	1	2	3	4	5	6
Age (yrs)	43	44	53	53	62	68
Duration of symptoms (yrs)	5	6	3	1.5	4	4
Severity	severe	severe	severe	moderate	moderate	severe
E_1 (pg/ml)	57.6	70.6	112.9	85.9	65.9	151.1
E_2 (pg/ml)	24	38.4	38.4	30.4	40	22.8
T (ng/dl)	8.4	7.1	11.0	4.9	12.0	n.e.
LH (mIU/ml)	25	14	44	16	18	44
FSH (mIU/ml)	8.1	10	55	6.8	12	20

TABLE 3

SERUM ESTROGENS AND OTHER SEX-RELATED HORMONES IN KW AND KAS

E_1, estrone; E_2, estradiol; T, testosterone; KW, Kugelberg-Welander disease; n.e., not examined; KAS, bulbospinal muscular disease of Kennedy-Alter-Sung type. Normal values are shown in Table 2.

Case	1	2	3	4
Age (yrs)	24	45	50	61
Disease	KW	KW	KAS	KAS
E_1 (pg/ml)	129.4	128.2	92.9	92.9
E_2 (pg/ml)	45.7	112.4	129.5	62.8
T (ng/dl)	8.0	9.8	n.e.	4.3
LH (mIU/ml)	18	13	26	23
FSH (mIU/ml)	8.8	3.8	9.1	15

Group I (8–11 yrs), Group II (12–15 yrs) and Group III (16–19 yrs). The results are shown in Fig. 4. In Group I, the baseline serum level of E_1 was significantly higher in the DMD patients than in normal subjects (40.5 ± 9.2 pg/ml vs. 30.5 ± 3.7 pg/ml; $P < 0.05$), whereas serum levels of E_2 in DMD (14.2 ± 2.8 pg/ml) and in normal

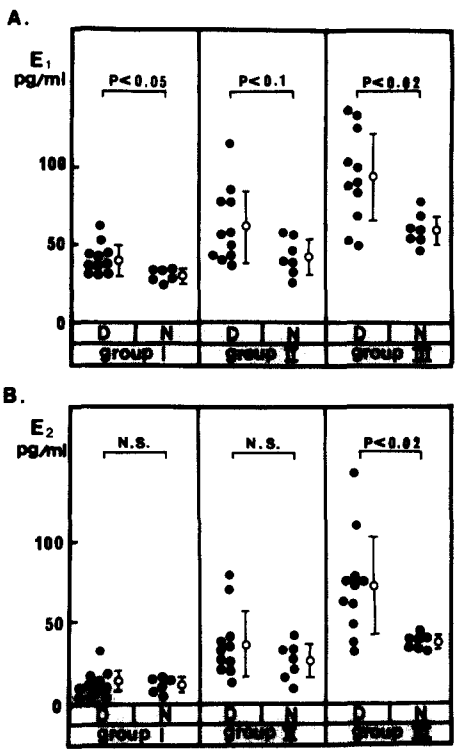

Fig. 4. Serum estrone (E_1) (A) and estradiol (E_2) (B) levels in patients with Duchenne muscular dystrophy (D) and normal men (N). After fractionation by Sephadex LH-20 column chromatography, estrogens were determined as in Fig. 2. The data were analyzed for each 4-yr age group: Group I (8–11 yrs), Group II (12–15 yrs) and Group III (16–19 yrs).

TABLE 4

LIVER FUNCTION AND THE LEVELS OF SERUM ESTROGENS AND OTHER SEX-RELATED HORMONES IN DMD WITH HYPERESTROGENEMIA

Group II: 12–15-yr age group; Group III: 16–19-yr age group. ICG, indocyanine green test; E_1, estrone; E_2, estradiol; T, testosterone; DMD, Duchenne muscular dystrophy. a: Group II = 43.0 ± 10.8; Group III = 59.8 ± 8.8. b: Group II = 26.4 ± 10.3; Group III = 38.9 ± 3.9. c: Group II = 7.4 ± 1.2; Group III = 7.6 ± 2.2. d: Group II = 13.2 ± 0.4; Group III = 21.0 ± 7.4. e: Group II = 11.0 ± 2.1; Group III = 10.1 ± 2.6. Values are means ± SD.

Case	Group	ICG % (15 min)	E_1 (pg/ml)	E_2 (pg/ml)	T (ng/dl)	LH (mIU/ml)	FSH (mIU/ml)
1	II	7.1	111.9	70.8	6.5	10	4.2
2	II	7.5	84.9	80.0	9.0	19	5.6
3	III	3.7	87.7	62.5	6.6	11	4.1
4	III	0.9	101.2	109.7	5.7	12	5.5
5	III	0	122.5	142.4	4.9	21	5.2
6	III	6.4	132	74.3	5.2	18	4.1
7	III	0	98.3	60.8	8.4	11	3.1
8	III	2.9	103.6	74.3	11.0	15	4.6
	Normal	0–10	a	b	c	d	e

subjects (12.7 ± 2.8 pg/ml) were not significantly different. In Group II, the baseline serum level of E_1 in DMD was 61.5 ± 22.1 pg/ml and that in normal subjects was 43.0 ± 10.8 pg/ml ($P < 0.1$), and the E_2 level in the former (36.4 ± 20.1 pg/ml) and the latter (26.4 ± 10.3 pg/ml) did not significantly differ. In Group III, the baseline serum levels of E_1 and E_2 in the control group were 59.8 ± 8.8 pg/ml and 38.9 ± 3.9 pg/ml, respectively. The DMD patients had significantly higher baseline serum E_1 (92.3 ± 27.0 pg/ml) and E_2 (59.8 ± 8.8 pg/ml) levels than normal subjects ($P < 0.02$). The serum estrogen levels in DMD patients did not show significant correlation with clinical stage or serum creatine kinase activity (data not shown).

The DMD patients with hyperestrogenemia showed normal hepatic clearance of the dye (indocyanine green). Their serum testosterone and LH levels were essentially normal, but their FSH levels were lower than normal ($P < 0.01$) (Table 4).

DISCUSSION

Some patients with muscle wasting diseases such as KAS, KW, ALS and DMD, showed higher serum E_1 levels than normal control subjects. Clearly, other neuromuscular diseases than KAS alone are also accompanied by hyperestrogenemia despite the absence of gynecomastia. These diseases may not share common pathogenetic mechanisms and we have found no clear-cut relationship between the serum estrogen levels and the clinical symptoms including gynecomastia. Hence, it is likely that the hyperestronemia in these muscle wasting diseases are secondary epiphenomena.

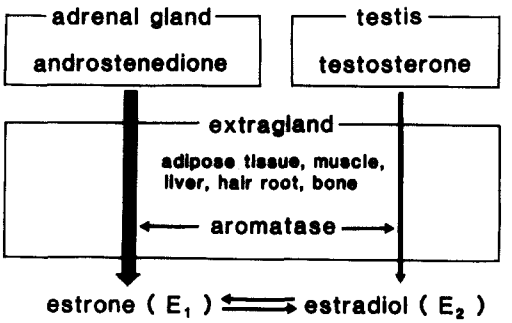

Fig. 5. Estrogen production in normal man. Estrone is converted from androstenedione, and estradiol from testosterone through extraglandular aromatization. Aromatase exists in adipose tissue, muscle, liver, hair roots and bone.

Estrogens and their analogues are metabolized nonspecifically by means of drug-metabolizing enzymes in the liver. In states of severe hepatic dysfunction, serum estrogen levels (E_1 plus E_2) increase. However, none of our patients had notable liver dysfunction and none had taken medications that could have affected the drug-metabolizing enzymes. Hence, we assume that the hyperestronemia in the neuromuscular diseases we studied was not due to any hepatic metabolic disturbance.

In males, estrogens are chiefly produced from the androgens through extra-glandular aromatization. Estrone is converted from androstenedione, and estradiol from testosterone. The conversion ratio is greater for androstenedione to estrone than for testosterone to estradiol (Longcope et al. 1969), and estrone and estradiol are interconvertible. The sites of peripheral aromatization are thought to be adipose tissues and muscles (Longcope 1978), liver, hair roots (Schweikert et al. 1975) and bones (Vittek et al. 1974) (Fig. 5). Since our patients had no glandular abnormalities, their high serum E_1 levels appear to be the result of increased peripheral androgen–estrogen conversion. Although it is known that the conversion of circulating androstenedione to estrone increases in hyperthyroidism (Southren et al. 1974), hepatic cirrhosis (Gordon et al. 1975) and obesity (Schneider et al. 1979), these possible causes of increased peripheral aromatization were ruled out in our cases.

The possibility that peripheral aromatization may occur in muscle or connective tissues is supported by the following facts: (a) hyperestronemia is a common phenomenon in the 4 kinds of muscle wasting diseases having different pathogenetic mechanisms; (b) both muscle and adipose tissue are important sites for aromatization.

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