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## **Muscle function in monozygotic female twin pairs discordant for hormone replacement therapy**

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**Running title:** Muscle function in HRT discordant twins

**Abstract**

Postmenopausal monozygotic twin pairs discordant for hormone replacement therapy (HRT) provide an advantageous study design controlling for genetic background for elucidating the relationships between aging, sex hormone levels, muscle strength, contractile capacity and fatigability. Thirteen postmenopausal monozygotic twin pairs discordant for HRT were measured for maximal voluntary torque (MVC) and twitch characteristics using electrical stimulation before and after intermittent dynamic plantarflexor exercise until exhaustion. Peak twitch torque was 32% higher in HRT users than in their non-using sisters ( $P=0.002$ ) but MVC did not differ. There were no differences in the activation level or twitch time characteristics between the co-twins. Fatigue caused decreases in MVC ( $P=0.001$ ), twitch torque ( $P=0.001$ ), time-to-peak ( $P=0.013$ ) and half relaxation time ( $P=0.001$ ) similarly in HRT users and non-users. In conclusion, in early postmenopausal women involuntary but not voluntary force-generating mechanisms of the plantarflexors are augmented by the use of HRT. This difference may result from central factors.

**Keywords:** estradiol, strength, twitch, fatigue, plantarflexor

## **Introduction**

Aging muscles experience loss of mass and strength that is attributed to factors such as type II muscle fiber atrophy, fiber necrosis and loss of motoneurons and lower maximal motor unit discharge rate<sup>1-4</sup>, but suggested to have a greater fatigue resistance especially in isometric protocols<sup>5</sup>. In women, the aging process is further affected by major changes in hormonal status during menopause, when the concentrations of estrogens decline dramatically.

Estrogen, as a pleiotropic hormone, can affect muscle function through the central nervous system or directly on the muscle tissue itself or both<sup>6</sup>. Especially, the notion that both estrogen receptors are expressed in human skeletal muscle cells of both genders<sup>7-8</sup>, implies that muscle is a target tissue for estrogen signaling. Estrogen has also been suggested to preserve type II muscle fibers in a study performed on ovariectomized rats<sup>9</sup>.

In aging women, hormonal replacement therapy (HRT) is quite commonly used to treat postmenopausal symptoms. HRT has been shown to have beneficial effects on voluntary muscle strength<sup>10-11</sup> but some reports have not shown this effect<sup>12-13</sup> (see also Greising et al.<sup>14</sup> for meta-analysis). Methodologically, it is important to understand that maximal voluntary contractions reflect the capacity of the whole neuromuscular system. To evaluate the effects of neural and muscular factors independently, electrical stimulation can be used to elicit muscle contraction, i.e. a twitch, without influence of central factors such as motivation and other factors associated with central nervous system. A twitch, produced by supramaximal electrical stimulus elicited to a peripheral nerve can activate all muscle fibers at once.

Since hereditary factors affect muscle strength and performance among older women<sup>15</sup>, a genetically controlled study design with postmenopausal monozygotic (MZ) twin sisters discordant for HRT use provides a powerful tool to study the role of estrogen containing replacement therapy in muscle's force producing capacity; differences between co-twins of discordant pairs cannot be ascribed to genetic effects. In the present study, we investigated the association between muscle force production and HRT by utilizing a comprehensive battery of tests involving both voluntary as well as electrically stimulated force assessments.

Ronkainen et al.<sup>13</sup> reported using the same MZ pairs that there were no significant differences in maximal isometric knee extension force between monozygotic twins discordant to HRT. The twins on HRT had, however, 16 % greater lower limb muscle extensor power compared to their sisters not on HRT suggesting that neuromuscular system may be affected by HRT. Thus, we hypothesized that the plantarflexor muscle torque, twitch and fatigability would differ between the twins.

## **Materials and Methods**

### *Design and subjects*

This study is part of a larger study “Sarcopenia – Skeletal Muscle Adaptation to Posmenopausal Hypogonadism and Effects of Hormone Replacement Therapy and Physical Activity in Older Women: a Genetic and Molecular Biological Study on Estrogen-related Pathways” (SAWEs). The study design and subject recruitment has been described previously<sup>13, 16</sup>. Briefly, after screening and confirmation of monozygosity by multiple genetic markers, a total of 15 MZ twin pairs, 54-62 years old, discordant for HRT were

assessed. Successful measurements for the current study were obtained from 13 MZ twin pairs. The mean age of the 26 subjects was  $57 \pm 2$  years (range 54-62 years). All measurements were done on the same day in a given twin pair. Pretest-posttest design was used to examine the neuromuscular function of calf muscles before and after intermittent submaximal voluntary contractions until fatigue. The study personnel were blinded to the HRT status.

Subjects' physical characteristics are given in Table 1. The duration of HRT usage was on average  $6.8 \pm 4.3$  years (range 2-16 years). Tibolone was the effective agent of the pills in four HRT-users, estradiol-progesterone in two and estradiol in seven subjects. Physical activity level, as assessed using 12 month and 7 day recall from which the mean total daily metabolic equivalent scores were calculated<sup>16</sup>, did not differ between the co-twins.

The Ethics Committee of the Central Finland Health Care District approved the study and it was conducted according to the guidelines in The Declaration of Helsinki. Written informed consent was provided by the subjects before participating in the measurements. Before measurements the subjects had a medical examination where contraindications for the maximal contractions and fatigue test were considered and for the safety of the subjects a physician was available during all measurements.

#### *Blood sampling*

Fasting blood samples were taken after 15 min rest in supine position between 0700 and 0900 before the other laboratory measurements. The sera were stored at  $-70$  °C after sampling. Sex hormone binding globulin (SHBG) levels were measured using solid-phase, chemiluminescent immunometric assays (Immulite 1000, Diagnostic Products Corporation,

Los Angeles, CA, USA). Serum  $17\beta$ -estradiol ( $E_2$ ) was determined in duplicate by extraction RIA as previously described<sup>17</sup> and testosterone levels as previously described<sup>18</sup>.  $E_2$  and testosterone were utilized together with SHBG in calculating the free  $E_2$  and free testosterone levels, respectively, according to a previously presented method<sup>19</sup>.

*Preparation procedure for the peak twitch torque assessments*

Bipolar EMG surface electrodes (Beckmann miniature skin electrodes, 650437, IL, USA) with 20 mm interelectrode distance were placed according to the SENIAM recommendations<sup>20</sup>. The EMG measurements were used to confirm that the stimulation was targeted to correct muscles and for analysis of frequency content of the signal (see below). The skin under the electrodes was shaved, abraded and cleaned with alcohol to reduce the inter-electrode resistance below 5 k $\Omega$ . Electrodes were placed on the right leg over m. soleus (SOL), m. gastrocnemius medialis (GM) and m. tibialis anterior (TA). A 60 X 38 mm pregelled electrode (Unomedical Ltd, Worcestershire, UK) was placed on the right lateral malleolus to serve as a ground to the abovementioned electrodes.

To stimulate plantarflexors, a 6.98 cm round self-adhering reusable carbon film anode coated with conductive gel (Mettler Electronics, Anaheim, CA, USA) was positioned proximal to the superior border of the patella and a cathode (1 cm<sup>2</sup>) on the skin overlying the tibial nerve in the popliteal fossa. The optimal stimulation point was searched while the subject was lying in prone position and defined as a location where the peak-to-peak amplitude and the shape of M-wave at the given intensity were most repeatable. The cathode was attached to the location with elastic bandage. A constant current stimulator (Digimeter model DS7A, Hertfordshire, UK) was used to deliver single pulses of 0.1 ms duration to the

tibial nerve to evoke superimposed twitch during maximal voluntary contraction (MVC) and a twitch to a relaxed muscle. Supramaximal intensity (125% Mmax) was used for both superimposed and twitches to a relaxed muscle.

We chose to stimulate the nerve because the percutaneous stimulation delivered from the skin surface to the muscle may not reach all the muscle fibers<sup>21</sup>. Repeatability of the twitch torque response was tested in 11 subjects with three stimulations with minimum of 10 s interval gave mean coefficient of variation (standard deviation/mean\*100) of 6% (range 1-26%).

#### *Maximal voluntary plantarflexion torque measurements*

Maximal voluntary isometric plantar flexion torque (MVC) was measured using a custom made adjustable dynamometer chair that has capabilities for both isometric and dynamic force measurements<sup>22</sup>. Right foot was secured on a footplate with two straps at an ankle joint angle of 90°, the knee was fully extended with a strap just above the patella and lower back was supported by backboard. This position provided firm support during the plantarflexions so that the calcaneus was not able to rise from the footplate. Hands were relaxed on thighs and left knee was flexed during the measurements. Subject practiced the isometric plantar flexion task with a feedback from torque signal provided on a computer screen. After 5-10 submaximal practice contractions, three trials of maximal voluntary contractions were performed with about 5 s rest intervals. The duration of MVC was ~2 s during which strong verbal encouragement and visual feedback of the torque level were given.



During MVC a supramaximal superimposed twitch was elicited at the time of peak torque in order to find out the maximality of the contraction. Before and two seconds after MVC a twitch was elicited to a relaxed muscles (Fig. 1).

### *Fatiguing exercise*

After MVC and twitch measurements the subjects practiced dynamic plantar flexion task with a low-load (10-30 kg). The load was provided by free weights (10-90 kg range with minimum increment of 5 kg). The footplate was rotated from 80° (dorsiflexed) starting position to 110° of plantarflexion and back to the starting position. The upper and lower limits of the ankle movement were shown in a screen in front of the subjects and they were requested to rotate the pedal between the limits with a frequency of 1 Hz. The researcher helped the subjects to maintain the rhythm by saying “push” every second. The practice took 5-8 minutes.

Exercise load was determined by the maximum repetition method. The number of heavy load plantar flexions that the subject was able to make was used to calculate the subject specific maximum load<sup>23</sup>. For fatiguing exercise 60% of maximal load was used. The subjects performed 10 plantarflexions followed by 10 s rest interval until: (1) they were not able to maintain the whole range of motion for five times, or (2) they did strong counter movements with increased dorsiflexion. During exercise, the subjects received verbal instructions, feedback and verbal encouragement to continue exercise until exhaustion. Immediately after the exercise the device was locked at an ankle angle of 90° and the MVC with superimposed twitch and twitches to relaxed muscle were remeasured.

*Data collection and analysis*

Torque was measured with a piezoelectric crystal transducer (Kistler, Switzerland) and was fed into a charge amplifier (Kistler, Type 5011). The angular movement of the ankle joint was measured using linear potentiometer. Surface EMG was collected with Eisa-system (Eisa 16-2, Freiburg, Germany) with bandwidth of 10 – 10 000 Hz per 3 dB and gain of 500. EMG, torque and angle signals were collected into a computer via analog-to-digital converter CED 1401 (Cambridge Electronics Design, UK) and stored using Signal 2.14 software with a sampling frequency of 2 kHz.

Peak torque from MVC (value just before superimposed twitch) and twitches were analyzed. The MVC value with the greatest torque and with the lowest amplitude of superimposed twitch was chosen for the analysis. The level of neural activation (LOA) was calculated using formula by Folland and Williams<sup>24</sup>:

$$\text{LOA (\%)} = \text{MVC}/\text{TMF} * 100, \text{ where } \text{TMF} = (1/(1-\text{Ts}/\text{Tc})) * \text{VolF}.$$

In the formula TMF is true maximal force, Ts superimposed twitch, Tc control twitch, VolF the level of voluntary force at which Ts is evoked, LOA (%) is the percentage of the muscle activation and MVC is the maximal voluntary force. Typically the peak torque of MVC occurred just before the twitch was elicited but it could have been different from VolF. From the twitch, time-to-peak and half relaxation times were analyzed (Fig. 1). From the EMG signal mean power frequency (MPF) and median frequency (MF) during MVC were calculated using FFT window width of 1024 data points. MPF and MF have relative advantages and disadvantages depending on the quality of the EMG signal and the shape of the spectrum. Thus the estimates of both MPF and MF provide an acceptably good

representation of the frequency shift of the myoelectric signal that occurs with fatigue process<sup>25</sup>. Maximum M-wave (Mmax) was measured as peak-to-peak amplitude (mV).

### *Statistical analysis*

Percentage changes in the variables after fatiguing task were calculated as  $\Delta\% = (\text{post value} - \text{pre value}) / \text{pre value} * 100\%$ . Intra-pair percentage differences (IPD%) were calculated as follows:  $\text{IPD} (\%) = (\text{HRT-user} - \text{non-user}) / \text{non-user} * 100$ . Mean IPD and 95 % confidence intervals (CI) were calculated. Normal distribution of the data was checked with Shapiro-Wilk test. Paired sample t-test was used to establish the intra-pair differences in the before fatigue condition. Wilcoxon Signed Ranks Test was used to calculate intra-pair differences for serum hormone concentrations because the data was not normally distributed. The effects of fatigue were tested using mixed model ANOVA (fatigue\*HRTuse). Intraclass correlation analysis between twins from key variables were performed to estimate the magnitude of genetic and environmental variance, under the assumption of no shared environmental effects as data from MZ pairs alone was available. These analyses were carried out using SPSS 15.0 for Windows (LEAD Technologies, Inc., USA) with significance level set at  $P < 0.05$ . Pearson's correlation coefficient together with linear regression analysis corrected for clustered sampling was used to investigate associations between variables (Stata 10.0, Stata Corporation, Texas, USA).

## **Results**

### *Intra-pair differences before fatigue*

As expected, the serum E<sub>2</sub> and free E<sub>2</sub> concentrations were higher ( $P < 0.01$ ) in the HRT users than non-users but that of testosterone and SHGB did not differ (Table 1). There was no significant intra-pair difference in maximal voluntary peak torque between users and non-users, but the twitch torque was 32% higher ( $P = 0.002$ ) in the HRT users (Table 2, Fig. 2). There were no differences in twitch time-to-peak or half relaxation times between the sisters. Neither did the EMG parameters differ between the twins before fatigue (Table 2).

The mean level of voluntary activation in the HRT users was  $85 \pm 11\%$  from the muscles' full capacity to produce force compared to  $90 \pm 15\%$  in the non-users ( $P = 0.3$ ). The pairwise intraclass correlation for maximal voluntary torque was 0.806 ( $P = 0.001$ ) but it was not significant for twitch torque ( $r = 0.314$ ,  $P = 0.3$ ). In the bivariate analysis adjusted for the dependency between the sisters the voluntary peak torque correlated positively with testosterone ( $r = 0.501$ ,  $P = 0.008$ ) and free testosterone ( $r = 0.605$ ,  $P = 0.003$ ) but twitch torque did not correlate with neither testosterone ( $r = 0.205$ ,  $P = 0.3$ ) nor estradiol ( $r = 0.177$ ,  $P = 0.4$ ).

#### *Effect of fatigue*

Duration and load of the exercise was similar in both groups: the HRT users exercised  $112 \pm 5$  s with load of  $57.3 \pm 9.5$  kg and the non-users  $137 \pm 9$  s with  $55.0 \pm 8.9$  kg ( $P = 0.3$  and  $P = 0.5$  for time and load, respectively). Also the work done during the exercise was similar ( $4390 \pm 1300$  J in users and  $4940 \pm 2060$  J in non-users,  $P = 0.4$ ). Maximal voluntary torque decreased  $13 \pm 17\%$  in the HRT users and  $8 \pm 13\%$  in non-users ( $P = 0.001$ ) without a significant intra-pair difference. Twitch torque ( $P = 0.001$ ), time-to-peak ( $P = 0.013$ ) and half relaxation time ( $P = 0.001$ ) decreased, but the decrement was similar both in the HRT users

and the non-users. However, the intra-pair difference in twitch torque observed before fatigue disappeared after the fatigue. GM MPF ( $P = 0.001$ ) and GM MF ( $P = 0.001$ ) decreased without intra-pair difference. The MPF and MF of the SOL did not change with fatigue.

## **Discussion**

The main finding was that the maximal voluntary torque did not differ between the HRT users and their non-using sisters, but the twitch torque elicited by electrical stimulation to the tibial nerve was 32% higher in the HRT users than in their non-using co-twins. Interestingly, we found high intraclass correlation of MVC between the twins suggesting a strong genetic control on voluntary force that has been previously documented in other muscles than plantarflexors<sup>15,26</sup>. In contrast, the lack of significant correlation of twitch torque suggests that the intrinsic, involuntary force producing capacity can be affected by HRT in early postmenopausal women.

The controversial finding that involuntary but not voluntary torque was affected by HRT can result from few reasons. First, the electrical stimulation used to elicit the twitch response parallels animal studies where contractions are involuntary and do not involve central factors that can play large role in voluntary contractions. In accordance to the present results, a recent animal experiment showed a direct positive effect of E<sub>2</sub> on twitch torque of rat genioglossus muscle<sup>27</sup>. This effect was at least partly mediated via regulation of estrogen receptor alpha, both at transcript and protein level. Also Hatae<sup>28</sup> has shown an immediate increasing effect of estradiol administration on twitch force in frog skeletal muscle fibers. Hatae suggests that the twitch potentiation may result from the unbalanced Ca<sup>2+</sup> turnover in cytoplasm with the rate of Ca<sup>2+</sup> release from sarcoplasmic reticulum being slightly faster than

Ca<sup>2+</sup> reuptake. On the other hand, Ca<sup>2+</sup> sensitivity may remain the same although isometric force is reduced in soleus fibers of estrogen deficient ovariectomized rats<sup>29</sup>. Besides changes in the calcium mechanism, E<sub>2</sub> can induce changes in the myosin filaments. Specifically, reduction in strong-binding structural state of myosin head leading to reduction in force has been reported in ovariectomized mice, while this effect is reversed in the presence of E<sub>2</sub><sup>30</sup>.

The second reason for the present controversial findings between MVC and twitch torque may be a different neural control between subjects<sup>31</sup>. Especially older adults and those who are not used to maximal contractions co-contract antagonist muscles with the end result that the torque or force does not reflect the full capacity of the muscle measured. In the present study, we measured TA EMG activity and it was found to be active to some extent in 19 subjects (Fig. 1). Unfortunately, we did not measure maximal dorsiflexion which would have enabled us to estimate the effect of the co-contraction to the plantarflexion torque. We do suggest that this effect should be taken into account in the future studies. This is one potential reason why the present study revealed association of HRT with twitch but not on maximal voluntary torque.

Previous research has either found<sup>10,11,32</sup> or not found effects of HRT on muscle voluntary force<sup>12,33,34</sup> (see also<sup>14</sup>). The reasons for the contradictions may come from several factors. In addition to the study design, methodology and study group, the dose and duration of HRT usage, and the time between menopause and data collection may be very important variables. In this study the self-reported mean time from menopause was 7.0 (range 3-13) years in the non-users and 8.3 (range 3-19) years in the HRT users with mean of 6.8 years (range 2-16) on the treatment.

Besides the length of HRT administration also the age is an important factor. In the present study group there was only one twin pair over 60 years of age and they had the largest intra-pair difference in twitch (113 %, Fig. 2) but not in MVC (3 %). These results gives us a possibility to put forth a hypothesis that the differences due to HRT are first expressed in twitch and only at later age the effect may be visible also in MVC.

Regarding the twitch time-to-peak or half relaxation times, we found no differences between the twins. Contrary to these results, an increase in twitch 70 % decay time<sup>27</sup> and half-relaxation time in rats having greater serum E<sub>2</sub> concentration have been reported<sup>35</sup>. Comparison of in vivo human studies with animal experiments is not, however, straightforward. For example, in humans the force measured is always a sum from several muscles while animal experiments are often made with isolated muscles or muscle fibers ex vivo. Further, maturation and age related effects in animals and also in human studies complicate the direct comparison between the studies.

#### *Effect of HRT on fatigability*

Torque and twitch characteristics were similarly affected by fatigue in co-twins regardless of their HRT status. While statistically not significant, the exercise time to fatigue was 17.5 % shorter and work done 11.2 % smaller in the HRT users compared to the non-users. In spite of the lack of significance in these variables there were also other indications of greater fatigability in HRT-users. The facilitated fatigue may be seen in the result that the twitch torque was greater in HRT users before fatigue but the users fatigued more so that the intra-pair difference disappeared after the fatiguing exercise.

These modest indications of greater fatigability with greater estradiol level are supported by animal experiments. Both Hou et al<sup>27</sup> in *in situ* rat muscles and Hatae<sup>28</sup> in single frog muscle fibers reported greater fatigability with E<sub>2</sub> administration.

During the exercise we monitored changes in EMG power spectrum in both SOL and GM muscles. The plantarflexor exercise caused fatigue that was seen in the spectral parameters in GM but not in SOL muscle. This may be because GM muscle is faster and more fatigable and because the experimental setting with extended knee left the GM in optimal position for work production<sup>36,37</sup>.

Since the present protocol primarily fatigued GM muscle, which contains more fast twitch fibers than SOL<sup>38</sup>, and resulted in greater, although non-significant decrease of the twitch in the HRT users, the findings may be speculated in the light of previous results regarding the effect of estrogen on type II fibers. More specifically, estrogen has been suggested to have a preservative effect on type II fibers whereas estrogen deficiency can induce a shift in the fiber types toward fatigue resistant type I fibers<sup>9</sup>. If this fiber type change in the GM of HRT users is prevented, it could be an alternative explanation why the twitch was higher before fatigue, and fatigue resistance was lower in the HRT users.

Hatae<sup>28</sup> suggested that with high levels of E<sub>2</sub> the reuptake of released Ca<sup>2+</sup> by sarcoplasmic reticulum could not be accomplished on time resulting in decreases in the following contraction. Thus, the same mechanism of E<sub>2</sub> action that is involved in potentiating twitch may contribute to faster fatigability. Importantly, the fact that twitch force was greater in the HRT users before fatigue must be noted when functional implications of HRT on fatigability are under consideration. The high initial capacity of muscles to produce force is essential in older age and if it comes with greater fatigability it may be a useful trade off.



**Conclusion**

In early postmenopausal women HRT is associated with higher intrinsic force-generating capacity of the plantarflexors. However, this HRT-related augmentation in involuntary force is not observed in voluntary contractions. This contradiction may result from central factors such as different antagonist co-activation during plantarflexion task.

**List of abbreviations**

CI Confidence interval

E<sub>2</sub> Serum 17β-estradiol

GM Musculus gastrocnemius medialis

HRT Hormone replacement therapy

IPD Intra-pair differences

LOA Level of neural activation

MF Median frequency

Mmax Maximum M-wave

MPF Mean power frequency

MVC Maximal voluntary torque

MZ Monozygotic

SHBG Sex hormone binding globulin

SOL Musculus soleus

TA Musculus tibialis anterior

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TABLE 1. Physical characteristics and hormone levels of the subjects (mean  $\pm$  SD). P-value shows the significance of the intra-pair difference (Paired t-test for physical characteristics and Wilcoxon Signed-Rank test for hormones and fiber types). Negative values of intra-pair difference (IPD) indicate that non-user had higher value.

| Variable                           | HRT- users          | Non-users           | IPD % (95% CI)           | P-value |
|------------------------------------|---------------------|---------------------|--------------------------|---------|
| Height (cm)                        | 163.6 $\pm$ 5.1     | 162.7 $\pm$ 4.6     | 0.6 (0.1 to 1.0)         | 0.028   |
| Weight (kg)                        | 68.7 $\pm$ 8.6      | 69.6 $\pm$ 13.5     | 0.9 (-9.2 to 11.1)       | 0.8     |
| BMI (kg/m <sup>2</sup> )           | 25.7 $\pm$ 3.1      | 26.4 $\pm$ 6.0      | -0.2 (-9.8 to 9.3)       | 0.6     |
| Body fat (%)                       | 30.7 $\pm$ 6.7      | 32.5 $\pm$ 8.4      | -2.9 (-15.3 to 9.4)      | 0.2     |
| 17 $\beta$ - estradiol (pmolL)     | 136.00 $\pm$ 198.56 | 29.54 $\pm$ 25.53   | 557.7 (-160.1 to 1275.4) | 0.004   |
| Free 17 $\beta$ -estradiol (pmolL) | 2.59 $\pm$ 3.23     | 0.69 $\pm$ 0.55     | 407.8 (-47.9 to 863.4)   | 0.007   |
| Testosterone (pmolL)               | 718.46 $\pm$ 278.65 | 796.92 $\pm$ 361.49 | -2.6 (-19.6 to 14.4)     | 0.7     |
| Free testosterone (pmolL)          | 9.94 $\pm$ 5.55     | 10.80 $\pm$ 5.04    | -6.3 (-23.7 to 11.1)     | 0.3     |
| SHBG (nmolL)                       | 59.28 $\pm$ 35.78   | 52.29 $\pm$ 22.51   | 30 (-22.2 to 82.6)       | 0.6     |

BMI = body mass index, SHBG = sex hormone-binding globulin

TABLE 2. Contractile and EMG signal characteristics of the twins before fatigue (mean  $\pm$  SD,  $N = 13$ ).  $P$ -value is from the paired t-test and percentage intra-pair differences (IPD%) with 95% confidence intervals are given. Negative values of IPD indicate that non-user had higher value.

| Pre variables                      | HRT-users        | Non-users         | IPD (%) 95 % CI      | P-value |
|------------------------------------|------------------|-------------------|----------------------|---------|
| Maximal torque (Nm)                | 116 $\pm$ 28     | 120 $\pm$ 27      | -3.5 (-13.2 to 6.2)  | 0.3     |
| Twitch (Nm)                        | 28.0 $\pm$ 6     | 21.9 $\pm$ 5      | 31.7 (14.2 to 49.2)  | 0.002   |
| Time-to-peak (ms)                  | 149 $\pm$ 18     | 147 $\pm$ 22      | 2.3 (-5.6 to 12.1)   | 0.6     |
| $\frac{1}{2}$ relaxation time (ms) | 129 $\pm$ 24     | 128 $\pm$ 45      | 6.6 (-9.4 to 23.0)   | 0.9     |
| Activation level (%)               | 85 $\pm$ 10      | 90 $\pm$ 15       | -1.5 (-17.8 to 14.8) | 0.3     |
| Mmax (mV)                          | 2.95 $\pm$ 1.47  | 2.79 $\pm$ 1.64   | 11.1 (-20.9 to 43)   | 0.8     |
| SOL MPF (Hz)                       | 125.7 $\pm$ 31.1 | 128.8 $\pm$ 288.7 | 2.7 (-17.1 to 22.5)  | 0.3     |
| SOL MF (Hz)                        | 104.2 $\pm$ 31.2 | 102.1 $\pm$ 29.8  | 7.5 (-14.4 to 29.3)  | 0.8     |
| GM MPF (Hz)                        | 137.0 $\pm$ 36.1 | 139.1 $\pm$ 29.1  | -0.5 (-14.5 to 13.5) | 0.8     |
| GM MF (Hz)                         | 115.5 $\pm$ 37.0 | 111.4 $\pm$ 29.9  | 4.3 (-11.7 to 20.3)  | 0.5     |

SOL = soleus, GM = gastrocnemius medialis, MPF = mean power frequency of EMG signal, MF = median frequency of EMG signal

**Legends to figures**

FIGURE 1. Raw recordings of torque and EMG of the SOL, GM and TA muscles during maximal voluntary contraction with superimposed twitch and twitch. The twitch was elicited by electrical stimulation to tibial nerve, timing of which is seen from the stimulation induced artefact in the EMG signal. Time-to-peak (TTP) and half relaxation times ( $\frac{1}{2}$ RT) were measured from the twitch.

FIGURE 2. Differences in twitch torque between the co-twins as a function of age.

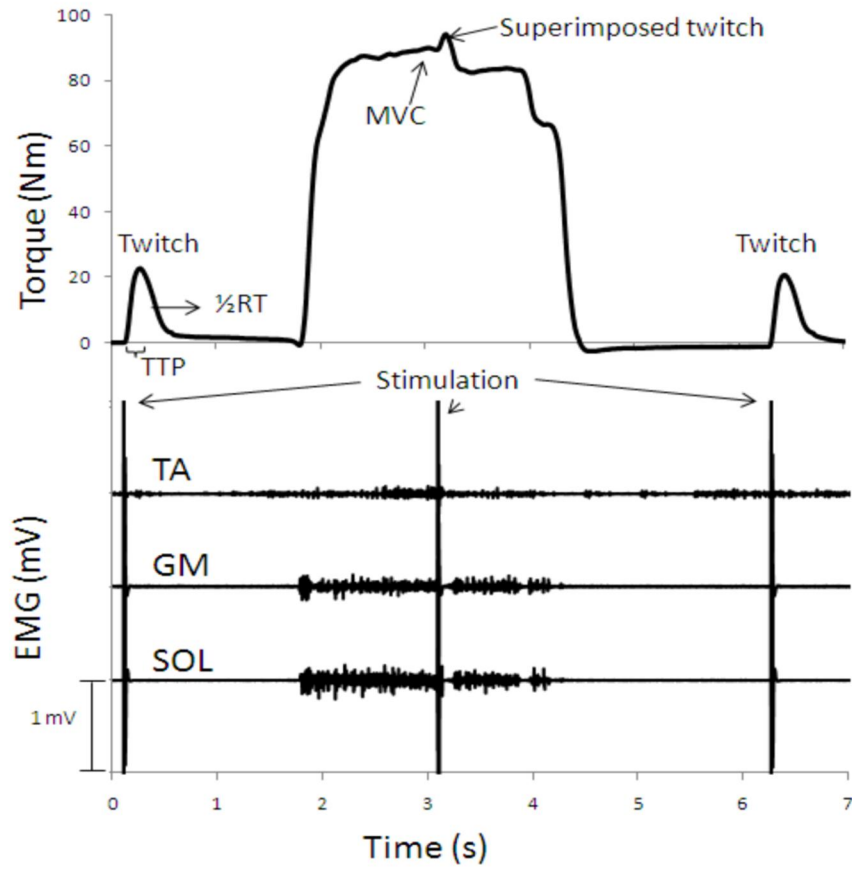


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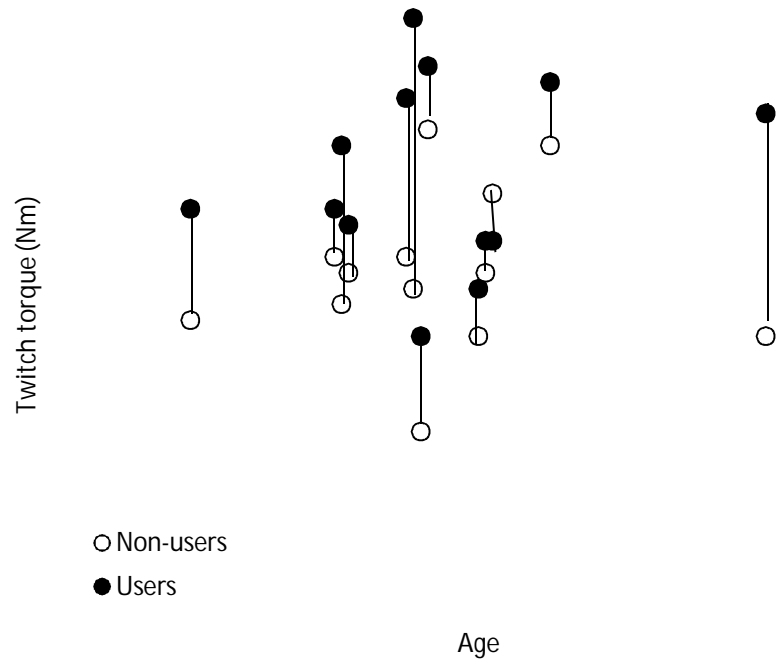


FIGURE 2 Differences in twitch torque between the co-twins as a function of age.