Effects of acute changes in oestrogen on muscle function of the first dorsal interosseus muscle in humans

J. P. Greeves, N. T. Cable*, M. J. M. Luckas†, T. Reilly and M. M. Biljan†

Centre for Sport and Exercise Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF and †Reproductive Medicine Unit, Liverpool Women's Hospital, Crown Street, Liverpool L8 7SS, UK

1. To investigate the effect of the female reproductive hormones on muscle function, patients undergoing *in vitro* fertilization were tested during two phases of treatment. The first was following the downregulation of pituitary gonadotrophin releasing hormone (GnRH) receptors and the second after 9 days of gonadotrophin injections.

2. Maximal strength and fatiguability of the first dorsal interosseus muscle were assessed when oestrogen and progesterone were low, and less than 2 weeks later when oestrogen production reached supraphysiological levels.

3. There were no significant changes in either strength or fatigue resistance during acute, massive fluctuations in oestrogen. These results occurred at a time when progesterone levels remained relatively low.

4. Contrary to previous work, the present results suggest that oestrogen does not affect muscle strength.

The menopause is defined as a loss of ovarian function, characterized by very low concentrations of oestrogen and progesterone (Whitehead & Godfree, 1994). This hormonal change has been associated with a significant reduction in maximal voluntary contraction per cross-sectional area (MVC/CSA) in postmenopausal women for the adductor pollicis muscle (Phillips, Rook, Siddle, Bruce & Woledge, 1993*b*; Phillips, Rowbury, Bruce & Woledge, 1993*c*) and the quadriceps (Rutherford & Jones, 1992). Administration of hormone replacement therapy prevents the loss of specific force associated with the menopause (Phillips *et al.* 1993*b*), indicating that this muscle weakness is related to changes in reproductive hormone status, rather than an age-related decrement in the force-generating capacity of muscle (Bruce, Newton & Woledge, 1989).

Both oestrogen and progesterone levels diminish at the menopause and thus the hormone responsible for the reduction of force-generating capacity is uncertain. In eumenorrhoeic younger females there are cyclical changes in strength of the adductor pollicis (Phillips, Gopinathan, Meehan, Bruce & Woledge, 1993*a*) and the quadriceps (Sarwar, Beltran Niclos & Rutherford, 1996) during the menstrual cycle. Maximum strength is reported to coincide with the mid-cycle peak of oestrogen and lowest force production occurs at the post-ovulatory trough of oestrogen concentrations (Phillips, Rutherford, Birch, Bruce & Woledge, 1995). Current evidence therefore supports the positive role of oestrogen for enhancing strength.

In the present study, the independent effect of oestrogen on muscle function was examined in young females undergoing *in vitro* fertilization (IVF). In this model, oestrogen levels are significantly reduced after 3 weeks of administration of gonadotrophin releasing hormone (GnRH) analogues, which downregulate pituitary GnRH receptors. Secretion of gonadotrophin and ovarian steroid hormones is subsequently suppressed. This is followed by 9–10 days of injected exogenous gonadotrophins, which hyperstimulate the ovaries to produce multiple follicles and consequently very high oestrogen levels.

Maximal voluntary contraction (MVC) and fatigue characteristics of a small muscle, the first dorsal interosseus (FDI), were measured following downregulation and during hyperstimulation of the ovary.

METHODS

Subjects

Fourteen volunteers undergoing *in vitro* fertilization treatment, with a mean ± S.D. age of 34·7 ± 4·2 years, height of 162·2 ± 4·5 cm and body mass of 61·1 ± 6·2 kg were recruited from the Reproductive Medicine Unit of the Liverpool Women's Hospital. Informed consent was obtained by the medical staff and the patients were referred to the laboratory. The cause of infertility in

* To whom correspondence should be addressed.

Table 1. Oestradiol concentrations and endometrial thickness of patients as determinants of adequate downregulation and hyperstimulation of *in vitro* fertilization treatment

Condition	Oestradiol (pmol l^{-1})	Endometrial lining (mm)
Hypoestrogenia	10–100	2·4–4·2
Hyperoestrogenia	1551–9935	6·9–14

all the subjects recruited in this study was damaged fallopian tubes. Their follicle stimulating hormone (FSH) levels at day 2 or 3 of the menstrual cycle were 2 to 6 u l^{-1} indicating normal ovarian function and reserve (Toner, 1993). Patients with abnormal follicle stimulating hormone (FSH): luteinizing hormone (LH) ratios, oligomenorrhoea or ultrasonic evidence suggesting polycystic ovarian syndrome were excluded from the study. Patients taking medication likely to affect muscle strength did not participate. Subjects gave their written informed consent to participate in the study, which was approved by the Ethics Committees of Liverpool John Moores University and The Royal Liverpool University Hospital.

Experimental protocol

Maximal voluntary contraction and fatigue resistance of the FDI were measured on two occasions, separated by 9 days. All tests were performed in the morning. The first measurement was undertaken during a hypoestrogenic state following a course of the GnRH analogue nafarelin (200 mg twice daily; Synarel®, Roche). Downregulation was confirmed by ultrasonic demonstration of an endometrial thickness under 4 mm. This is associated with plasma oestrogen levels under 100 pmol l^{-1} (Santolaya-Forga, Ramakrishnan & Scommegna, 1992). The second measurement was performed after 9 days of gonadotrophin injections, which hyperstimulate the ovaries, producing very high oestrogen levels. Plasma oestradiol was measured in all patients at both stages of treatment (Table 1).

Measurement of muscle strength

Force production was measured using a dynamometer designed to isolate the FDI muscle. The hand was pronated and positioned on a metal plate mounted on a wooden platform. The forearm, which rested on the diagonal slope of the platform, was securely strapped at the wrist, mid-forearm and lower portion of the elbow joint. The lateral side of the distal interphalangeal joint of the index finger was aligned with the force transducer attached to a strain gauge (Model UL4000, Maywood Instruments Limited, Basingstoke, UK). The thumb was fully abducted and secured with a strap around the proximal phalange. The remaining fingers were strapped together and secured onto Velcro webbing to reduce force production from other muscles (Fig. 1). Upward movement of the index finger was prevented by a clamp tightened at the base of the phalange. The position of the hand was standardized for each session to ensure the muscle length was consistent between trials. The hand and forearm were initially immersed in warm water at 40 °C for 10 min to increase blood flow, and throughout the

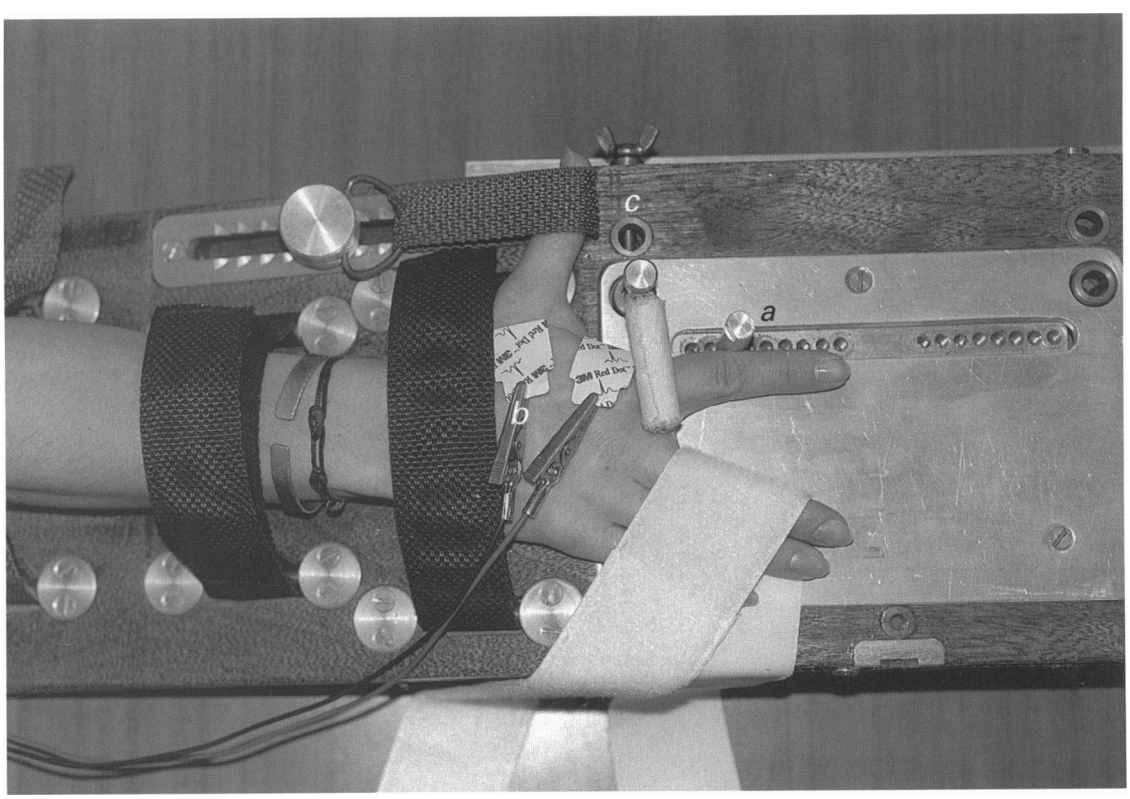

Figure 1

Dynamometer showing the index finger in relation to force transducer (*a*), electrodes (*b*) and thumb (*c*).

experiment a reading lamp was positioned at a standard distance over the muscle. Whilst muscle temperature was not measured, this procedure was repeated on both occasions in an attempt to standardize muscle temperature.

The FDI was stimulated percutaneously with self-adhesive surface electrodes (3M Healthcare, St Paul, MN, USA). The cathode was positioned on the belly of the FDI and the anode placed near the carpometacarpal joint of the thumb. The muscle was stimulated with 1 and 40 Hz tetani to confirm accurate location of the electrodes. Electrical impulses were applied at 150 V at a pulse width of 100 μs duration with a computer-driven Digitimer stimulator (Model DS7, Digitimer Ltd, Welwyn Garden City, UK). The force output was amplified and visually displayed on an Apple Macintosh computer, interfaced with a data acquisition system (Biopac MP100WS, Santa Barbara, CA, USA).

Maximal voluntary contraction

Maximal volunatary contraction of the FDI was measured whilst fully abducting the index finger. This is the only muscle involved in producing this movement. Superimposed percutaneous electrical stimulation was employed to ensure maximal activation of the FDI. Disappearance of the 1 Hz impulses confirmed maximal volitional force. Each trial was preceded by a 60 s rest interval. The highest of three trials was recorded. The coefficient of variation (c.v.) from repeated tests using this technique in our laboratory is 9·6% with limits of agreement ranging from −8·54 to 8·43 N.

Fatigue characteristics

Fatigue resistance of the FDI was assessed using a modified Burke protocol (Burke, Levine, Tsairis & Zajac, 1973). This involved repeatedly stimulating the muscle for 3 min at 40 Hz with a 1 s interval between each tetanic contraction. Patients were not able to tolerate voltages that were sufficient to elicit maximal stimulation of the muscle, although the current was constant for individual subjects between tests. Forces of up to 20% of maximal voluntary contraction (MVC) were recorded. Figure 2*A* and *B* displays typical myograms of a tetanic contraction in a fresh and fatigued state. The fatigue index (FI) was calculated as the percentage loss of force over the 3 min (Fig. 2*C*). Speed of relaxation was measured as the time taken for peak force to reach half-peak force. A 2 min rest was allowed between the MVC and before commencing the fatigue test. Fatigue results are presented for seven subjects; the remaining patients did not tolerate the 40 Hz electrical impulse.

Statistical analysis

Differences in MVC and fatigue characteristics (force loss, mean time to peak tension and relaxation rate expressed as percentage of initial force) between the two test conditions were assessed using Student's paired t tests. The significance level was set at 5%.

RESULTS

Maximal voluntary contraction

There were no significant differences in maximal voluntary contraction of the FDI between the low (27·9 ± 1·6 N) and high (27·5 ± 1·5 N) oestrogen conditions (means ± S.E.M.) ($P > 0·05$). This is seen in Fig. 3.

Fatigue test

A typical myogram of the fatigue test is shown in Fig. 2*C*. There were no statistically significant differences in any of the fatigue parameters measured during the fatigue test between the two trials ($P > 0·05$). There was a loss of peak

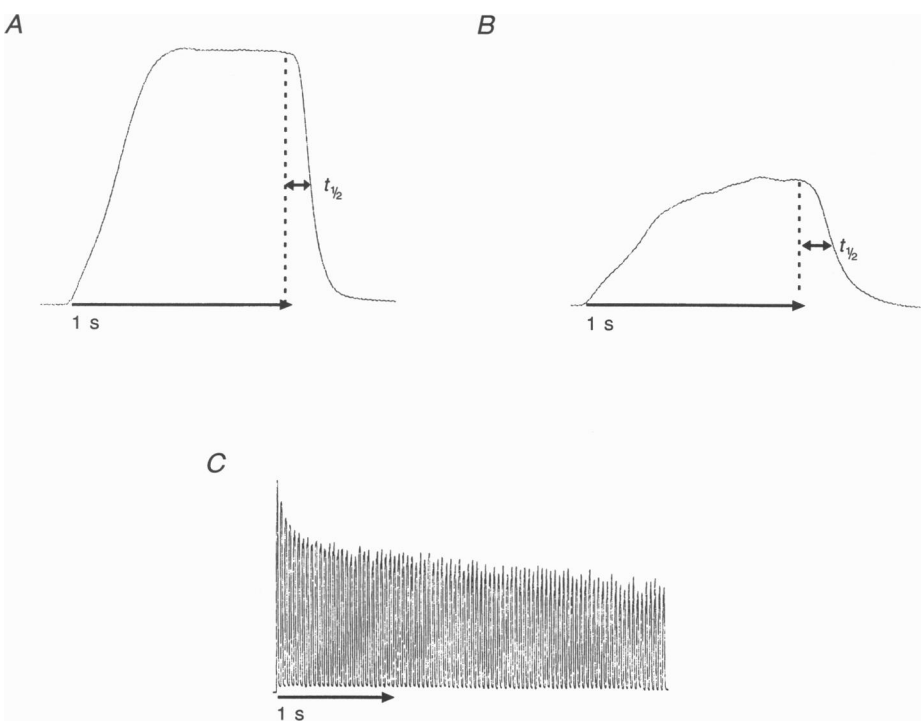

Figure 2. Typical myograms from the fatigue test

A, tetani in fresh muscle showing half-relaxation time ($t_{½}$). *B*, tetani in fatigued muscle illustrating slowing of relaxation. *C*, trace from a fatigue test (1 s, 40 Hz twitch for 3 min).

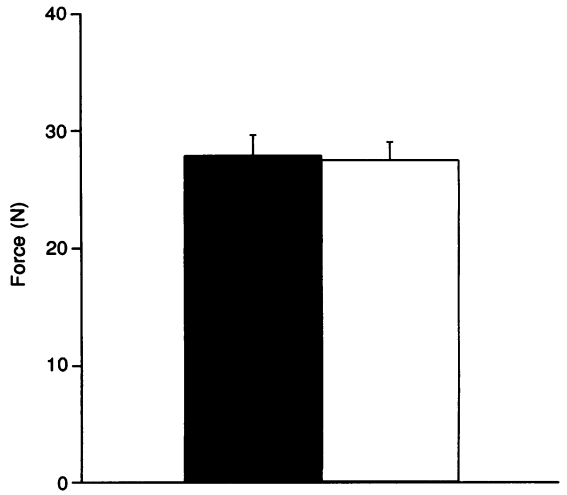

Figure 3

Maximal voluntary contraction (MVC; means ± S.E.M.) of the first dorsal interosseus (FDI) muscle in hypoestrogenic (■) and hyperoestrogenic females (□). There was no significant difference between treatments.

tension over the 3 min of 36·4 ± 6·8% in the hypoestrogenic and 42·5 ± 15·6% in the hyperoestrogenic condition. Mean twitch tension also diminished to 58·9 ± 7·6 and 57·5 ± 9·2% of its initial value following the low and high oestrogen concentrations, respectively. Half-relaxation time increased by 52·9 ± 16·4 and 81·4 ± 20·1% during the fatigue test in hypo- and hyperoestrogenic conditions, respectively.

DISCUSSION

Muscle function was measured in IVF patients following (1) downregulation of pituitary GnRH receptors and (2) administration of exogenous gonadotrophins. The hormonal consequences of this treatment are low oestrogen levels due to a lack of pituitary stimulation and subsequently massive increases in oestrogen concentrations due to development of multiple follicles. Under these conditions, we did not observe any change in maximal strength or fatigue resistance of the FDI.

These results suggest that oestrogen does not influence force. However, muscle weakness reported in postmenopausal women is prevented in females taking hormone replacement therapy (Philllips *et al.* 1993*b*) implicating the role of reproductive hormones in this loss of strength. Evidence from studies undertaken during the menstrual cycle also indicates oestrogen is responsible for these changes in strength, as maximal strength is greatest around midcycle, at the peak of oestrogen production (Phillips *et al.* 1993*a*; Sarwar *et al.* 1996).

In the above models, oestrogen is accompanied by fluctuations in the other main reproductive hormone, progesterone, and peptide gonadotrophins follicle stimulating hormone (FSH) and luteinizing hormone (LH), this makes it impossible to isolate the hormone responsible for a strengthening effect. In IVF patients, pituitary down-regulation suppresses both oestrogen and progesterone. Oestrogen increases with subsequent gonadotrophin administration while progesterone remains low. Although progesterone concentrations were not measured in the present study, progesterone values during this procedure remain low until human chorionic gonadotrophin (hCG) is given prior to egg collection (Harada *et al.* 1995). Patients in this study were tested before hCG was given. Consequently,

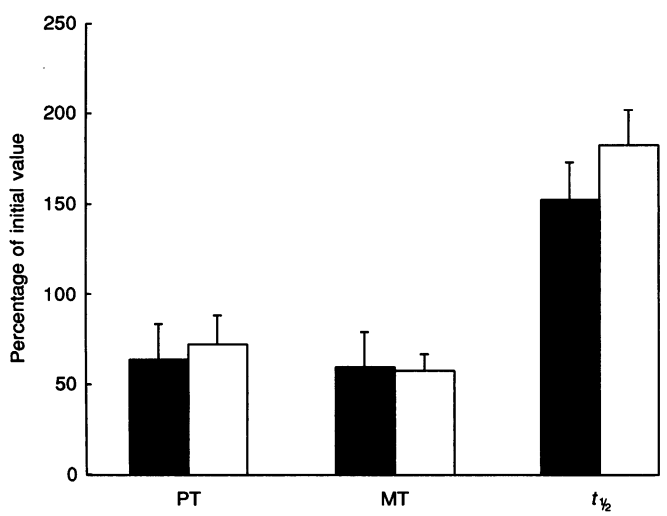

Figure 4. Fatigue characteristics of the FDI muscle in hypoestrogenic and hyperoestrogenic females

■, hypoestrogenia; □, hyperoestrogenia. Values at the end of the fatigue test for peak tension (PT), mean tension (MT) and half-relaxation time ($t_{½}$) are reported as a percentage of initial value. There were no significant differences between treatments for any variable.

muscle strength was examined when both oestrogen and progesterone concentrations were very low and again with high oestrogen and low progesterone concentrations.

To our knowledge, this is the first study to assess the strength-related effects of oestrogen independently from other reproductive hormones. Furthermore, downregulation of GnRH receptors at the stage of IVF treatment inhibits gonadotrophin secretion. The subsequent administration of these peptide hormones, initiating follicular growth, results in high concentrations of the gonadotrophins concurrent with the increasing levels of oestrogen. These results therefore also suggest that muscle strength is not influenced by fluctuations in LH and FSH which occur at this time.

Since the present study argues against an independent role of oestrogen on muscle strength, the alterations in force production cited in previous work (Phillips *et al.* 1993*a*, *b*, *c*; Sarwar *et al.* 1996) may be influenced by progesterone, or may be a consequence of an interaction between oestrogen and progesterone. This possibility is supported by the observation that the force-generating capacity of the FDI in the IVF patients was much lower than age-matched females at the same stage of the menstrual cycle in our laboratory (J. Greeves, unpublished observations). Due to the nature of subject recruitment in the present study, it was not possible to establish baseline values of MVC prior to pituitary downregulation. The possibility therefore exists that the MVCs measured after downregulation may be reduced in response to a decline in progesterone concentrations. The fact that MVC values remained constant following hyperstimulation of the ovary (producing large increases in oestrogen but little change in progesterone) further implicates progesterone as a moderator of muscle strength. This hypothesis warrants further investigation.

Our findings against a positive effect of oestrogen have been demonstrated in previous work. Bassey, Coates, Culpan, Littlewood, Owen & Wilson (1995) reported that oestrogen has a negative influence on strength. In young, eumenorrhoeic females, oestrogen concentration was inversely related to handgrip strength, which declined by 5% with a 200 pmol l^{-1} rise in oestrogen. Furthermore, in older subjects (aged 45–54 years) of varying menstrual status, no differences in strength of the quadriceps or in handgrip strength have been observed (Bassey, Mockett & Fentem, 1996).

Few studies have used fatiguability as a parameter for assessing muscle function in relation to hormonal changes. Electrical stimulation was employed to determine the fatigue resistance of the FDI via electrically evoked impulses. There were no significant differences in fatigue characteristics between the hypo- and hyperoestrogenic conditions. These observations are contrary to those reported previously. Sarwar *et al.* (1996) measured the quadriceps during five stages of the menstrual cycle, early and mid-follicular, ovulatory and mid- and late luteal. The muscle was slower and more fatiguable at ovulation, compared with the late-luteal phase. No changes were observed in women on the combined oral contraceptive pill as a control group.

The fatigue protocol used in the present study differed from that employed by Sarwar *et al.* (1996). The frequency of stimulation was the same although contraction time and relaxation were longer in duration. As a consequence, the FDI did not fatigue to the same extent as the quadriceps. This difference was not the result of stimulating a larger muscle, since the fatigue index of the adductor pollicis and the FDI are also higher using a similar protocol to previous studies (Tanaka McDonagh & Davies, 1984; Rutherford & Jones, 1988; Sarwar *et al.* 1996). Since the FDI muscle in the present study was less fatigued, it is possible that any hormonally induced strength changes were less apparent. However, given the precise manipulation in the hormonal milieu, it is far more likely that the lack of change in FI reflects minimal change in concentration of progesterone. This hypothesis is supported by the observation that fatiguability relates to changes in basal body temperature secondary to increases in progesterone concentration during the luteal phase (Sarwar *et al.* 1996).

In conclusion we have failed to detect an independent effect of oestrogen on muscle function. Given that muscle strength remained constant when both LH and FSH also changed markedly, the present results suggest that changes in progesterone alone, or in combination with other reproductive hormones, may be responsible for the changes in strength previously reported, both postmenopausally and during the menstrual cycle.

Bassey, E. J., Coates, L., Culpan, J., Littlewood, J. J., Owen, M. & Wilson, K. (1995). Natural variations in oestrogen and FSH levels in eumenorrheic women in negative association with voluntary muscle strength. *Journal of Physiology* **489.P**, 28*P*.

Bassey, E. J., Mockett, S. P. & Fentem, P. H. (1996). Lack of variation in muscle strength with menstrual status in healthy women aged 45–55 years; data from a national survey. *Journal of Physiology* **491.P**, 84*P*.

Birch, K. M., McFadyen, I. R., Reilly, T. & Sinnerton, S. (1993). Menstrual cycle effects on isometric and dynamic lifting. In *Contemporary Ergonomics*, ed. Lovesy, E. J., pp. 308–312. Taylor & Francis, London.

Bruce, S. A., Newton, D. & Woledge, R. C. (1989). Effect of age on voluntary force and cross-sectional area of human adductor pollocis muscle. *Quarterly Journal of Experimental Physiology* **74**, 359–362.

Burke, R. E., Levine, D. N., Tsairis, P. & Zajac, F. E. (1973). Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *Journal of Physiology* **234**, 723–748.

Harada, T., Yoshida, S., Katagiri, C., Takao, N., Ikenari, T., Toda, T., Mio, Y. & Terakawa, N. (1995). Reduced implantation rate associated with a subtle rise in serum progesterone concentration during the follicular phase of cycles stimulated with a combination of a gonadotrophin-releasing hormone agonist and gonadotrophin. *Human Reproduction* **10**, 1060–1064.

PHILLIPS, S. K., GOPINATHAN, J., MEEHAN, K., BRUCE, S. A. & WOLEDGE, R. C. (1993*a*). Muscle strength changes during the menstrual cycle in human adductor pollicis. *Journal of Physiology* **473**, 125*P*.

PHILLIPS, S. K., ROOK, K., SIDDLE, N., BRUCE, S. & WOLEDGE, R. C. (1993*b*). Muscle weakness in women occurs at an earlier age than in men, but strength is preserved by hormone replacement therapy. *Clinical Science* **84**, 95–98.

PHILLIPS, S. K., ROWBURY, J., BRUCE, S. & WOLEDGE, R. C. (1993*c*). Muscle force generation and age: the role of sex hormones. In *Sensori-motor Impairments in the Elderly*, ed. STELMACH, G. E. & HOLMBERG, V., pp. 129–141. Kluwer Academic.

PHILLIPS, S. K., RUTHERFORD, O. M., BIRCH, K., BRUCE, S. A. & WOLEDGE, R. C. (1995). Hormonal influences on muscle force: evidence for an inotropic effect of oestrogen. *Sports Exercise and Injury* **1**, 58–63.

RUTHERFORD, O. M. & JONES, D. A. (1988). Contractile properties and fatiguability of the human adductor pollicis and first dorsal interosseus: A comparison of the effects of two chronic stimulation patterns. *Journal of the Neurological Sciences* **85**, 319–331.

RUTHERFORD, O. M. & JONES, D. A. (1992). The relationship of muscle and bone loss and activity levels with age in women. *Age and Ageing* **21**, 286–293.

SANTOLAYA-FORGA, S., RAMAKRISHNAN, V. & SCOMMEGNA, A. (1992). The menstrual cycle: Relations of biophysical and hormonal determinations in normal women of reproductive age. *Fertility and Sterility* **58**, 1230–1233.

SARWAR, R., BELTRAN NICLOS, B. & RUTHERFORD, O. M. (1996). Changes in muscle strength, relaxation rate and fatiguability during the human menstrual cycle. *Journal of Physiology* **493**, 267–272.

TANAKA, M., MCDONAGH, M. J. N. & DAVIES, C. T. M. (1984). A comparison of the mechanical properties of the first dorsal interosseus in the dominant and non-dominant hand. *European Journal of Applied Physiology* **53**, 17–20.

TONER, T. P. (1993). The significance of elevated FSH for reproductive function. *Baillieres Clinical Obstetrics and Gynaecology* **7**, 283–295.

WHITEHEAD, M. I. & GODFREE, V. (1994). *Hormone Replacement Therapy: Your Questions Answered.* Churchill Livingstone, Edinburgh.

Acknowledgements

We would like to acknowledge the help of Dr W. Buckett and Dr I. Aird for their technical assistance with subject recruitment and Mr C. Kingsland for the continued support of the Reproductive Medicine Unit at Liverpool Women's Hospital.

Author's email address

N. T. Cable: t.cable@livjm.ac.uk

Received 20 August 1996; accepted 6 December 1996.