THE EFFECT OF REPRODUCTIVE HORMONES ON MUSCLE FUNCTION IN YOUNG AND MIDDLE-AGED FEMALES

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A thesis submitted in partial fulfilment of the requirements of Liverpool John Moores University for the degree of Doctor of Philosophy

May 1997

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ABSTRACT

The menopause is associated with a deficiency of reproductive hormones, and accompanied by a significant loss of bone mass. This bone loss is accelerated within the first five years post-menopause. Muscle strength at this time would have important clinical implications for correcting imbalance and preventing falls. The aim of the studies within this thesis were to 1) determine the rate and time course of force loss of the quadriceps muscle group over 12 months in three groups of women with varying hormonal status 2) establish the role of oestrogen in this weakness and 3) investigate the effectiveness of hormone replacement therapy (HRT) in maintaining muscle function.

The reliability of an isokinetic dynamometer and a strain gauge assembly was examined initially to determine the inherent variability of muscle function assessment. Strength of the knee extensors measured on the isokinetic dynamometer was deemed reliable in middle-aged women, although at 1.05 rad/s more practice trials were needed to attain peak torque. Measurements of the knee flexors were highly variable. Maximal voluntary isometric contractions were repeatable using the strain gauge system, for both the knee extensors and first dorsal interosseus (FDI) muscle. There was greater variability in force production generated from electrically stimulated contractions.

Maximal strength of the knee extensors declined by 9.3±4.6 and 10.3±3.1% (mean±SE) for dynamic (1.05 rad/s) and isometric strength respectively over 9 months in hypoestrogenic post-menopausal women. There were no changes at higher angular velocities, or for handgrip strength. These results support the role of reproductive hormones in influencing force production, which is further endorsed by the observation that females on HRT did not experience a reduction in strength over this time. The force loss was significant only when the post-menopausal and HRT group were compared ($p<0.05$). The post-menopausal group were within 1 to 3 years past the menopause, the time period in which bone loss is rapid. This rapid loss of strength would therefore be expected to level out, similarly to bone.

The menopause is an oestrogen-deficient and progesterone-deficient endocrinopathy. It is not possible to identify which hormone, if not both, is responsible for these observed changes in strength. To explore the relationship between acute changes in oestrogen and progesterone and strength, maximal force production of the quadriceps and first dorsal interosseus (FDI) was measured across the menstrual cycle. Maximal strength of the quadriceps was lowest prior to the surge in luteinizing hormone (LH) and reached its peak mid-luteal, a difference of 12.6±4.3% (mean±SE). These changes were significantly different ($p<0.05$). From these results, there does not appear to be a role of unopposed oestrogen influencing force production but the pattern of strength changes implicates progesterone. There were no corresponding fluctuations in strength of the FDI, which remained relatively stable across the menstrual cycle. The contractility and fatigue resistance of the quadriceps did not differ significantly between any phase ($p>0.05$). The difficulty in isolating oestrogen during the menstrual cycle does not render this a good model to assess its effects upon force production. Maximal strength and fatiguability of the FDI were examined in young women undergoing *in vitro* fertilisation (IVF) treatment when acute, massive changes in oestrogen are induced. There were no differences in muscle function of the FDI when assessed under very low or high oestrogen changes ($p>0.05$). The independent effects of oestrogen upon muscle function were not demonstrated here.

Hormone replacement therapy is the most efficacious treatment for preventing menopausally-related bone loss. The results from the longitudinal study suggest that HRT confers protection against muscle weakness as a consequence of ovarian failure. Whether HRT maintains or restores strength was examined in the FDI of post-menopausal women ($n=9$). The oestrogen only and oestrogen-progestogen phases were compared with baseline measurements. A positive change in strength was observed, although this did not reach significance ($p<0.1$). The increase in strength (15.2±20.6%) between baseline and the oestrogen-progestogen phase of HRT corroborates the involvement of progesterone in determining muscle function.

The findings suggest that the menopause is associated with a loss of strength, prevented by the administration of HRT. Oestrogen alone does not influence force production, although progesterone is implicated. This has important ramifications in hysterectomised women who are prescribed preparations containing oestrogen only.

ACKNOWLEDGEMENTS

I extend my sincere gratitude to Dr. N.T. Cable and Prof. Tom Reilly for their guidance and advice during the supervision of my PhD. I would also like to express a **special thanks** to Dr. Tim Cable whose continued support, motivation and understanding during the experimental work and writing up of this thesis has been appreciated.

Much of the work within this thesis was undertaken in collaboration with The Women's Hospital, Liverpool. Without the cooperation of staff at the Reproductive Medicine Unit, this would not have been possible. I would like to thank Mr Charles Kingsland for allowing access to his patients, Dr. M. Biljan for his enthusiasm and ideas and for the help of Dr. M. Luckas, who was involved with three of the studies within this thesis (studies 4.2, 4.3, 4.4). The assistance of Dr. G. Atkinson and Dr. A. Nevill with the statistical analyses is gratefully ackowledged.

I would like to thank Una Creagh, Diana Leighton, Barry Drust and Lee Nolan for their friendship and the support and encouragement they have offered. I hope this will continue.

I would finally like to acknowledge my parents for their love and support throughout my life and their belief in my abilities. My sister, Lynda, for her constant source of encouragement. To Simon, for his patience, love and constant faith in me. Without this support from my family I would never have reached this far. I therefore dedicate this thesis to them.

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ETHICS APPROVAL

All experimental work conducted in the methodology and experimental studies were approved by the relevant Ethics Committee either at Liverpool John Moores University, Liverpool Women's Hospital or both. For subject consent forms see appendix 5.

CHAPTER ONE

INTRODUCTION

1.1. INTRODUCTION

Osteoporosis (*brittle bone disease)* is a contemporary disease which causes extreme morbidity and mortality in post-menopausal women. Statistics from the National Osteoporosis Society (1995) estimate that by 80 years of age, approximately 40-50% of women will have developed osteoporosis, and one woman in eight will have sustained a fracture. This places considerable strain on health care costs. Whilst osteoporosis also affects men, it is far more prevalent in their females counterparts since oestrogen deficiency is the single most significant factor in postmenopausal osteoporosis (Riggs et al., 1982). Bone loss begins to accelerate immediately after the menopause at a rate of 1 to 7% per annum depending on skeletal site (Krolner and Nielson, 1982; Lindsay et al., 1976). Hormone replacement therapy (HRT) is the most efficacious treatment for preventing osteoporosis (Aitken et al., 1973) and also protects against the risk of cardiovascular disease (Paganini et al., 1988). This treatment is most effective when taken before or shortly after menopause at a time when bone loss is accelerated (Riggs and Melton, 1986).

A loss of bone mass after the menopause is accompanied by a loss of force per cross-sectional area (force/CSA) of skeletal muscle. A significant reduction in specific force of the adductor pollicis muscle (AP) has been reported in women around 50 years of age, coinciding with the menopause (Phillips et al., 1993b), implicating an additional role of reproductive hormones in regulating muscle function. This has previously been indicated by Winner et al. (1989) who found a greater incidence of falling in perimenopausal women.

Muscle strength is positively related with functional activities such as walking speed and stair climbing ability and is negatively related to the incidence of hip fractures (Aniansson et al., 1983). Muscle weakness and fatigue will therefore impair the ability to undertake everyday activities. Muscle weakness is more pronounced in the proximal, lower limb muscles, predisposing postmenopausal women to the risk of falling and sustaining fractures (Wickham et al., 1989). Since muscle strength is compromised in females suffering from osteoporosis (Rutherford and Jones, 1992) this

poses a major health problem in vulnerable females. Impaired functioning of a muscle group such as the quadriceps, which has an important role in performing activities of daily living will have significant clinical implications in the ageing population. Given that the specific force of the AP muscle is lower in hypoestrogenic post-menopausal women, other muscles may be similarly affected. The sites at most risk from osteoporotic fractures are the hip, wrist and spine, and therefore there is a need to investigate the effect of hypoestrogenia/hypoprogesteronia on the strength of muscles associated with these areas.

Specific muscle force is greater in post-menopausal women taking hormone replacement therapy compared to age-matched controls (Phillips et al., 1993b). Understanding the onset and rate of a reduction in muscle strength associated with the menopause is therefore a requisite for the administration of HRT. How reproductive hormones mediate their effects on muscle is currently unknown, and it is still highly speculative which hormone — oestrogen or progesterone — is responsible for preserving strength. There are tenuous suggestions from studies of the endogenous fluctuations during the menstrual cycle that oestrogen is responsible for increases in strength of the AP muscle (Phillips et al., 1996), although findings of a negative correlation between oestrogen and hand grip strength (Bassey et al., 1995) conflict with these reports. If progesterone is implicated, this would have significant clinical consequences in hysterectomised post-menopausal women. A progestogen component is added to HRT preparations to reduced endometrial hyperplasia in females with an intact uterus and thus treatment for this group of women may need to be revised.

The purpose of this thesis will be to investigate the effects of reproductive hormones on muscle strength in middle-aged women. The rate of strength loss of multiple muscle groups, the quadriceps and handgrip, will be assessed longitudinally between three groups of menopausal and post-menopausal females of varying hormonal status. Other models will be employed to determine the possible mechanisms and hormone responsible for strength changes. From these findings, it is hoped that the hormonal milieu within which a loss of muscle function occurs will be elucidated, so that

preventative measures can be enforced in the growing population of post-menopausal females.

1.2. AIMS AND OBJECTIVES

The investigation of the effects of reproductive hormones on muscle function will be undertaken through the following aims:-

1) Determine the rate of force loss of the quadriceps and palmer flexors in menopausal and post-menopausal women.

2) a) Compare the effects of acute changes in reproductive hormones on muscle strength of a large muscle group, the quadriceps, and a small muscle, the first dorsal interosseus (FDI).
   b) Assess the effects of acute changes in reproductive hormones on contractile properties of the quadriceps.

3) a) Investigate the role of oestrogen in influencing strength changes in young women
   b) Examine the role of oestrogen on the fatigue resistance of the FDI.

4) a) Examine the efficacy of hormone replacement therapy (HRT) as a prophylaxis to muscle weakness
   b) Establish the effects of HRT on the contractile properties of the FDI.

These primary aims cannot be fulfilled until a series of methodological steps have been taken. These are concerned with the assessment of reliability of the equipment and protocols employed in the experimental studies:

i) Establish the day-to-day reliability of the LIDO Active® isokinetic dynamometer across a range of increasing angular velocities.

ii) Determine the reliability of performance of leg and handgrip strength in middle-aged women.

iii) Quantify inherent variability of measuring maximal voluntary contraction (MVC) of the quadriceps and FDI using a strain gauge system.

iv) Assess the reliability and repeatability of electrically stimulated contractions of the quadriceps.

v) Establish the reliability of the hand dynamometer used to measure maximal strength and electrically stimulated contractions of the first dorsal interosseus (FDI) muscle.

Fulfillment of these aims will elucidate the hormonal milieu in which muscle weakness occurs and the role of HRT in preventing the reduction in the force generating capacity of skeletal muscle. The effects of hormonal status on volitional and electrically stimulated contractions will also be determined.

The following means will be employed in fulfilling these aims:

1) Employment of the LIDO Active® dynamometer to measure isometric and concentric strength isokinetically across a range of increasing angular velocities.

2) Application of the electrical stimulator to: a) confirm maximal activation of muscle b) electrically stimulate muscle at increasing frequencies and c) induce fatigue.

3) Utilise a strain gauge system for measurements of volitional and electrically stimulated isometric contractions.

4) Construction and use of the hand dynamometer to assess muscle function of the first dorsal interosseus muscle with use of the electrical stimulator.

CHAPTER TWO

REVIEW OF THE LITERATURE

2.0. REVIEW OF THE LITERATURE

Over the past decade, there has been an increase in the participation of women in sports and regular exercise programmes. As a consequence of this, much scientific interest has focused on both the females' responses to exercise and the effects of exercise on reproductive functioning.

The increase in longevity of women in contemporary society compared to their female counterparts earlier this century is probably due to an enhanced quality of life, aand women now tend to live one third of their life in the infertile, post-menopausal period. The health implications of the menopause and loss of reproductive hormones are a growing concern since hormonally related insidious diseases are associated with increased morbidity and mortality. These are offset with the availability of hormone replacement therapy (HRT). Although there is much concern over its 'safety', research is ongoing to reduce the side effects and enhance the therapeutic role of HRT.

To understand these issues fully, this chapter will provide a theoretical background of the regulation and functions of the female reproductive system, focusing on endocrinological changes during the menstrual cycle and menopause.

2.1. THEORECTICAL BACKGROUND OF THE HUMAN REPRODUCTIVE SYSTEM. FROM MENARCHE TO MENOPAUSE.

2.1.1. Endocrinology of the human female menstrual cycle

The onset of reproductive function, termed *menarche*, is reported to occur between the 10th and 16th year in 95% of European girls (Abraham, 1978). Reproductive cycles begin at pubery when the hypothalamic pulse generator is activated. Stimulation of the hypothalamic-pituitary-ovarian axis initiates the first menstrual flow. From menarche to the end of reproductive life, *menopause,* a series of coordinated events occur within the ovaries and endometrium called the menstrual cycle. These events are

controlled by a finely tuned interaction of hormones, regulated by the brain. An interrelationship between the hypothalamus, anterior pituitary, and the ovaries lead to the periodic maturation and extrusion of the ovum (egg) from the ovary, which is then transported to the fallopian tubes to be fertilised. Meanwhile, the endometrium undergoes histological changes in preparation for the fertilised egg.

The normal menstrual cycle established after puberty averages 28 days, although most cycles range between 23-35 days. The cycle can be divided into four phases: day 1 of the cycle is the onset of *menstruation*, which lasts 4-5 days; *follicular phase* of the ovary corresponds to the proliferative of the endometrium; *ovulation*, which lasts about 36 hours and the *luteal phase* which corresponds to the secretory phase of the endometrium. The hypothalamus and pituitary gland control timing within a menstrual cycle, but the ovary regulates its phases and duration as ovarian steroids exert "negative" and "positive" feedback effects on the hypothalamus. Variability in cycle length of women is determined by the duration of the follicular phase. The luteal phase is usually constant, lasting 14 days (Vollman, 1977), but it may be shortened in highly trained females athletes (Prior et al., 1982).

2.1.1.1. Structure and function of the ovary

The function of the ovary is to nurture the growth and development of an ovum in preparation for ovulation and subsequent fertilisation. The activities within the ovary are cyclical and involve a complex process of steroid synthesis, negative and positive feedback signals and interaction with exogenous hormones. A morphological account of the ovary will be given to assist in the understanding of the events of the menstrual cycle.

2.1.1.1.2. Morphology of the ovary and follicular development

The ovary consists of three regions, the outer cortex, inner medulla and hilum. The cortex contains the functional units of the ovaries, i.e. the follicles, in different states of

development and occupy the main body of the ovary. The medulla forms the stromal cells and the hilum yields the entry point of the nerves and blood vessels (Fig. 2.1.1).

Fig. 2.1.1. Microscopic view of the human ovary. From Ojeda, 1992, pp. 135.

At birth, each ovary contains up to one million follicles, each one enclosing a primary oocyte. The oocyte is surrounded by a flattened layer of epithelial cells separated by the basal lamina. This is the *primordial follicle.* Several layers of cuboidal granulosa cells evolve from these stromal cells to form the *primary follicle.* During the early part of the menstrual cycle, 20 to 25 primary follicles begin to produce low levels of oestrogen.

Around day 5 of the menstrual cycle, a cohort of these follicles develop into secondary follicles, characterised by concentric layers of granulosa cells and the cultivation of outer theca cells, separated by the basal lamina. A glycoprotein band is formed between the oocyte and granulosa cells called the zona pellucida. Thecal cells differentiate into theca interna and theca externa layers; theca interna become cuboidal and fill with lipid droplets, indicative of steroidogenesis. The granulosa cells secrete follicular fluid containing steroids, pituitary hormones — luteinzing hormone (LH) and follicle stimulating hormone (FSH) — and local growth factors which fill the follicular cavity or *antrum,* forcing the oocyte to the edge of the follicle. As the oocyte is displaced, it becomes surrounded by granulosa cells called the cumulus oophorus (Fig.

2.1.2). These follicles become *antral* or *graafian follicles* and reach a diameter of 5 mm (Ojeda, 1992).

Fig. 2.1.2. Schematic representation of the development of an ovarian follicle. From Ojeda, 1992, pp. 145.

Growing antral follicles need gonadotropins to reach their ovulatory size. Of the selection of follicles which have progressed beyond the antral stage, only one "dominant" follicle reaches ovulation. The LH surge usually occurs after the leading follicle exceeds 16 mm (Edwards and Brody, 1995). Under the influence of LH, the oocyte matures and ovulation occurs 36 to 40 hours later. Following the expulsion of the oocyte, the collapsed follicle is reorganised to form the corpus luteum. Granulosa and thecal cells are 'luteinized', and fibroblasts and capillaries invade. If fertilisation does not occur, the corpus luteum remains functional for 13 to 14 days and then undergoes luteolysis. Endocrine function is lost rapidly and the corpus luteum is replaced by scar tissue called the corpus albicans (Ojeda, 1992).

2.1.1.2. Hypothalamic 'neural' and pituitary control

Reproductive function is regulated by two peptide hormones, called the gonadotropins, secreted from the anterior pituitary gland. Follicle stimulating hormone (FSH) stimulates follicular maturation and production of oestrogen. Luteinizing hormone

(LH) is responsible for ovulation, luteinization and ovarian steroidogenesis. Prior priming with FSH is essential for LH to exert its effects (Shearman, 1986). Prolactin is also released, although its physiological role in ovarian control is unclear (Shearman, 1986). The gonadotropins, particularly LH, are released into the bloodstream every 60-90 minutes in a pulsatile manner. This is called a "circhoral" rhythm. If this pulse frequency declines oestrogen output becomes low and inconsistent (Edwards and Brody, 1995) and ovarian follicles fail to develop.

The release of these hormones are, in turn, regulated by a pulsatile secretion of gonadotropin releasing hormone (GnRH) from the arcuate nucleus of the hypothalamus. The frequency of release of this decapeptide is suppressed by increasing progesterone concentrations produced by the ovary, and stimulated by the mid-cycle rise in oestrogen, preceding the increase in serum LH (Miyake et al., 1983).

2.1.1.3. Ovarian control

Oestrogen and progesterone are the two principal ovarian steroid hormones which regulate events within the ovary. These sex hormones are under the control of the gonadotropins. The formation of oestradiol is dependent upon both LH and FSH, whereas the synthesis of progesterone is enhanced by LH alone. The secretion of progesterone however, can be stimulated by both gonadotropins. Oestrogen and progesterone exert "negative" and "positive" feedback mechanisms on both the GnRH pulse generator and on the secretion of FSH and LH from anterior pituitary. This is shown in Fig. 2.1.3.

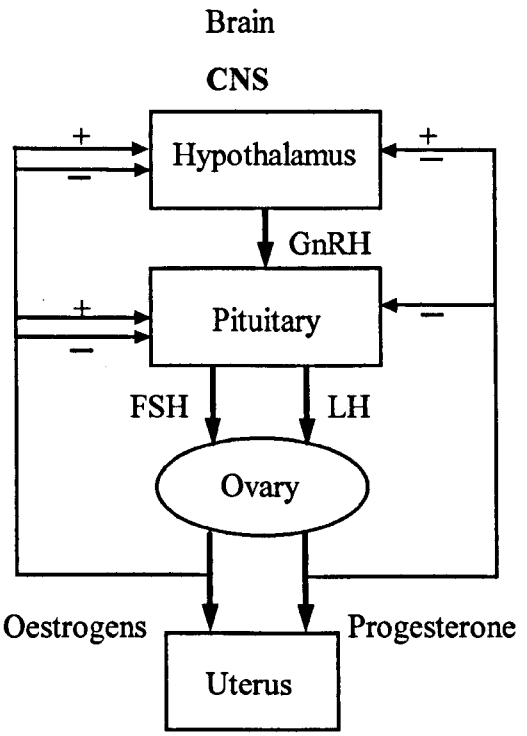


Fig. 2.1.3. Interaction of hypothalamic-pituitary-ovarian axis and regulation of steroid hormones oestrogen and progesterone.

2.1.1.4. Follicular (proliferative) phase

The onset of the menstrual flow is considered as the first day of the cycle. A reduction in oestrogen and progesterone causes a sloughing off, or shedding of the structural functionalis layer of the endometrial lining along with small amounts of blood ~ 40 ml from supporting blood vessels, which lasts 4 to 5 days. The negative feedback of oestrogen on gonadotropin release is relieved and FSH levels begin to rise. Under the influence of FSH, the ovarian cycle is initiated with the development of the primary follicles. It has been suggested that *activin,* the ovarian FSH-releasing protein, has a physiological role in the maintenance of FSH secretion (Ojeda, 1992).

The developing follicles produce increasing levels of oestrogen which is more pronounced with the maturation of antral follicles late in the follicular phase. This is facilitated by the increased sensitivity of the follicle to FSH by the oestrogen it

synthesises (Richards and Midgley, 1976). The secretion of FSH declines in response to high oestrogen concentrations and inhibin. Inhibin is a peptide hormone secreted by the ovary which exerts selective inhibitory control over the secretion of FSH. The production of inhibin is stimulated by FSH itself (Ojeda, 1992). The inhibition of LH by low oestrogen concentrations during the early follicular phase is relieved at high oestrogen levels in the late follicular phase. In the presence of an oestogenic milieu, the endometrium undergoes "proliferative" histological changes (Shangold, 1988). Progesterone and 17 α-hydroxyprogesterone levels remain unchanged until the ovulatory phase (Ojeda, 1992).

2.1.1.5. Mid-cycle dynamics

The mid-cycle hormonal events involve a complex relationship between gonadotropins LH and FSH, and the sex hormones oestradiol (E_2) and progesterone (Fig. 2.1.4). The pre-ovulatory elevation of E_2 characterising the ovulatory phase, triggers a surge in gonadrotropins. A rapid rise in LH and a less pronounced increase in FSH are elicited from the positive feedback of E_2 on the pituitary. The gonadotropin surge lasts approximately 24 hours and is induced only if the oestradiol threshold exceeds 250 pg/ml for longer than 36 hours (Edwards and Brody, 1995) (This surge is in concert with the dominant follicle reaching >16 mm). The peak in LH is denoted as day 0 in the diagram (Fig. 2.1.4). As gonadotropins begin to rise, there is a concomitant increase in 17α-hydroxyprogesterone (17-OH) and a smaller rise in testosterone and androstendione.

Progesterone increases concomitantly with E_2 prior to the LH surge, which is proposed to act synergistically to induce the progressive increase in basal LH (Hoff et al., 1983). This increase in progesterone has also been implicated in regulating the pre-ovulatory gonadotropin surge *in vitro*. In the ovariectomized rat, oestradiol alone failed to generate the full gonadotropin surge and progesterone was required to restore the levels to the same magnitude and duration seen in the proestrus rat (Mann and Barraclough, 1973). The rise in progesterone may also be responsible for the mid-cycle elevation of FSH.

The rise in progesterone continues after the initiation of the LH surge, although this is not accompanied by an increase in E_2. Soon after the LH surge, E_2 falls precipitously (Fig. 2.1.4), despite increases in androstenedione and testosterone. This may be due to the rapid rise in progesterone within the follicle inhibiting aromatase activity, resulting in a decline in E_2 formation (Hoff et al., 1983).

The most mature, dominant follicle, produces greater amounts of E_2 than the growing follicles and is most sensitive to gonadotropin stimulation. On selection of this follicle, the other growing follicles undergo atresia. Oocyte maturation begins in response to the LH surge and is mature at ovulation 10 to 12 hours later, 24 to 36 hours after the E_2 peak. Oestradiol and androgen levels have fallen at this time, although progesterone increases further and 17α-hydroxyprogesterone remain elevated. At ovulation the oocyte is expelled from the follicle and transported to the fallopian tubes in preparation for fertilisation.

After ovulation, under the influence of LH the supporting cells of the follicle form the corpus luteum (*yellow body*). The corpus luteum produces progesterone in significant amounts (and E_2 to a lesser extent) and influences secretory changes in the uterine endometrium. If fertilisation does not occur, the corpus luteum degenerates within two weeks, resulting in a sudden loss of progesterone. Withdrawal of progesterone and E_2 initiates another menstrual flow. The events of the cycle are shown in Fig. 2.1.5.

Fig. 2.1.4. Mean values (±SE) of LH, FSH, progesterone, oestradiol (E2) and 17-hydroxyprogesterone (17αOH prog) in daily serum samples of 9 women during ovulatory menstrual cycles. Days from mid-cycle LH surge (0). From Ojeda, 1992, pp. 150.

Fig. 2.1.5. Menstrual and ovarian cycles with hypothalamic and anterior pituitary gland hormones. From Tortora and Anagnostakos, 1989, pp. 902.

2.1.2. Biosynthesis of reproductive steroid hormones

Oestrogen and progesterone, like all steroid hormones, are derived from the precursor cholesterol (chol = *bile* and steroes = *solid*), a 4-ring hydrocarbon molecule with a side chain (Fig. 2.1.6). Cholesterol is transported to the ovary via the blood stream with low-density lipoproteins (LDL), and is released from its LDL-receptor complex inside the ovary through hydrolysis with lysosomes. Excess cholesterol is esterified and stored in lipid droplets for later use.

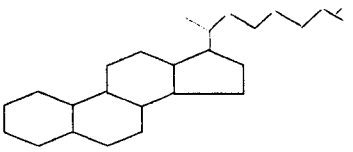


Fig. 2.1.6. Structure of cholesterol, a precursor to steroid synthesis.

Cholesterol (C 27) is initially converted to pregnenolone (C 21) through the sidechain cleavage of the 6-carbon isocaproic acid molecule. This reaction occurs in the mitochondria mediated by the cytochrome P-450. This is the rate limiting step of the steroid biosynthetic pathway (Al-Azzawi, 1992). Pregnenolone is then converted into progesterone or into 17 α-hydroxypregnenolone. These, in turn, can be metabolized to 17 α-hydroxyprogesterone (See Fig. 2.1.7).

Fig. 2.1.7. Biosynthetic pathway for the oestrogens. From Ojeda, 1992. pp 136.

Further metabolism to androgens and oestrogens results in an additional reduction in the number of carbons to C 19 of androgens and C 18 to oestrogens. Androstenedione and testosterone are immediate precursors for the aromatisation of oestrogen production. Luteinizing hormone is responsible for stimulating the production of androgens used to synthesise oestrogens, and receptors are found on steroidogenic cells of the stroma and thecal cells of the follicles. A gradual increase in plasma LH levels promote the differentiation of thecal cells and induces the synthesis of 17,20-lyase enzyme, increasing the availability of androgens for oestrogen biosynthesis. Oestradiol is metabolized from testosterone in granulosa cells of antral follicles through an FSH-mediated aromatisation enzyme complex (Fig. 2.1.8). Oestrone and oestriol are synthesised from androstenedione. Progesterone is secreted by all steroidogenic cells of the ovary regardless of their localisation.

A "two cell-two gonadotropin" hypothesis has been devised to explain the gonadoptropic control of ovarian steroidogenesis. Under the influence of LH, thecal cells produce androgens that upon diffusion to the granulosa cell compartment of the follicle are converted to oestrogens via an FSH-supported aromatization reaction (Fig. 2.1.8).

Fig. 2.1.8. Control of ovarian oestrogen, progesterone and androgen production by LH and FSH. Luteinizing hormone acts on thecal and granulosa cells. Follicle stimulating hormone acts on granulosa cells only. From Ojeda, 1992. pp 139.

Oestradiol is the predominant oestrogen in women of reproductive age and exists in equilibrium with oestrone in a ratio of 1:2 to 1:4. The ovary secretes oestradiol directly into the bloodstream. After the menopause the main oestrogen, oestrone, is derived from the peripheral conversion of androstenedione or oestradiol. Oestrone is further metabolised in the liver to oestriol. More than 70% of circulating oestrogens are bound to proteins in the blood stream, preferably albumin for which they have a low affinity, or testosterone-binding globulin (TeBG) for which they have a high affinity. Since oestrogens have a lower affinity for TeBG than testosterone, there are more circulating oestrogens available to tissues. Only free steroids can be transported to their target cells. Steroids are dissociated from their binding molecule at the target cells, and allowed to enter the cell membrane to bind to high affinity, low capacity receptors located in the cytoplasm. The steroid receptor complex migrates to the nucleus and bind to a specific segment of deoxyribonucleic acid (DNA). This reaction modifies its transcription and the synthesis of specific types of messenger ribonucleic acid (mRNA) (Fig. 2.1.9).

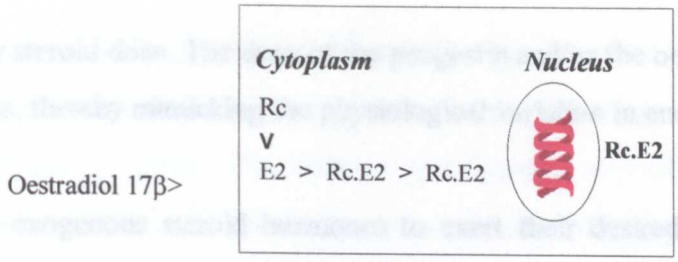


Fig. 2.1.9. Oestrogen-receptor complex: RC=cytoplasmic oestradiol 17β complex.

2.1.3. Oral contraceptives

The prevalence in the use of exogenous steroid hormones in women of reproductive age is increasing. The principal use of steroid hormones are for contraceptive purposes, although they are also used medically e.g. relieve dysmenorrhoea. There are reports that a growing number of elite athletes take steroid hormones for cycle control or as a form of hormonal replacement to ameliorate the effects of amenorrhoea on the skeletal system (Shangold, 1988). The widespread use of oral contraceptives (OC) is accompanied by a growing concern of the side-effects of the constituent steroid hormones in these agents. There are further implications for the athlete of OC's upon exercise performance given the effects of OC's on metabolic function (Lebrun, 1994).

Oral contraceptive agents operate by inhibiting gonadotropin-releasing factors from the hypothalamus through a negative feedback effect, similarly to the mechanism of endogenous hormones. Gonadotropin secretion is subsequently suppressed preventing ovulation (Bingel and Benoit, 1973). Oral contraceptives provide a constant dosage of synthetic hormones, in contrast to the cyclical fluctuations in naturally occurring hormones. The dosage of current combined preparations are lower (<35μ oestrogens and < 1 mg progestins) compared to original formulations introduced in 1960 (Kaunitz et al., 1995) in an attempt to minimise the risk of cardiovacular disease and other side effects associated with high doses of the synthetic hormones (Baird and Glasier, 1993). In monophasic OC's, a constant dose of oestrogen and progestin is provided in each of the 21 active tablets of the cycle pack. This is followed by seven pill-free days. The development of phasic OC's to reduce the metabolic side-effects have lower overall

monthly steroid dose. The dose of the progestin and/or the oestrogen is varied over the pill cycle, thereby mimicking the physiological variation in endogenous hormones.

For the exogenous steroid hormones to exert their desired pharmacological effects, these components must be converted to more potent synthetic derivatives. Current OC formulations contain the metabolically active syntheticoestrogen ethinyloestradiol derived from the addition of a 17-α ethinyl radical to oestradiol. Mestranol, the less biologically active syntheticoestrogen, must be converted to ethinyloestradiol in the liver before exerting its oestrogenic effects (Whitehead and Godfree, 1992) and must therefore be administered in higher doses. Ethinyloestradiol is administered in low doses of 20 to 35 μg and are now contained in all OC preparations. Progestins are characterised according to the structure of the steroid from which they were derived. Most progestins are derivatives of 19-nortestosterone (Bembem, 1993), subclassified as gonanes or estranes (Crook et al., 1988). The progestational potency of gonanes are ten fold higher, with greater androgenic effects than estranes. Progestins of a progesterone derivative (17-α hydroxyprogesterone) are no longer available as a result of its association with breast cancer in dogs (Daniel, 1970). Table 2.1.1. lists the progestin components of current OC preparations.

Table 2.1.1. *Oral contraceptive oestrogens and progestins.*

Oestrogens	Ethinyloestradiol
19-Nortestosterone-related progestins	**Estranes** Norethisterone[a]* Norethisterone acetate* Noretynodrel Lynestrenol Ethynodiol diacetate **Gonanes** Levonorgestrel[a]* Norgestimate[b]* Gestodene[b]* Desogestrel[b]*

a denotes the metabolically active steroid
b denotes new generation of progestins

* = progestins used by subjects in study 4.2

The effects of OC's are dependent upon the type and dose of progestins administered (Bembem, 1993). Progestins may have androgenic, oestrogenic, antiandrogenic or antioestrogenic activity. The balance of oestrogen and progestin components in a specific preparation will also be important for determining their physiological effects. The effects of OC's on muscle strength will be discussed in section 2.2.

2.1.4. The menopause and climacteric

Circamensal reproductive cycling is not a perpetual rhythm, but diminishes during middle-age as a consequence of the ageing process. The cessation of menses due to ovarian follicular failure is termed *the menopause* — the transition from a reproductive to non-reproductive stage of life. This biological phenonemon is not an abrupt event but may take up to 10 years for amenorrhoea to occur. A 12 month interval of amenorrhoea is characteristic of the menopause (Khaw, 1992). Different terms are used interchangeably to categorise the stages of endocrinological changes surrounding the menopause. For the purpose of the thesis, the conventional terms pre-menopause, peri-menopause and post-menopause will be used in reference to the following definitions:

Pre-menopause: *The stage of reproductive function prior to the climacteric.*

Peri-menopause: *Also called the climacteric, it may begin 5-10 years before the menopause and is associated with the endocrinological, biological and clinical features of approaching menopause, and at least the first year after the menopause.*

Post-menopause: *Following the menopause, although it cannot be determined until after a period of 12 months of spontaneous amenorrhoea has occurred.*

2.1.4.1. Epidemiology of the menopause

The increase in the female population over the past century (Fig. 2.1.10) is considered to be the result of an increase in the number of births and a reduction in the mortality rate. At present, the highest number of females in the population are aged between 16-39 years, with proportions decreasing thereafter. The Office of Population Census and Surveys (OPCS) predict this pattern to change by 2031 where the greatest population

of women will be aged 40-64 years. The increase in the older population is attributable to the number of women surviving over 80 years. They have been predicted to comprise 50% of the females population over the next 2-3 decades (Population trends 25, 1995).

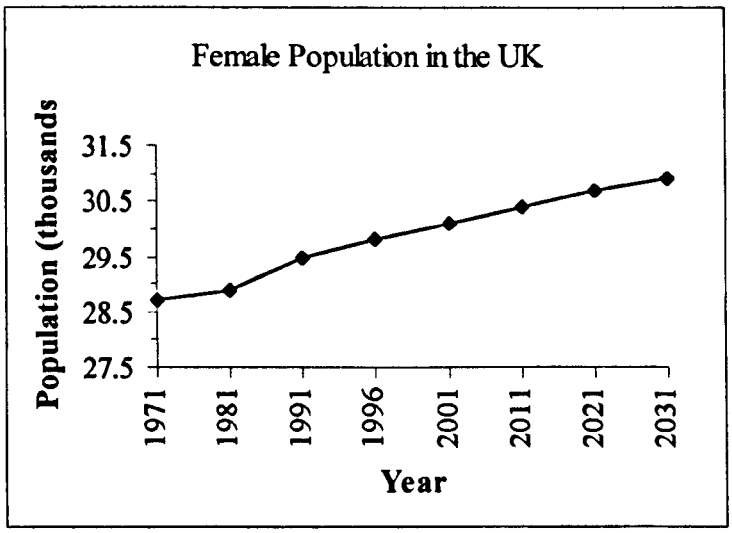


Fig. 2.1.10. The female population of the United Kingdom (UK) over 45 years, estimated into the next century: From SOCIAL TRENDS 24, 1994.

The current life expectancy of females in the UK is 79 years. This is estimated to increase to 81 years by the year 2000. In contrast to the increase in longevity, age at menopause has remained constant. The average age at menopause is 49.8 - 50.8 years in developed countries (Khaw, 1992), and has not reported to have changed over the past century (See Fig. 2.1.11). Women can therefore expect to live one third of their lives in the postmenopausal state. Unlike menarche, age at menopause does not appear to be affected by factors such as race, weight, skinfold thickness or socio-economic status. Even oral contraceptive use, which acts by suppressing ovulation, and parity do not delay menopause (Brambilla and McKinlay, 1989). However, smoking (McKinlay et al., 1985) and possibly malnourishment (Scragg, 1973) accelerate the menopause by 1-2 years.

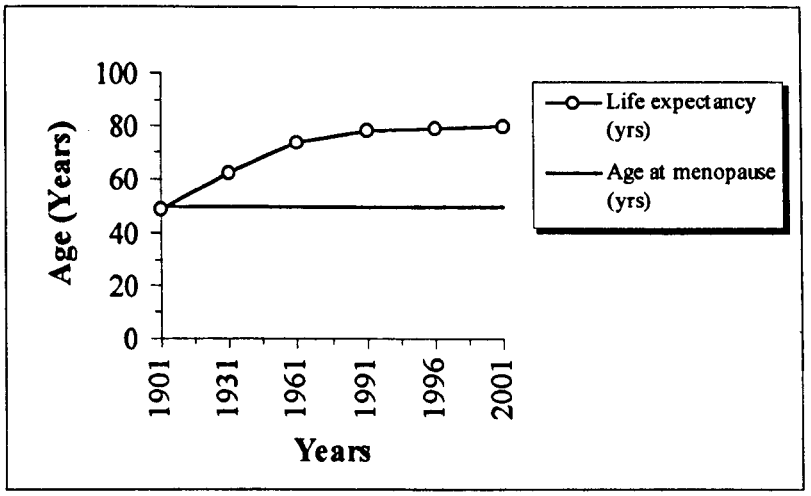


Fig. 2.1.11. Average life expectancy and age at menopause in females from 1901-2001. From: POPULATION TRENDS No 23, 1993. OPCS.

2.1.4.2. Aetiology of the menopause

The menopause is the result of a depletion of primordial follicles or oocytes in the ovary and the consequent fall in oestrogen and progesterone secretion. Since pregnancy and oral contraceptive use do not influence menopausal age, it has been suggested that these follicles are subject to a 'programmed cell death' (Al-Azzawi, 1992).

Throughout life, oocytes undergo growth, ovulation or atresia. Atresia is degenerative process of the ootytes, characterised morphologically by the necrosis of the oocyte and the granulosa cells. Prior to birth, at 24 weeks of gestation, there are about 7 million oogonia contained within the primitive gonad. At birth, only about 2 million of these mature into primary oocytes, the others degenerate and die. At puberty this is reduced to around 400 000. The loss of follicles during reproductive life are not replaced, and hence the continual ovulation and atresia exhausts the 'ovarian capital'.

Follicles decline exponentially throughout life at two different rates (Edwards, 1995). As shown in Fig. 2.1.12, follicular loss up to 37 years of age is relatively slow until follicular numbers reach 25 000. Follicles are subsequently lost at a rapid rate and at

the menopausal transition, at around 51 years, the typical follicle number is 1 000 (Edwards and Brody, 1995).

Fig. 2.1.12. Biexponential model of declining follicle numbers in women aged between 0 and 51 years, showing a sharp decline in older ages. From Edwards and Brody, 1995, pp 165.

2.1.5. Endocrinology of the menopause

The progressive failure in ovarian function begins 5-10 years prior to the menopause. Oestrogen and progesterone production decline due to depleting oocytes and an increase in gonadotropin stimulation compensates for this ovarian unresponsiveness. Oestradiol levels fall below a critical threshold and endometrial stimulation no longer occurs, resulting in amenorrhoea. Endocrine changes are not restricted to reproductive hormones. Other ovarian, pituitary and hypothalamic hormones such as inhibin, prolactin and catecholoestrogens are involved.

The following phases are characteristic of the endocrine changes at the climacteric:

- *Hypothalamic pituitary hyperactivity* - this starts 5-10 years before the menopause and continues after the menopause
- *Ovulatory and corpus luteum failure* - starts 5-10 years before the menopause
- *Ovarian follicular failure* - begins at the menopause

These events will be clarified below.

2.1.5.1. Endocrine changes before the menopause

The change in ovarian function resulting in the menopause begins *in utero* where there is a progressive decline in oocytes from 24 weeks of gestation. Whilst this continues through reproductive life, ovulation only contributes to a small proportion of the loss. As the number of follicles decrease, the oocytes that remain are those which are most resistant to simulation by gonadotropins. Consequently, oestrogen levels begin to decline in the older pre-menopausal women. Sherman et al. (1976) found that oestradiol (the main oestrogen of reproductive age synthesized by the ovary) concentrations are reduced in pre-menopausal women aged 46-56 years with regular menstrual cycles compared with younger women at the same stage of the cycle. In response to reduced oestradiol production, the negative feedback mechanism of the hypothalamus and pituitary causes FSH levels to increase (Sherman et al., 1976). This is the most characteristic feature of endocrine changes at the climacteric.

The hyperactivity of the hypothalamic-pituitary axis initially compensates for the increasing resistance of follicles to gonadotropin stimulation. The rise in LH occurs later and is less marked than that in FSH. This differential increase in FSH, compared with LH, in older regularly menstruating women was first reported by Sherman and Korenman (1975). They also postulated that a non-steroidal negative feedback factor of FSH, called *inhibin,* is reduced leading up to the menopause, consequent to a diminished number of follicles. Inhibin is found in ovarian follicular fluid and considered a biological marker of ovarian function relating to the size of the ovarian

follicular pool. Whilst oestradiol also regulates FSH, in isolation it is not sufficient to account for gonadal feedback (Chetkowski et al., 1986).

As oestradiol levels continue to fall as a result of follicular deficiency, the menstrual cycle shortens. Ovarian unresponsiveness becomes more marked and the cycle lengthens, and becomes increasingly anovulatory. In conjuction with defective corpus luteum formation, progesterone secretion is significantly reduced resulting in unopposed oestrogen secretion. This may give rise to dysfunctional endometrial bleeding, hyperplasia and carcinoma. Progesterone is the first hormone to become deficient at the climacteric.

2.1.5.2. Endocrine changes at the menopause

The reduced follicular development results in inadequate oestrodiol secretion to stimulate endometrial growth. Menstruation does not occur, and thus amenorrhoea ensues. The cessation of menses marks a change from cyclical to continuous hypothalamic, pituitary and ovarian function. The menopause is the last menstrual period and is the only constant feature of the climacteric. The main steroid hormone changes at the menopause are shown in Table 2.1.2.

Table 2.1.2. *Circulating sex steroid hormones (μ/mol) before and after natural menopause and oophorectomy.*

	Oestrone (E_2)	Oestradiol (E_1)	Progesterone (P)	Testosterone	Androstenedione
Premenopause					
EF	25-50	25-75		200-400	1600-1750
LF	150-200	200-600		300-800	1850-2000
ML	70-100	100-300	100-500	300-600	
OP†	20-40	15-25	50	75-150	600-1500
Postmenopause					
Natural	20-40	9-15	100-200	200-300	600-900
OP‡	20-40	9-15	100-200	100-150	500-800

EF=Early follicular *OP†=Oophorectomy before menopause*
LF=Late follicular *OP‡=Oophorectomy after menopause*
ML=Mid-luteal

2.1.5.3. Endocrine changes after the menopause

An important hormonal marker of the menopause is serum FSH which increases 10-15 fold post-menopause, compared to levels found during the follicular phase in women of reproductive age. Circulating LH levels only increase 3-5 fold in comparison. Both gonadotropins reach a peak 2-3 years after the menopause (Chakravarti et al., 1976) and then begin to decline until levels equivalent to those found prior to menopause are reached 20-30 years later (Fig. 2.1.13.).

Fig. 2.1.13. Mean (±1SD) plasma FSH and LH values in pre-, peri- and post-menopausal women. FP = follicular phase, PO = peak ovulatory value, LP = luteal phase. A and B are women still menstruating with apparent symptoms. B are women complaining of vasomotor symptoms. (Yrs = years). From Whitehead and Godfree, 1992, pp 9.

The main circulating oestrogen following the menopause is oestrone, synthesised from the peripheral conversion of androstenedione. Aromatization of androstendione to oestrone in adipose tissue accounts for 98% of the total oestrone production in post-menopausal women. The plasma levels of both oestrone and oestradiol correlate with body weight (Armstrong et al., 1996) and excess body fat post-menopausally (Judd et al., 1976). Even though it appears that post-menopausal women are not totally deficient in oestrogen, oestrone has only one tenth of the biological activity of

oestradiol. Plasma oestradiol levels in the immediate post-menopausal period range 10-15 μ/ml, as compared with 25-27 μ/ml and 20-600 μ/ml in the early follicular phase and late follicular phase respectively in pre-menopausal women.

The ovary continues to secrete androgens — androstenedione, testosterone, dehydroepiandrostenedione (DHA) and its sulphate (DHAS) — after the menopause. Androstenedione is derived from the ovary (30%) and the adrenal cortex (70%). The main proportion of testosterone is derived from the adrenal cortex (50%), then the ovary (35%). Peripheral conversion of androstendione accounts for 15% of testosterone produced (Fig. 2.1.14). Following bilateral oophorectomy, plasma androstenedione and testosterone fall by 50% in both pre- and post-menopausal women. Chakravarti et al. (1976) reported a 20% fall in concentrations of androstenedione, oestrone and oestradiol within a year after the menopause. After 5 years, androstenedione increased and testosterone levels had fallen significantly.

Progesterone (P) and 17-hydroxyprogesterone (17 HOP) levels in post-menopausal women are derived exclusively from the adrenal gland. They are suppressed by dexamethasone and increased 50% by adrenocorticotropin releasing hormone (ACTH). Bilateral oophorectomy does not affect progesterone concentrations.

Fig. 2.1.14. Diagrammatic representation of the source of oestrogens in post-menopausal women. From Anderson, 1979.

2.1.6. Physiological and pathological changes

Oestrogen exerts widespread effects on many tissues of the body, of both intra- and extra ovarian origin. The decline in oestrogen prior to, and hypoestogenia at the menopause are therefore associated with many physiological and pathological changes. It is important to note that the climacteric is as much a progesterone deficiency as an oestrogen deficiency syndrome. In addition to oestrogen, the effects of progesterone and other endocrine changes will be addressed in this section.

The symptoms of the climacteric are classified from their time of onset and aetiology and range from temporal, vasomotor disturbances to chronic and insiduous diseases which result in physical and pathological changes that pose health hazards to middle-aged women. These are listed in Table 2.1.3.

Table 2.1.3. *Acute, intermediate and long-term symptoms associated with oestrogen deficiency. From: Whithead and Godfree (1992).*

2.1.6.1. Acute responses *(vasomotor symptoms)*

Acute responses to the climacteric arise when menstruation is still ongoing. These symptoms are characterised by vasomotor disturbances, manifest as sensations of heat which travel from the face, neck and chest outwards. The 'hot flush' is associated with sweats, palpitations, dizziness and fainting. Vasomotor symptoms affect approximately 75% of females during the climacteric (McKinlay and Jeffers, 1974; Studd et al., 1990), 70% of of which are affected for 2 years, 25% for 5 years and 5% long-term indefinitely.

The endocrine and physiological mechanisms responsible for flushes are not fully understood. The occurrence and intensity of symptoms have not been correlated with plasma E_2 levels, although it has been postulated that the concentrations of 'free' E_2 (those not bound to plasma protein) are involved. Some studies have shown that episodic discharge of the gonadotropin LH is responsible for these symptoms (Casper, 1979; Tataryn et al., 1979). Meldrum et al. (1981) found that inhibiting LH secretion does not mitigate the frequency or severity of symptoms, and thus some other mechanism is involved which may originate from the hypothalamus.

Sweating often coincides with the onset of a hot flush. The sensation of a flush precedes an initial increase in skin conductance and vasodilation ensues. A fall in core temperature — up to 1°c — and a rise in peripheral skin temperature from 1° to 5° have also been proposed (Tataryn et al., 1979). This suggests an initial change within the central nervous system which includes an acute resetting of the thermoregulatory centre. Cardiovascular changes have been detected, with a marked increase in heart rate of up to 20 beats per minute (Silverman et al., 1981), suggesting an increase in sympathetic drive.

2.1.6.2. Intermediate responses

Oestrogen has many regulatory functions on reproductive tissues and thus prolonged oestrogen deficiency affects the reproductive tract. Indeed, within 3-4 years post-menopause, atrophic vaginitis, vaginal dryness and dyspareunia will begin to cause problems in 10-20% of the female population (Studd et al., 1990). At 5-8 years, 40-50% will be inflicted with genital tract atrophy. Moreover, loss of collagen from the skin and connective tissue may induce thinning and muscular aches and pains respectively. Approximately 30% of collagen is lost during the first 5 years after menopause.

2.1.6.3. Chronic responses

Long-term consequences of ovarian failure may have more serious implications on health and quality of life in post-menopausal women. The incidence of cardiovascular heart disease (CVD) and osteoporosis increase with age. These are two major causes of morbidity, which have been suggested to be exacerbated by the menopause. Female mortality due to diseases associated with hypoestrogenia across all ages show that coronary disorders are the main cause of death (Mortality statistics, 1992, Fig.2.1.15.).

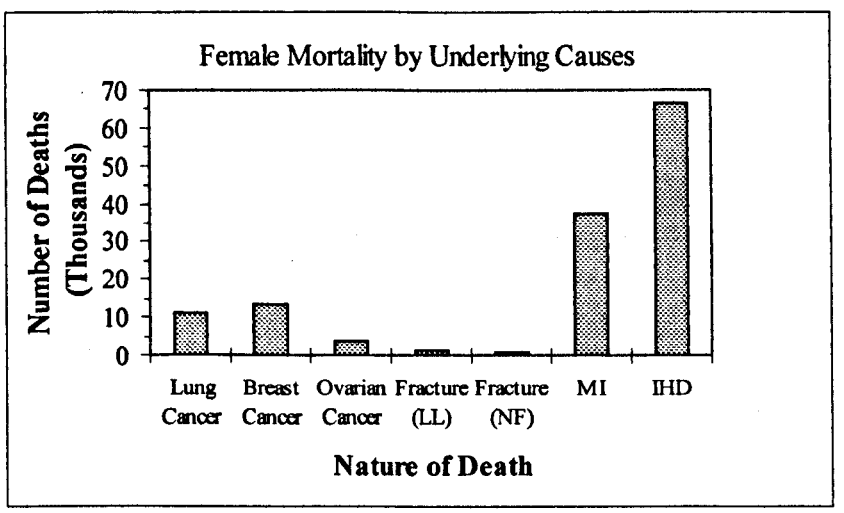


Fig. 2.1.15. Female mortality (thousands) in England and Wales by underlying causes in 1992. LL = lower limb; NF = neck of femur; MI = myocardialinfarction; IHD =ischaemic heart disease (MI and IHD = cardiovascular disease). From Mortality Statistics: Cause. Series DH2 No19 OPCS.

Mortality of different age categories for disorders associated with the menopause are shown in Fig. 2.1.16. Despite an increase in the incidence of gynaecological and breast cancer after the menopause (50 years plus), heart disease is accountable for significantly more deaths. This is exacerbated with ageing.

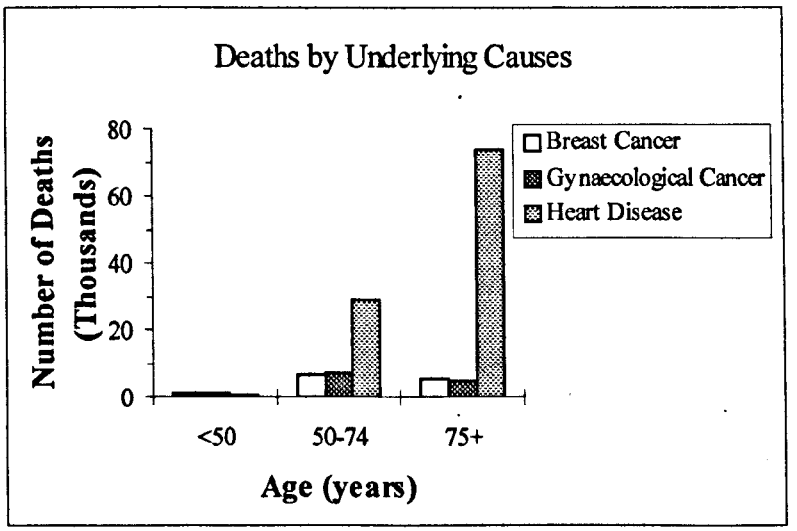


Fig. 2.1.16. Deaths in women from breast cancer, gynaecological cancer and heart disease in 1992. From: Mortality Statistics: Cause. Series DH2 No19 OPCS.

2.1.6.3.1. Cardiovascular disease

Low oestrogen levels have been associated with the high incidence of cardiovascular disease in post-menopausal women (Witteman et al.,1989). The low mortality rate associated with CVD in pre-menopausal females compared to males of the same age support these suggestions, and figures from the British Heart Foundation show that cardiovascular mortality is 2.7 times greater in males than females below 55 years.

Oestrogen deficiency is believed to cause unfavourable changes in lipid metabolism, including a decline in high density lipoproteins (HDL), an increase in low density lipoproteins (LDL) and higher serum cholesterol levels from pre-menopausal controls (Wahl et al., 1983). Hence, the protective role of oestrogens on the cardiovascular system is reversed postmenopause. However, oestrogen status is not the only risk factor of CVD. The confounding effects of hereditary predisposition, obesity,

hypertension, diabetes mellitus, smoking, hyperlipidaemia, inactivity and stress causes problems in isolating oestrogen status as a risk factor.

Whilst controlling for age and smoking, Colditz et al. (1987) claimed that women aged 30-55 years who had undergone a natural menopause and had never taken oestrogen replacement were not at an increased risk of CVD. Cross-cultural studies corroborate these findings. Reduced absolute death rates of CVD in Japanese women despite lower oestrogen levels compared with caucasian women (Godsland et al., 1987) cannot be explained by differences in oestrogen status. Colditz et al. (1987) did report that women who had surgical removal of the ovaries (bilateral oophorectomy) with no subsequent hormone replacement therapy, had an increased risk of CVD. Oestrogen replacement in this group appeared to reduce the risk of CVD (Colditz et al., 1987).

Many epidemiological studies have shown that the administration of oestrogen reduces the incidence of CVD by 50-70% (Knopp, 1988). Furthermore, hormone replacement therapy (HRT) also protects post-menopausal women against stroke (Paginini-Hill et al., 1988), probably mediated through its changes in lipid metabolism. Oestrogen replacement causes a decrease in total cholesterol and LDL and an increase in HDL (Wahl et al.,1983), reversing lipid profiles to pre-menopausal values. The effect of oestrogen on triglycerides and LDL depends on the type and dose of oestrogen. Oestradiol valerate used in many HRT preparations causes a fall in these lipids whereas ethinyloestradiol, used in oral contraceptive preparations, increases the LDL and triglycerides concentrations. In addition to the progestogen component, this explains the increased risk of myocardial infarction and thromboembolic disease in premenopausal women (Meade et al., 1980). Since many progestogens antagonise the effect of oestrogen, to obtain optimal benefits from HRT women with an intact uterus should be administered a progestagen of a pregnane derivative

A meta-analysis survey in post-menopausal oestrogen users and non-oestrogen users suggests that this hormone is a significant (but not the only) protective factor against CVD (Manson, 1992). However, the mechanism of the action of oestrogen is still uncertain. In pubescent females, the large changes in oestrogen do not alter lipid ratios

which has led researchers to focus on possible non-lipid mediated actions of oestrogens (Bourne et al., 1990; Lieberman et al., 1994). Bourne et al. (1990) treated 10 post-menopausal women with transdermal oestrogen (Estraderm®) and sequential oral norethisterone acetate. Using transvaginal ultrasound and pulse doppler, they found a reduction in arterial impedance and vascular tone of the uterine artery. Lieberman et al. (1994) reported improvements in flow-mediated endothelium-dependent vasodilation in post-menopausal women after 9 weeks of oestrogen replacement therapy. Additonally, Padwick et al. (1989) may have elucidated a further mechanism. They identified a protein related to the oestrogen receptor in smooth vessels and suggested that oestrogen may affect arterial status through a conventional sex hormone-receptor mechanism.

2.1.6.3.2. Skeletal changes

The loss of endogenous oestrogen at the menopause is known to disturb the homeostatically maintained process of bone remodelling. The consequential changes in skeletal intergrity can lead to a metabolic bone disease called *osteoporosis*. The association between bone loss and the menopause was first recognised in 1941 by Fuller Albright (Albright et al., 1941) and is now axiomatic (Lindsay et al., 1976; Horsman et al., 1977; Lindsay et al., 1978b). Oestrogen deficiency has since become established as the single most important factor in the aetiology of osteoporosis (Stevenson et al., 1989).

Osteoporosis is the most common metabolic bone disorder in Western countries and is becoming a serious, yet preventable public health problem. Osteoporosis is characterised by a significant reduction in bone density per unit volume, leading to increased susceptibility to fractures. Lifetime risk of fractures in women is greatest for the vertabrae at 32%; risk of hip and Colles' fracture is 16% and 15% respectively (Compston, 1992).

(i) Bone loss

The skeletal system is composed of cortical and trabecular bone. Compact plates of cortical bone are located in the *peripheral* skeleton. Bones of the central or *axial* skeleton consist of trabecular bone, a honeycomb of vertical and horizontal plates filled with red marrow (Marcus, 1991). Age-related bone loss affects cortical and trabecular bone in both sexes and may lead to "senile" osteoporosis (Riggs and Melton, 1986). Women experience an accelerated loss of trabecular bone after the menopause at a rate of 1-6% per annum (Morgan, 1973; Lindsay et al., 1976; Krolner and Nielson, 1982). Trabecular bone is more metabolically active than cortical bone and is more responsive to oestrogen deficiency. Common sites for fracture following the menopause are therefore localised at the wrist, spine and hip. Studies reporting bone loss at different skeletal sites are conflicting. In a longitudinal study of 139 healthy post-menopausal women, bone loss of the lumbar spine occurred before the menopause which amounted to half of overall bone loss, and was accelerated after the menopause at 1% per annum. No significant change was found for the midradius (99% cortical bone) pre-menopausally (Riggs et al., 1986). Krolner and Nielsen, (1982) did not report any change in bone of the lumbar vertebrae before the menopause but documented a 6% loss per annum after the menopause. Oestrogen deficiency has been shown to be a major contributor to a reduction in bone density of the proximal femur (Stevenson et al., 1989), although this hasn't been found in previous studies (Riggs and Melton, 1986). Rates of loss differ between cortical and trabecular bone, at different skeletal sites (Riggs et al., 1981; Stevenson et al., 1987) although it is agreed that the spine is the main site of disease in post-menopausal women (Riggs et al., 1982).

Fracture risk is dependent on two factors — peak bone mass and subsequent rate of loss. Peak bone mass is attained by mid-thirties and is mainly under genetic control (Lindsay et al., 1983). Lifestyle factors such as physical activity and diet, smoking, excess caffeine and alcohol intake have also been implicated in the redution of bone density (Stevenson et al., 1989). For instance, daily consumption of caffeine can reduce BMD of the hip and spine if at least one glass of milk is not consumed each day (Barrett-Connor et al., 1994). Maximising peak bone mass in adulthood is therefore

important in preventing bone density falling below the critical threshold for fracture risk in later life. Fig. 2.1.17 illustrates the effect of peak bone mass on fracture risk.

Fig. 2.1.17. Effect of bone mass on fracture risk. The upper broken line represents BMD in women with a high peak bone mass who lose 20% of their bone density after the menopause. The lower line represents bone density in women with low peak bone mass who only lose 10% of their bone density post-menopausally, yet become osteoporotic. From Stevenson, 1990.

(ii) Bone mineral density and muscle strength

Bone mineral density is not only dependent on systemic (hormonal) factors. Mechanical mechanisms are also in operation through external (gravity) and internal (muscular contraction) forces. The adapatation of bone to the force placed upon it was first recognised by Wolfe (1872 - Wolfe's law). It has since been recognised that muscle is an important determinant of bone mass (Doyle et al., 1970; Carter and Hayes, 1977; Zimmerman et al., 1990). Indeed, weight-bearing activities have been shown to increase the load to bone although activities such as swimming, which do not incorporate gravity stimulation, do not produce the same effect (Wolman, 1990). This is further supported in changes following immobilisation, in which significant bone losses of up to 1-2% per week in trabecular bone have been reported (Whedon, 1984). Many assessments have been undertaken assessing muscle strength and bone density at local sites. Exercise exerts a local effect on the skeleton, where greatest increases in BMD occur at the site of maximum stress. A 30% difference in BMD of the playing

arm compared to the non-playing arm has been reported in tennis players (Huddleston et al., 1980) and is greater in the os calcis of runners (Williams et al., 1984).

Muscle strength has been implicated in predicting bone mineral density in post-menopausal women (Snow-Harter et al., 1990; Kyllonen et al., 1991) at functionally-related antomical sites (Pocock et al., 1989; Rutherford and Jones, 1992; Zimmerman et al., 1990; Madsen et al., 1993). Correlations have been found between muscle strength of the back and bone density of the vertebral bodies (Sinaki et al., 1986; Halle et al., 1990) and quadriceps strength and BMD at the proximal tibial (Madsen et al., 1993) and proximal femur (Pocock et al., 1989). According to Zimmerman et al. (1990) muscle strength is not a predictor of BMD, but may be a factor in determining BMD. The association between muscle function and bone density is further substantiated from findings of strength in osteoporotic patients. Muscle weakness is more pronounced in senile osteoporotic sufferers compared to healthy age-matched controls (Rutherford and Jones, 1992). Meena et al. (1973) revealed that osteoporotics had lower bone mass of the proximal radius site, but they did not have smaller muscles. However, the muscle weakness reported with osteoporosis may be independent of muscle mass, similiarly to that reported by Rutherford and Jones (1992), and has the same aetiology as the significant reduction in bone.

(iii) Pathogenesis of post-menopausal osteoporosis

Bone is a dynamic tissue and is constantly under repair through a process of remodelling. Bone resorption is governed by *osteoclasts* and bone formation by *osteoblasts*. This dynamic homeostatic process is regulated through the action of hormones and growth factors. A disruption in hormone balance may result in a significant loss of bone (or an increase in bone mass). Oestrogen deficiency has been implicated in increasing the rate of bone resorption through indirect influences on other regulatory factors and/or direct effects on bone.

The mechanisms by which oestrogen alters bone turnover are still much debated, although it has been suggested that oestrogen acts indirectly by affecting the secretion

of parathyroid hormone (PTH). In hypoestrogenic post-menopausal women, reduced calcitonin levels enhance bone resorption to stimulate increased serum calcium levels. Parathyroid hormone is subsequently suppressed which reduces the synthesis of 1,25-dihydroxyvitamin D_3 (Compston,1992). Recent proposals have focused on the role of the immune system. Oestrogens inhibit cytokines interleukin 1 (IL-1), IL-6 and tissue necrosis factor (TNF), indicators of bone resorption. Cytokines also increase the sensitivity of bone to resorptive effects of PTH (Arnaud, 1993). Evidence of a direct effect of oestrogen on bone have been reported in *in vitro* studies. Eriksen (1988) and Komm (1988) both found that oestrogens have specific receptors in osteoblasts and therefore probably affect bone metabolism directly through a receptor-mediated mechanism. Even though it is recognised that oestrogen deficiency is responsible for increased bone resorption, the precise mechanisms need confirmation.

Whilst oestrogen has been reported the most important factor associated with bone metabolism, the role of progesterone in bone remodelling has been questioned. Considering the close relationship of both these hormones in the ovulatory cycle, it is reasonable to assume that progesterone may be the 'other hormone' which balances the activity of oestrogen in bone resorption. Indeed, *in vitro* studies have shown that progesterone acts directly on bone by engaging osteoblast receptors (Eriksen et al., 1988) and indirectly, by competing for glucocorticoid receptors on osteoblasts (Feldman et al., 1975). Further evidence emanates from hormonal treatment and bone markers. A reduction in calcium and hydroxyproline excretion, markers of bone resorption, following oestrogen treatment (Gallagher and Nordin, 1975) has been observed with progesterone treatment (Lobo et al., 1984). However, it has been reported that progestagen combined with oestrogen has a different effect on bone markers than oestrogen treatment alone. Christiansen et al. (1985) noted an increase in osteocalcin and alkaline phosphatase after progestogen administration on days 13-22 of a 28-day oestrogen therapy, compared with oestrogen alone in which urinary calcium and hydroxyproline excretion diminished. This would suggest that progesterone and oestrogen combined are implicated in increased bone formation as opposed to decreased bone resorption with oestogen therapy alone.

(iv) Progesterone and bone mineral measurements

Preliminary findings have implicated progesterone in the changes of bone mineral density. In a prospective study, Prior et al. (1989) measured BMD using single-energy quantitative computed tomograpy (QCT) of 66 healthy pre-menopausal women during one year. Changes in the menstrual cycle were monitored using basal body temperature readings. The mean length of the luteal phase was positively correlated with percent annual change in BMD of the vertebrae (thoracic 12 to lumbar 13). The authors have suggested that anovulation, with subsequent low or absent progesterone, might explain the low bone mass in peri-menopausal women.

Data assessing the effects of progesterone treatment on BMD are limited. Work from animal studies has demonstrated an increase in femoral width and ashed mineral in progestogen-treated rats (Lindsay et al., 1972; Aitken et al., 1978). In humans, Prior et al. (1987) reported an increase in vertebral density with QCT in 11 post-menopausal women treated with medroxyprogesterone for one year. Furthermore, medroxyprogesterone administered cyclically to pre-menopausal women with secondary amenorrhoea resulted in a significant increase in bone density in 3 women taking 10 mg/month; for those who took less progestogen, there was evidence of a dose-response relationship of bone changes. In clinical trials, Lee (1990) found that bone density increased markedly with the administration of 'natural' progesterone applied in a cream, which was greater than synthetic progestagens. In 67 post-menopausal women, bone density increased by 10% in the first six to twelve months, followed by an annual increase of 3 to 5%. Oestrogen slowed down bone loss, but no increases were observed (Fig. 2.1.18.).

Fig. 2.1.18. Bone mineral denisty changes with progesterone (P), oestrogen only (E_2) or controls. From Lee, 1990.

Osteoporosis can be prevented through different approaches, but needs to be recognised from an early age so that attainment of peak bone mass can be optimised. Exogenous oestrogens have proven to be the most efficacious treatment for preventing osteoporosis (Albright et al., 1941; Aitken et al., 1973; Stevenson et al., 1990) even if given many years after the menopause (Quigley et al., 1987) although treatment should begin soon after ovarian failure. It has been recommended that duration of therapy should last approximately 7 years after menopause (Felson et al., 1993).

2.1.7. Hormone replacement therapy

The use of oral contraceptives (OC) as a form of hormone replacement in amenorrhoeic athletes has been mentioned previously in section 2.1.3. Hormone replacement therapy (HRT) is the most efficacious treatment for menopausally-related symptoms but differs to OC's in dose and composition. Another important difference is the therapeutic application of HRT, formulated to produce a physiological, not a pharmacological effect. Hormone replacement therapy replenishes the loss of endogenous reproductive hormones as a consequence of the menopause or other disorders associated with a hypoestrogenic and hypoprogesteronic state. There is substantial evidence to suggest that hormone replacement therapy mitigates the acute menopausal symptoms such as vasomotor disturbances (Hargrove et al., 1989) and

confers protection against bone loss (Lindsay et al., 1976; Weiss et al., 1980; Felson et al., 1993) and cardiovascular disease (Colditz et al., 1987; Paganini-Hill et al., 1988).

2.1.7.1. Formulations

The majority of HRT preparations contain an oestrogen and progestogen component. The oestrogen component of HRT is not synthetic (i.e. not structurally dissimilar to naturally occurring oestrogen) like ethinyloestradiol of OC formulations. Conjugated equine oestrogens for example, most widely prescribed in HRT preparations in the U.K (Whitehead and Godfree, 1992), comprise 50-65% oestrone sulphate with the remainder constituting equine oestrogens which are structurally similar to oestrogens. The oestrogen component of HRT is believed to be responsible for mitigating acute vasomotor symptoms and reducing the risk of osteoporosis and cardiovascular disease. Progestogens are only added to prevent endometrial hyperplasia caused by unopposed oestrogen, and are therefore not routinely prescribed to hysterectomised women (without an intact uterus) (Whitehead et al., 1990). Progestogens of HRT preparations are the same as those of OC formulations, and are mostly derived from the 19-nortestosterone group. Progesterone is the precursor of many other steroids and thus the admininstration of 'natural' progesterone results in its rapid metabolism. Very high doses (200 mg) are needed to elicit an endometrical effect which has to be administered twice daily (Padwick et al., 1986) with accompanying sedative effects.

Synthetic progestogens may cause adverse side effects which may detract from the benefits of HRT. These side effects range from physical symptoms of breast tenderness and bloating to anxiety, irritability and depression of a psychological origin (Whitehead et al., 1990). Side effects are dose related, and depends on the type of progestogen administered. Probably the most significant of these adverse effects are the unfavourable changes in lipid profile. The beneficial effects of oestrogen are offset with the addition of a progestogen which causes HDL to fall and LDL cholesterol to increase (Ottoson et al., 1985; Siddle et al., 1990).

Progestogens taken sequentially in combined preparations also induce a withdrawal bleed which may be unacceptable in post-menopausal women. A gonadomimetic, tribolone, has been developed to prevent withdrawal bleeding associated with conventional HRT. Tribolone is a derivative of a C-19 nortestosterone compound which possesses oestrogenic, progestogenic and androgenic properties (Ellerington et al., 1992). Tribolone has been demonstrated to relieve vasomotor symptoms as effectively as conjugated equine oestrogens and oestradiol valerate (Crona et al., 1988; Volpe et al., 1986). Tribolone has been shown to be more effective in preventing bone loss than a placebo (Lindsay et al., 1980) although comparisons with HRT preparations have not been made. Their effects on lipid metabolism remain inconclusive.

2.1.7.2. Route of administration

The oral and transdermal routes for oestrogens and progestogens are the most popular methods of administration. Other parenteral applications such as creams are also advocated. The main disadvantage with oral administration of hormones is their metabolism in the gastrointestinal tract and 'first pass' effect of the liver (Ellerington et al., 1992). Oestradiol administered orally undergoes rapid conversion to oestrone in the gut mucosa. Hence, all oestrogens prescribed orally are absorbed into the portal venous system as oestrone, which passes through to the liver and is further metabolised and inactivated. Between 30 to 90% of the administered dose is inactivated by the liver prior to reaching the systemic circulation. Transdermal patches were developed to overcome the hepatic first-pass effect. The efficacy of this method appears to equal oral administration, with relief of hot flushes and maintenance of bone density (Haas et al., 1988). There is little intermediate metabolism through the epidermis (Stumpf, 1990) and thus the initial dose of oestogen in oral preparations is higher than that of the non-oral route. Progestogens are usually added sequentially in oral preparations, mimicking the endogenous hormonal fluctuations during the menstrual cycle. However, the adverse side effects of higher doses of progestogens in oral preparations, and the unfavourable changes in the lipid profile, have prompted the development of non-oral administration of the progestogen norethisterone acetate.

2.2. MUSCLE FUNCTION

The endocrinology of the female reproductive system has been reviewed in the previous section, so that reference throughtout the thesis to the hormonal status of young and middle-aged women and the effects that reproductive hormones exert will be fully understood.

The purpose of this section is to review aspects of muscle function and to establish a link with reproductive hormones. It is necessary at first to brief the reader of the structure and characteristics of the skeletal muscle to clarify the mechanisms of the actions of hormones.

2.2.1. Introduction

The ability to undertake any activity, from subliminal actions of blinking to strenuous exercise, is possible through the functioning of the muscular system. A highly specialised contractile machinery, skeletal muscle is characterised by its physical location, histology and nervous mode of control (Tortora and Anagnostakos, 1990). Cardiac (heart) tissue and smooth muscle (e.g. blood vessels) are under involuntary control, whereas movement of skeletal muscle is voluntary.

Muscle is a responsive tissue, adapting to external stimuli to enhance its mechanical and physiological functioning. Like many living tissues, muscle is not resistant to disease. Muscle strength, the capacity to generate force, is impaired in diseases such as myasthenia gravis, mytonia congenita and muscular dystrophy. The accompanying sensations of weakness and fatigue result in diminshed capacity to undertake 'simple' activities without undue strain. Muscle function is also compromised in the elderly and muscular atrophy, localised in the proximal muscles of the lower limb, results in a significant reduction in strength. This has important implications for enhancing the functional capacity in the elderly population.

Measuring indices of muscle function can determine the individual changes in strength and identify weaknesses in muscular performance. Much of the equipment used in the past has been subject to measurement error and limited to select muscle groups. Contemporary dynamometers allow the measurement of different muscle groups during different contractions and through a wide range of movements. Information derived from these tests provide more 'accurate' and realistic information, a sound basis from which to study functional changes in the population.

2.2.2. Structure of skeletal muscle

Skeletal muscle is responsible for movement and support of the skeleton. The human body contains over 215 pairs of skeletal muscle. For muscle to produce movement, the individual muscle fibres must contract. Contraction is initiated through a complex interaction originating from the higher centres of the brain, which relays messages along the central nervous system (CNS), peripheral nerves to the neuromuscular junction. This culminates in the transmission of electrical activity to muscle fibres for contraction to occur (Tortora and Anagnostakaos, 1990).

Muscle is comprised of individual muscle cells called muscle fibres, which run along the longitudinal axis of the muscle. They are enclosed in a plasma membrane called the sarcolemma. Muscle fibres are arranged in bundles, and contain smaller subunits of myofibrils. These are the contractile elements of skeletal muscle. Each myofibril contains numerous sacromeres, the basic functional unit of a myofibril. They are arranged along the myofibril and gives the muscle fibre a striated appearance (Billeter and Hoppeler, 1992). The magnified striations represent dark (A bands) and light (I bands) regions, occupied by contractile proteins myosin and actin filaments (Fig. 2.2.1.).

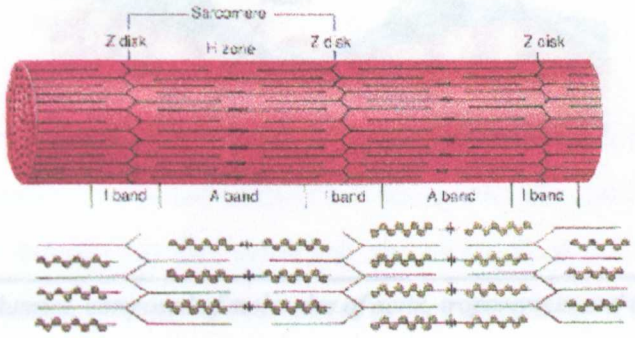


Fig. 2.2.1. A sacromere, the functional unit of the myofibril

Two thirds of skeletal proteins in each myofibril consist of myosin filaments. Myosin is characterised by two intertwined strands termininating in globular heads. The adenosine triphosphate (ATP) cleaving site is located in the myosin head, where the hydrolysis of ATP to adenosine diphosphate (ADP) provides energy for contraction. When the myosin head interact with actin molecules, ATPase is activated several hundred-fold (Fig. 2.2.2.).

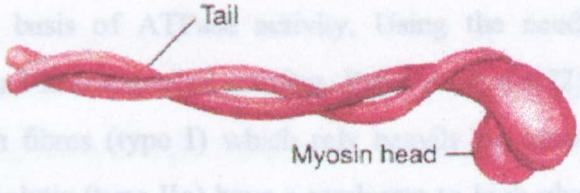


Fig. 2.2.2: A molecule of myosin

Actin, a double helical strand, is attached to two other proteins, tropomyosin and troponin. Tropomyosin is a long, rod-shaped protein spanning the length of seven actin residues. The troponin complex is carried on the tropomyosin and consists of globular shaped troponin C, troponin I and an elongated troponin T. Troponin C is the binding site for calcium (Fig. 2.2.3).

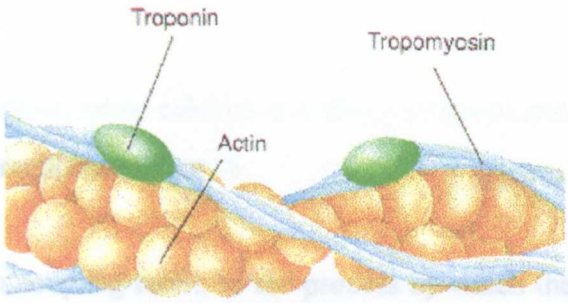


Fig. 2.2.3. An actin filament, composed of molecules of actin, tropomyosin and troponin

A system of membranous channels called the sarcoplasmic reticulum (SR) run longitudinally to the myofibril, providing a storage site for calcium in preparation for muscle contraction. The transverse tubules, which extend from the sarcolemma, transports extracellular fluid to individual muscle fibres. Muscle fibres are innervated by a motor neuron, which along with its axon and the fibres it supplies is collectively called the motor unit (Billeter and Hoppeler, 1992).

Characteristics of motor units differ, and early reports of Ranvier demonstrated that speed of contraction differed between muscles of the same individual (Ranvier, 1873: cited by McComas and Thomas, 1968). The nomenclature of muscle fibres depends on the different isoforms of the myosin. Three distinct isoforms have been identified, which differ on the basis of ATPase activity. Using the needle biopsy technique (Bergstrom, 1962) and histochemical staining, Peter et al. (1972) classified the fibres types as slow twitch fibres (type I) which rely heavily on aerobic metabolism; fast twitch-oxidative glycolytic (type IIa) have a moderate to high glycolyic activity with a higher capacity for aerobic metabolism and fast twitch glycolytic fibres (type IIb) are characterised by a high glycolytic capacity. Faster myosin heads split ATP about 600 times per second, double the speed of slower myosins (Billeter and Hoppeler, 1992). In most everyday contractions, slow motor units (type I) are the first to be recruited. Greater power output recruits fast (type II) fibres and fast glycolytic fibres (type IIb) are preferentially activated in fast movements.

2.2.3. Excitation-contraction coupling

Excitation-contraction coupling refers to the process by which the nerve signal results in muscular movement. Nerve impulses travel along the axon at a speed of several metres per second and activate the motor unit and all the fibres in innervates. The site of signal transduction from the motor nerve to the muscle fibre's membrane is the synapse. This is separated from the muscle membrane by a cleft or the *neuromuscular junction* (NMJ). The arrival of a nerve impulse at the nerve's endings (motor end plates) stimulates the release of a neurotransmitter, acetylcholine (ACH), into the synaptic cleft and binds to receptors on the muscle cell membrane. The binding of ACH increases the permeability of sodium (Na^+) and initiates depolarisation of the postsynaptic muscle membrane generating an action potential. The action potential travels across the sarcolemma and through the T tubules and the sarcoplasmic reticulum (SR) to the interior of the muscle fibre. The electrical charge elicits the release of large quantities of stored calcium ions from the SR into the myoplasm, where they bind to troponin C on actin filaments.

2.2.3.1. Cross-bridge cycling

Muscle force is generated by the interaction of myosin heads with actin, which detach, and slide further along the molecule - a process of cross-bridge cycling. Prior to the binding of calcium, myosin heads are detached from actin, and adenosine triphosphate (ATP) is bound in the head of myosin. The ATP is split into adenosine diphosphate (ADP) plus phosphate (Pi) but is not released from the ATPase (adenosine triphosphotase) site. The binding of calcium to troponin C on actin filaments causes a conformational change in troponin C and subsequently troponin I, T and tropomyosin. The myosin heads attach to the active sites of actin, forming cross-bridges and the phosphate is released. This causes the head to tilt and pulls the actin filament towards the middle of the sacromere. This is referred to as the 'power stroke' and is the main regulatory step in cross-bridge cycling. After the 'power stroke' the cross-bridge is detached from the thin filament when ATP binds to the myosin head and a new cycle

begins. The muscle relaxes when calcium is actively removed and transported back to the SR, another energy dependent action.

The four states during cross-bridge cycling have been proposed to exist as two bound and two unbound states (Eisenberg et al., 1980). According to this model, the initial crossbridge attachment is in a weakly-bound state. Phosphate release results in the transition to a strongly-bound actin-myosin-ADP state. Pate and Cooke (1989) have developed a mathematical model similar to that of Eisenberg et al.(1980), including a state in which myosin binds weakly to actin prior to release of Pi. Under conditions of high concentrations of Pi, the model predicts that tension decreases. Force is lowered by altering the equilibrium between the two attached cross-bridge states (Pate and Cooke, 1989).

2.2.4. Force-velocity relations

The strength of a muscle is determined by the amount of force it can generate. Muscle can produce force whilst shortening, when it is static and during lengthening. The contractile behaviour of muscle can be characterised by the force-velocity relation, which describes the relationship between muscular tension and shortening velocity. The inverse relationship existing between force or load and shortening velocity was first demonstrated *in situ* in the early work of Fenn and Marsh (1935) and Hill (1938). Hill devised a formula in which the force (F) and shortening velocity (V) represent a hyperbola:

$$(F + a)(V + b) = (F_0 + a)\, b$$

where F_0 is the force exerted in an isometric contraction, and a, b are constants.

At zero velocities, the muscle is contracting whilst the joint angle remains constant. The myosin cross-bridges are formed and recycled, although the external force is too great for actin filaments to be moved (Wilmore and Costill, 1994). This action is termed *isometric*. The force-velocity relationship obtained from one joint angle for a

single muscle during an isometric contraction may not necessarily apply to other joint angles, since isometric force differs over a range of joint angles, as reviewed by Kulig (1984).

When the force developed by a muscle is greater than the external load, the muscle will shorten. During shortening or *concentric* contractions the thick (myosin) and thin (actin) filaments overlap, thereby exerting a positive force. This has been described above (section 2.3.2.1.). At increasing velocities, there is a non-linear decrease in force due to a reduced number of attached cross-bridges. This is due to the shorter time in which the myosin bridges are exposed to a potential binding site (Edman, 1992). Maximum shortening velocities are produced under zero load with lowest force production.

When the external load exceeds the isometric force F_0 the muscle is stretched against its external forces (Gülch, 1994). This *eccentric* action exerts negative force due the lengthening of the muscle. It has been well documented that force increases with velocity during "lengthening contractions" (Katz, 1939; Edman, 1988; Lombardi and Piazzesi, 1990). Increases in velocity above the optimum length, however, do not result in higher forces (Lombardi and Piazzesi, 1990). When muscle fibres are overstretched, the actin and myosin filaments are pulled further apart, hence fewer cross-bridges interact (Wilmore and Costill, 1994). The number of attached cross-bridges increases by approximately 10% from isometric contractions (Lombardi and Piazzesi, 1990), and thus the increase in force is likely to arise from the higher force developed per cross bridge.

The development of isokinetic dynamometry has enabled the characteristics of human muscles to be determined *in vivo*. The curvature of the force-velocity relationship is related to the fibre type composition of the muscle (Thortensson et al., 1976). Force output is greater for fast twitch fibres which are selectively recruited during fast contractions.

2.2.5. Assessment of muscle function

Objective measurements of muscle function are important for the quantification of skeletal disorders and symptoms of weakness and fatigue (Edwards and Hyde, 1977). The developments of equipment such as the strain gauge and isokinetic dynamometers have provided tools for measuring muscle force. The needle biopsy technique, used for histochemical analysis of muscle samples (Edwards et al., 1980) and electromyography employed to measured electrical properties of muscle (Stephens and Taylor, 1972; Moxham et al., 1982; Cooper et al., 1988) have allowed the functioning of muscle to be examined in greater detail. This has proven invaluable for the identification of the properties of muscle and the aetiology of skeletal disorders.

2.2.5.1. Maximal voluntary muscle force

The function of muscle is to generate force. Strength is therefore an important determinant of muscle function. The chain of commands which lead to a voluntary contraction (Edwards, 1978) is shown in Fig. 2.2.4. Impairment in muscle function may occur at any one of these 'links' and result in a reduced capacity to produce maximal strength.

Strain gauge systems have been employed to measure maximal voluntary isometric force in the quadriceps (Edwards et al., 1977b), the first dorsal interosseus muscle (Stephens and Taylor, 1972; Milner-Brown et al., 1973; Tanaka et al., 1984; Rutherford and Jones, 1988) and the adductor pollicis muscle (Merton, 1954; Edwards et al., 1977b; Phillips et al., 1993b,c). Measurement of maximal volitional force is dependent upon the motivation of the subject. Studies which have examined motor unit activation in human muscle have employed the twitch interpolation technique, the superimposition of electrical impulses, to compare the force generated from a voluntary contraction with that elicited from involuntary electrical stimulation. In the absence of fatigue, disappearance of the superimposed impulses confirming maximal activation has been reported for the quadriceps (Rutherford et al., 1986), soleus

(Bellemare et al., 1983) and the dorsiflexor (Belanger and McComas, 1981) muscles. Certain muscle groups are easier to activate than others, and appear to be dependent on their level of recruitment. Rutherford et al. (1986) reported that 30% of subjects, male and females of different ages, who were able to activate their quadriceps fully could not generate maximal activation of their biceps. This has also been found for the plantar flexors (Belanger and McComas, 1981), indicating that differences in muscle activation probably depends on the extent of their use and recruitment in maximal movements.

Stimulation of the motor nerve is impractical for muscles such as the quadriceps. Innervated by the femoral nerve, supramaximal stimulation of this muscle group is painful and can be dangerous. Percutaneous stimulation using surface electrodes is therefore used to assess the recruitment of the quadriceps during voluntary contractions (Chapman et al., 1984; Rutherford et al., 1986).

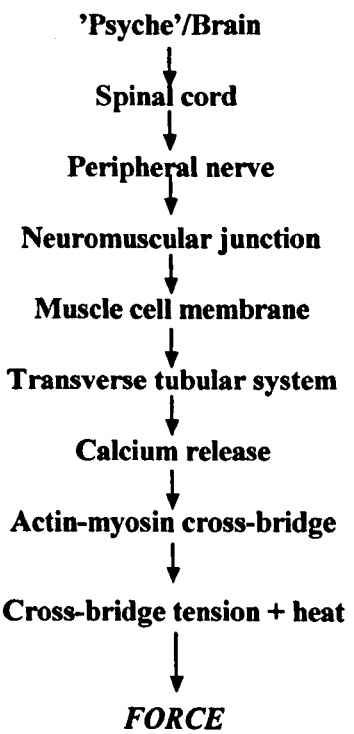


Fig. 2.2.4. Voluntary contraction of human skeletal muscle: From Edwards (1978).

2.2.5.2. Electrical stimulation

Twitch interpolation is one of several applications used in the assessment of muscle function by electrical stimulation. In addition to twitch interpolation, electrically stimulated contractions provide information of contractility, relaxation rate and rate of force fatigue of skeletal muscle. Duchenne in the last century was the first to use electrical stimulation to examine the actions of normal and diseased muscle (Edwards and Hyde, 1977), and also pioneered the development of the needle biopsy technique (Edwards et al., 1980). Much of the earlier work employing electrical stimulation made use of small peripheral muscles such as the first dorsal interosseus (Stephen and Taylor, 1972), the adductor pollicis (Merton, 1954; Edwards et al., 1977b) and the abductor digiti minimi (Burke et al., 1974) with accessible motor nerves. These muscles have little functional significance compared to large muscle groups, such as the quadriceps, which are mostly affected by skeletal disorders (Edwards et al., 1977b). Maximum tetanic stimulation of the femoral nerve innervating the quadriceps is painful and so only a portion of the muscle (20 to 40%) is routinely stimulated. Percutaneous stimulation via superficial branches of the nerve is delivered through large surface electrodes placed proximally and distally over the anterior side of the thigh (Edwards et al., 1977b). The validity of this method has been questioned, with claims that percutaneous stimulation is voltage dependent (Davies and White, 1982). This has not been demonstrated in the quadriceps except at low voltages (Edwards and Newham, 1984) or in either fresh or fatigued sternomastoid muscle (Edwards et al., 1984).

Characteristics of contractile properties are assessed through delivering trains of stimuli in a set pattern of frequencies e.g. 1, 10, 20, 50, 100 and 1 Hz, to the muscle. The is called a programmed stimulation myogram or 'PSM' (Cooper et al., 1988). The force-frequency relationship is illustrated below (Fig. 2.2.5.).

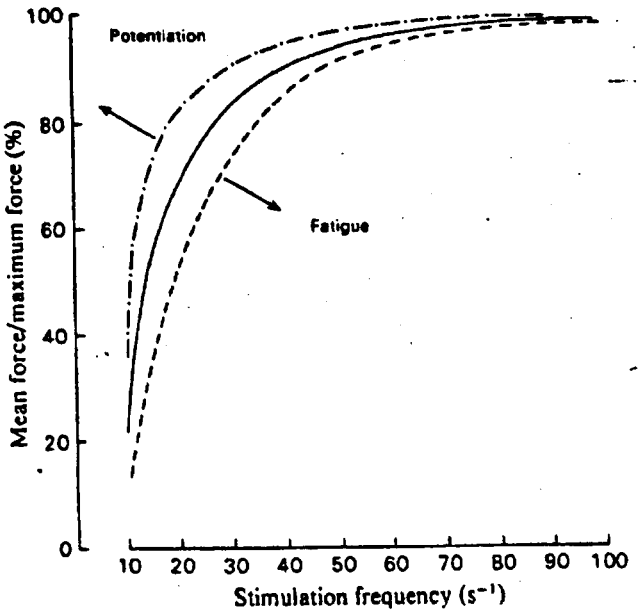


Fig. 2.2.5. Force-frequency curve in fresh and fatigued muscle.

Contractile properties do not necessarily determine fibre composition of different muscles. Round et al. (1984) examined the fibre composition of the human adductor pollicis muscle during postmortem. The muscle was composed predominantly (80%) of type I fibres although the contractile properties were similar to those obtained for the quadriceps which have approximately equal amounts of type I and II fibres. The twitch characteristics of the AP were different to the soleus, despite the higher proportion of type I fibres of this muscle (Round et al., 1984). Edwards et al. (1977b) reported similar force-frequency curves for the quadriceps and adductor pollicis muscle. Contractile properties obtained *in vivo* are agreeable with those obtained in isolated muscle preparations (Faulkner et al., 1979; Moulds et al., 1977).

2.2.6. Muscle weakness

Muscle weakness is a common symptom of neuromuscular disorders, manifest as the inability to perform everyday activities. It is defined as the failure to generate force which may be due to lack of motivation (neural drive), neurological disorders, immobilisation of a limb after fracture or ageing (Edwards, 1978). Muscle weakness is most pronounced in lower limb performance, and is of great concern due to its

principle role in everyday activities. Several mechanisms responsible for weakness have been identified through the development of different methods. So far, the inability to produce maximum force output has been attributed to the failure of electromechanical activation, depletion of fuel supply or degeneration of the contractile apparatus. In light of these factors, Edwards (1978) devised a schematic representation which may be used as a practical approach for diagnosing weakness (see Fig.2.2.6).

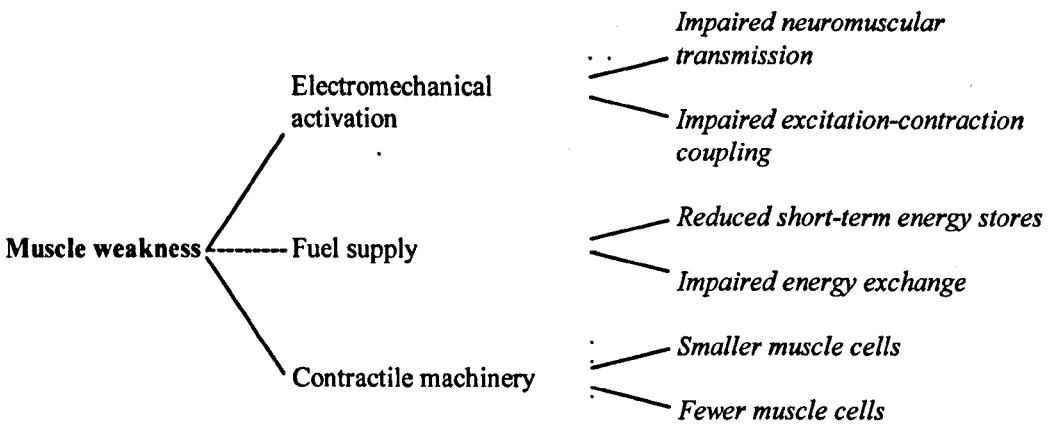


Fig. 2.2.6: Practical scheme for analysis of muscle weakness

2.2.7. Muscle fatigue

Muscle fatigue, which leads to muscular weakness, is defined as the inability to sustain expected force output (Edwards et al., 1981). Fatigue can occur anywhere between the central nervous system (CNS) to cross-bridge cycling, an impairment at any one (or more) site listed in Fig. 2.2.4. It is therefore difficult to identify one site of fatigue. Physiological changes are also manifest, such as the slowing of relaxation. In clinical assessments, muscle fatigue is quantified using volitional and electrically stimulated tests. Each technique is subject to criticism; volitional contractions are reflective of the functional capacity of muscle representative of daily activities, but are dependent upon the motivation and functional central drive of the subjects (Binder-Macleod and Snyder-Mackler, 1993). Electrical elicited fatigue is independent of motivation and determines whether failure to maintain the generated force is the result of lack of

neural drive ('central fatigue') or processes occurring within the muscle ('peripheral fatigue') (Edwards, 1978).

2.2.7.1. Central fatigue

Comparison of the volitional forces with electrically stimulated contractions has provided a measure of 'central fatigue'. The contribution of central mechanisms in eliciting fatigue was reported as early as 1892, when Lombard found that the work capacity of the finger muscle was maintained during electrical stimulation, but reduced during maximal voluntary contractions (MVC) (Fitts, 1994). It has been claimed that muscle fibre recruitment between volitional contractions and electrical stimulation differs and only those muscle fibres beneath the electrodes are activated during electrical stimulation (Fitts, 1994). Volitional contractions produce more work resulting in a higher rate of fatigue.

Several authors have used smoothed rectified electromyography (s.r.e) to record the electrical activity of the muscle during a maximal voluntary contraction (MVC) and electrical stimulated contractions (Stephens and Taylor, 1972; Bigland-Ritchie et al., 1978). Sustained maximal voluntary contraction of the first dorsal interosseus muscle and s.r.e fell proportionatly to loss of force during the first 60s of the contraction, resulting in a constant s.r.e/force.With time, however, force declined more rapidly. This was perceived as a failure of neuromuscular drive (Stephens and Taylor, 1972). These findings differ to those performed on the quadriceps. Bigland-Ritchie et al. (1978) found that s.r.e/force ratio remained constant even when force was decreasing. Even in well-motivated subjects the tendency of central fatigue was overcome during a brief extra effort.

The causes of muscle fatigue are multifactorial, and can take place at various sites within the central nervous system, the neuromuscular junction and several locations within the muscle fibres (Edwards, 1978). Under laboratory conditions, when subjects are well-motivated, muscular fatigue has been shown consistently to have peripheral origins.

2.2.7.2. Peripheral fatigue

Merton (1954) was the first to demonstrate the importance of peripheral fatigue when measuring maximal voluntary effort of the adductor pollicis muscle superimposed with stimulations of the ulnar nerve. Fatigue was reported to be of peripheral origin, occurring beyond the neuromuscular junction and attributed entirely to failure of the muscle contractile system. These conclusions were based on the observations that stimulation of the motor nerve failed to restore the initial force output and recovery from fatigue was not restored when circulation of the muscle was occluded. Subsequent studies have corroborated these findings, maintaining that events proximal to the neuromuscular junction (NMJ), or transmission of information across the NMJ, are not responsible for the the significant limitation to muscular performance (Bigland-Ritchie and Woods, 1984).

The peripheral failure to generate force is due to metabolic factors involving a reduction in ATP, accumulation of hydrogen ions (H^+) or the depletion of glycogen stores. Two other forms of peripheral fatigue have been recognised, depending on the site of fatigue. Low frequency fatigue is characterised by a loss of force when tested at low frequencies of stimulation (10 to 20 Hz), measured as a lowered 20/50 Hz ratio i.e. the force generated at 20 Hz as a percentage of the force at 50 Hz. This is attributed to the excitation-contraction failure. Force which is reduced at high frequencies of stimulation (50 to 100 Hz) is referred to as high frequency fatigue resulting from the impairment of transmission at the neuromuscular junction or conduction of the action potential also inhibits force production.. These forms of fatigue are identified by changes in the force-frequency relationship.

2.2.7.2.1. Metabolic causes of fatigue

Whilst controversy exists as to the aetiology of fatigue, it is well acknowledged that metabolic factors contribute, although may not be entirely responsible, for the reduction in force output. The accumulation of hydrogen ions (H^+), ammonia (NH_3)

and inorganic phosphate (P_i) impair force generation, whereas the depletion of ATP, phosphocreatine (PCr) and glycogen elicit fatigue.

The association between lactic acid, formed in muscle under anaerobic conditions, and force led to the belief that this by-product of glycolysis causes fatigue. Following much debate (see Fitts, 1994 for a review), it has since been established that the free H^+ derived from the dissociation of lactate adversely affects force production through its effects on glycolysis and/or on the contractile mechanism (MacLaren et al., 1989). This is most significant at work loads above 50-60 % of maximal aerobic capacity (Hermansen, 1971) and preferentially affects fast type II fibres. High intensity exercise also results in the accumulation of ammonia and acts as a possible inhibitory metabolite (Fitts, 1994). Similarly to lactic acid formation, ammonia accumulation is pronounced in fast twitch fibres. An increase in the concentration of inorganic phosphate is also implicated in the aetiology of fatigue, and is considered to have an inotropic effect on force production. The binding of P_i to myosin alters the cross-bridge to a weak-binding state (Pate and Cooke, 1989) hence reducing the tension generated by the cross-bridge.

Whilst the increase in P_i is associated with fatigue, the lack of intracellular phosphate may result in the reduction of PCr resynthesis affecting the availability of this immediate energy store (Fitts, 1994) and the regeneration of ATP. A reduction in ATP is a predominant factor involved in fatigue during short-term anaerobic work. Whilst the exhaustion of glycogen influences the capacity to undertake high intensity activities, this source of fatigue is important during submaximal exercise where slow oxidative fibres are recruited. Using muscle biopsy, the depletion of glycogen stores has been reported to be closely related to fatigue during sustained exercise at 65-75% of maximal oxygen uptake (Hermansen et al., 1976).

It is impossible to isolate any one metabolic factor in the cause of fatigue, although the contribution from different metabolites is controlled by the intensity of exercise and the fibre composition of the muscle involved.

2.2.7.2.1. High frequency fatigue

Fatigue at the neuromuscular junction (NMJ) is the first site of fatigue resulting from high frequencies of stimulation. Jones et al. (1979) reported a reduction in force following stimulation at high frequencies of around 80 Hz after a few seconds of 10 to 20% of the initial value. This rapid force loss is accompanied by a change in EMG, suggesting a failure of action potentials along the surface membrane of the muscle fibre (Moxham et al., 1982). Stephens and Taylor (1972) proposed that during a maximal voluntary contraction of the FDI, fatigue originates initially from failure at the NMJ. Conversely, Bigland-Ritchie et al. (1982) found that NMJ failure is not responsible for fatigue during the first 60 seconds of a maximal voluntary contraction and is therefore not a significant factor.

The development of high frequency fatigue has been attributed to the accumulation of potassium in the t-tubules and extracellular spaces, which results in the slowing of the action potential (Jones et al., 1979, 1981). Observations on calcium gradients support these propositions (Westerblad et al., 1990), although the effects of potassium on high frequency fatigue is supported by Sacco et al. (1994). The tibialis anterior was stimulated under ischaemic conditions at optimum and shortened lengths of different frequencies. Delivery of 30 Hz to the shorter muscle generated the same characteristics as stimulating the muscle at an optimum length at a higher frequency of 60 Hz. Recovery was most rapid when the muscle was shortened. This is possibly due to the restriction from shortened muscle in the movement of potassium which accumulates in the t-tubules, and when the muscle is lengthened, the potassium can diffuse freely out of these areas (Sacco et al., 1994).

The importance of high frequency fatigue is questionable since the firing rates of motor units during voluntary contractions are within the range of 5 to 30 Hz. The fall in firing rate from 30 Hz during sustained isometric contractions (Bigland-Ritchie et al., 1986) appears to reduce the tendancy of fatiguing from high frequencies and thus preventing the accumulation of potassium.

2.2.7.2.2. Low frequency fatigue

Low frequency fatigue is characterised by a loss of force at low frequencies of stimulation, and a right-hand shift in the force frequency curve (Fig. 2.3.5). Adequate excitation of the muscle membrane suggests that failure of exitation-contraction coupling occurs. This form of fatigue has been found to occur after dynamic exercise, and may last up to 24 hours. Edwards et al. (1977a) have examined electrically stimulated force generation of the quadriceps following box-stepping and cycling exercise. They reported a reduction in 20 to 50 Hz tetanic tensions of 50% following box-stepping and in one instance, cycling. This loss in force reflected the occurrence of low frequency fatigue due to excitation-contraction uncoupling. This form is fatigue is characterised by the slow recovery rate of up to 24 hours.

Davies and White (1982) investigated three types of dynamic exercise, level running, uphill walking and box-stepping on low frequency fatigue. Twitch and tetanic tensions following the box-stepping was reduced at 10 and 20 Hz, and 20/50 Hz ratio was lower, which lasted 22 hours. Even though their results were similar to Edwards et al., (1977a), Davies and White (1982) claimed that their subjects were weaker, but not more fatiguable following exercise. They also questioned the use of percutaneous stimulation in generating force at 20 and 50 Hz, suggesting that the shape of the force-frequency curve is voltage dependent. Edwards et al. (1984) have subsequently reported that the 20/50 Hz ratio is a reliable indicator of force-frequency relationship of the sternomastiod muscle using percutaneous electrical stimulation in fresh and fatigued muscle.

The mechanisms involved in the loss of force resulting from a failure of excitation-contraction coupling could be due to the impairment of sarcoplasmic calcium (Ca^{2+}) release or myofibrillar Ca^{2+} sensivity (Cooper et al.,1988). Force loss induced by increases in myoplasmic inorganic phosphate (P_i), causing myofibrillar Ca^{2+} insensitivity, cannot explain the persistance of low frequency fatigue since P_i returns to normal within minutes of cessation of activity. Metabolic or ionic changes are therefore unlikely to cause low frequency fatigue characterised by a long recovery rate. It is

possible that damage to the muscle fibre is responsible for this form of fatigue (Jones 1981), since it is most evident in muscles following eccentric or lengthening contractions (Newham et al., 1983) and when the muscle is exercised isometrically at a long length (Jones et al., 1989).

2.2.7.3. Relaxation rate

The slowing of relaxation following tetanic contractions is characteristic of fatigued skeletal muscle. The reduction in relaxation rate is believed to be the contribution to the maintenance of force (Jones, 1981), and prevent the muscle from performing rapid continuous movements. Metabolic changes are suggested to regulate the relaxation rate, although precise mechanisms are inconclusive. A reduced rate in the dissociation of cross-bridges may be involved (Edwards et al., 1975), a process requiring the removal of calcium by sequestration in the sarcoplasmic reticulum (Westerblad and Allen, 1996). In a comparison of normal subjects and patients with myophosphorylase deficiency, Cady et al. (1989) demonstrated that whilst H^+ accumulation is responsible for slowing of relaxation, there is another process which is independent of H^+. Wiles et al. (1979) reported similar relaxation rates from electrically stimulated and volitional contractions, but were not able to find a relationship between relaxation rate and fibre composition, determined from muscle biopsy.

Whilst the purpose of the slowing of relaxation is apparent, the precise mechanisms involved remain inconclusive. There is evidence to support the role of hydrogen ion accumulation, although current evidence suggests that other factors are also involved which have not been confirmed. A reduction in the rate of cross-bridge dissociation, an ATP-dependent process, is implicated in the slowing of relaxation. Therefore, a combination of metabolic factors which are difficult to isolate are responsible for the slowing of relaxation, which occurs independently of fibre-type and the mode of stimulation (electrical versus volitional contraction).

2.2.8. Factors affecting muscle strength

The factors which affect muscle strength are numerous and to review them all would exceed the scope of this thesis. The two most relevant factors appertaining to this work are ageing and reproductive hormones. References to isometric, dynamic and eccentric actions will be made.

2.2.8.1. Ageing

As life expectancy increases, so does the percentage population of elderly people. Understanding the effects of ageing in skeletal muscle has therefore become an increasingly important area of research. Muscle strength is correlated with the capacity to undertake activities such as walking speed and stair climbing (Aniasson et al., 1983). A reduction in muscle strength will therefore compromise the functional status of the elderly.

2.2.8.1.1. Muscle strength

Maximal strength is attained in young adulthood around 20-30 years, and declines with age. This was first reported as early as 1835 by Quetelet, who found loss of strength from 30 years (Larsson et al., 1979; Vandervoort and McComas, 1986; Narici et al., 1991). The onset of weakness has since been proposed to develop later, although there is controversy as to when this occurs. Larsson et al. (1979) found that maximal isometric and dynamic leg strength measured on an isokinetic device is attained at 20-29 years, and remains constant until 40-49 years. After 50 years, strength declined by 28.1% at 70 years compared with a younger group. These findings are in agreement with Asmussen and Heebøll-Nielsen, (1961), who reported peak isometric strength of the knee extensors at 30 years in males. In females, strength peaked at 20 years and decreased at an accelerated rate from 40 years. Aniansson et al. (1983) found a significant decrease in isometic and isokinetic torque in both sexes for the knee extensors between 70 and 75 years, whereas Borges (1989) reported a significant decrease in isometric strength at 60-70 years in males and females. A further 30% loss

in peak torque of the knee extensors in both sexes was documented in 78-81 year olds (Danneskiold-Samsoe et al., 1984). Strength losses with age are more pronounced in lower limb proximal muscles (Larsson, 1978). However, reductions in strength of other muscles are also manifest. Under isometric (Vandervoort and Hayes, 1989) and isokinetic (Cunningham et al., 1987) conditions, maximal strength of the plantar flexors declined with age.

Eccentric strength is not compromised to the same extent as concentric strength for knee extensors in elderly males and females (Vandervoort et al., 1990; Poulin et al., 1992), although the mechanism for this is not known. Narici et al. (1991) measured maximum strength in a distal upper limb muscle, the adductor pollicis (AP), in males aged 20-91 years and found that strength declined significantly from 59 years, and by the eighth decade strength was 57.6% of males in the second decade. Frontera et al. (1991) reported strength of the elbow extensors and flexors at 22.2 and 16.7% lower respectively in older women. In a longitudinal, Kallman et al. (1990) found that grip grip increased into the fourth decade, with an accelerated loss of strength thereafter. However, they also found that many older subjects maintained their strength throughout the duration of the 9 year study, whereas middle-aged and younger subjects lost strength. These findings demonstrate that great inter-individual variation in strength loss exist across all ages.

There is evidence to suggest that isometric strength is better maintained than dynamic strength in the elderley. An increased speed-dependent loss of force with age has been documented in several studies (Larsson, 1978; Larsson et al., 1979; Murray et al., 1985; Harries and Bassey, 1990; LaForest et al., 1990). Aniansson et al. (1983) reported a loss of force with increasing angular velocities which was more marked in males and females aged 70 years. Since peak torque was recorded at a knee angle of 0.52 rad (60°) there was probably insufficient time to allow for maximal activation at faster velocities. Borges (1989) reported that the time course, or onset, for the significant reduction in isokinetic torque occurs between 40-50 years in females across velocities of 0.21, 1.57 and 2.62 rad/s. This selective loss in dynamic leg strength measured isokinetically at increasing angular velocities is attributed to a reduction in

fast twitch fibres with age (Grimby and Saltin, 1983). This may be secondary to a decrease in physical activity recruiting these muscle fibres.

2.2.7.8.2. Muscle mass

Muscle strength is proportional to the "active cross-sectional area" of the muscle (Larsson, 1978). The relationship between strength and cross-sectional area (CSA) (Ikai and Fukunaga, 1968) would therefore indicate that the decrease in strength is attributable to a reduction in muscle mass. Indeed, substantial atrophy has been detected in the quadriceps of old women, which was 33% smaller than young women (Young et al., 1984) and 25% smaller in old compared to young males (Young et al., 1985).

Changes in the components of muscle account for the decrease in muscle mass. Atrophy of type II muscle fibres (Larsson et al., 1979; Lexell et al., 1988), a loss of muscle fibres (Grimby and Saltin, 1983; Lexell et al., 1988) and a loss of functioning motor units (Campbell et al., 1973) have been proposed. It has been claimed that the loss of fibres begin at 25 years, and accelerates thereafter (Lexell et al., 1988). These latter results support the increase in the speed-dependent loss of force with age.

2.2.8.1.3. Specific force

While a certain percentage of strength loss is attributable to a reduction in muscle mass, there is growing evidence to suggest that there are qualitative changes in muscle such that weakness is greater than loss of muscle mass. This specific decrease in force, has been reported in mice (Brooks and Faulkner, 1988; Phillips et al., 1991) and human (Bruce et al., 1989) muscle. Brooks and Faulkner (1988) detected a decrease in force per cross-sectional area (force/CSA) of around 11% in the soleus muscle and 20% of the extensor digitorum muscle (EDL) for isometric and shortening velocities. Phillips et al. (1991) reported a similar magnitude in loss of 13.3% for the soleus muscle in aged mice.

This weakness in animals has also been detected in humans. Using a method validated for measuring CSA of the AP muscle (Bruce et al., 1989), a 27% reduction in normalised force was found in elderly muscle compared with young controls, confirming the occurrence of a specific loss of force in human muscle not attributable to atrophy (Bruce et al., 1989). Young et al. (1984, 1985) reported a reduction in maximal voluntary force/CSA for elderly males (Young et al., 1985) but not for elderly females (Young et al., 1984). However, the problems inherent in accurately assessing the CSA of a large, multi-pennated muscle group such as the quadriceps, particularly when a large amount of atrophy has occurred, may have resulted in the failure to detect such losses. Some ageing studies which have estimated muscle mass have failed to detect a loss of specific strength. Frontera et al. (1991) proposed that muscle weakness of knee and elbow extensors and flexors in the elderly was proportional to muscle mass, as estimated from urinary creatinine excretion. Davies et al. (1986) found that specific tension of the triceps surae was 40% lower in the elderly compared to young subjects, although the error of calculating CSA from anthropometric measurements probably disguised the 'real' change in specific force. Measurement error will always confound accurate readings of CSA. However, there are improved methods of measuring muscle size available, albeit expensive (i.e. nuclear magnetic imaging, computed tomograghy). Using computed tomography, Overend et al. (1992) measured the ratio of CSA and isometric (90°) and dynamic strength (2.09 rad/s) of knee extensors and flexors. The authors did not report any age-related difference in isometric strength/CSA, although the concentric strength ratio was significantly lower in elderly men, indicating a loss of strength greater than the decrease in muscle mass. In humans, a reduction in specific strength has only been documented for isometric strength.

2.2.8.1.4. Contractile properties

Ageing of skeletal muscle is not only characterised by changes in strength: alterations in the contractile properties have also been found to occur. Electrically evoked impulses of whole muscle demonstrate that time to peak tension and relaxation time/rate are slower in elderly muscle compared with young for the triceps surae

(Davies et al., 1986), adductor pollicis (Narici et al., 1991) and quadriceps (Beltran-Niclos et al., 1995). Davies et al. (1986) reported not only significantly weaker muscles in the elderly, but greater fatiguability. In contrast, Narici et al. (1991) found the AP more resistant to fatigue in older subjects, possibly due to the decrease in size and number of type II fibres and an increase in the fatigue resistance of the existing large proportion of type I fibres. The inconsistency in findings are probably due to the different muscles tested and the variation in fatigue protocols. A leftward shift in the force-frequency curve (Narici et al., 1991) is supported in other studies of the human quadriceps (Beltran-Niclos et al., 1995) and aged mice (Brooks and Faulkner, 1988). This was suggested to occur as a result of significant muscle atrophy.

2.2.8.2. Reproductive hormones

A vast number of hormones have direct or indirect effects on skeletal muscle. For a review, see Florini (1987). The role of female sex hormones on muscle function has received relatively little attention. This is surprising given the widespread use of oestrogens to enhance meat production in farm animals (Florini, 1987). The most frequently used model for the examination of reproductive hormones on muscle performance has been the human menstrual cycle. Lately, the endocrinopathology of the menopause has also generated interest in its effects on muscle strength.

2.2.8.2.1. The human menstrual cycle

With increased participation of females in sporting activities, much of the earlier work investigating the menstrual cycle on muscular performance was prompted from speculation of a possible detrimental effect of the cycle phase on athletic performance. Athletes have supported these claims, perceiving that the premenstrual and first two days of the menstrual phase impairs performance (Erdelyi, 1962). However, it is the studies which measure muscular performance objectively which have practical applications. Wearing et al. (1972) measured hip flexion/extension and performances from the standing broad jump, and reported that the poorest performance occurred during menses, consistent with self-reports from athletes (Erdelyi, 1962), but peaked

pre-menstrually. Quadagno et al. (1991) measured performance of weight-lifters and swimmers at three phases, pre-menstrual, menstrual and post-menstrual across three cycles. They did not report any difference over the three phases. The problems associated with comparing explosive sports is the variability in the nature of the activity, and fitness of the subjects who are prone to disruptive cycles with increasing levels of fitness (Loucks and Horvath, 1985).

Studies in which dynamic strength performances have been assessed under laboratory conditions have failed to detect any changes in leg strength measured isokinetically across angular velocities of 1.05 to 4.18 rad/s (Dibrezzo et al., 1988, 1991, 1994; Richardson and George, 1993). Conversely, there have been several report of changes in isometric strength across the menstrual cycle for different muscles (Wirth and Lohman, 1982; Davies et al., 1991; Phillips et al., 1993a, 1996; Sarwar et al., 1995). These studies, however, have not yielded consistent findings. Wirth and Lohman (1982) reported greatest handgrip strength during the follicular phase compared with the luteal phase. In another study, handgrip was highest during menses with respect to the follicular and luteal phase (Davies et al., 1991).

Petrofsky et al. (1976) measured isometric grip strength in 7 females, 3 of whom were taking oral contraceptives (OC). Whilst there were no changes in force production between the pre-ovulatory and luteal phases in non-OC users, endurance time at 40% of maximal isometric strength was lower during the luteal phase. The low number of subjects tested may be accountable for this discrepancy with previous studies. Allen and Bailey (1982) assessed grip strength in two groups of subjects, one group was motivated and the other group was a control, across four phases — pre-menstrual, menstrual, post-menstrual and mid-cycle — and found no significant change in strength across the cycle between groups.

Motivating subjects does not guarantee maximal effort during a voluntary contraction. Furthermore, the palmar flexors may be more difficult to maximally activate compared with the quadriceps which are probably recruited more frequently at high intensities. Sarwar et al. (1995) found that handgrip strength peaked mid-cycle compared with

early follicular, mid-follicular, mid-luteal and late-luteal phases. Whilst maximal activation of the palmar flexors was not determined, the pattern of force change emulated those of the quadriceps, in which twitch interpolation confirmed that strength changes were peripherally modulated.

These findings so far support a role of reproductive hormones influencing force production. The variability in cycle phase and research design masks the hormone milieu responsible for these changes. The most detailed study to date of the hormonal fluctuations during the menstrual cycle is the work on the adductor pollicis (AP) muscle. In a preliminary report, a peak in force of the AP was found during the follicular phase with a rapid reduction around mid-cycle of 20% (Phillips et al., 1993a) In the full study, Phillips et al. (1996) made 8 measurements of force production of the AP over 3 cycles, and interpolated missing values. The results supported their preliminary findings of an ovulatory dip in force, preceded by a follicular peak in strength.

These authors further advocate the role of oestrogen in exerting a positive inotropic effect on skeletal muscle. The change in strength from peak to trough occurred within 2 days. Since the pre-ovulatory transient surge in oestrogen lasts ~24 hours (Ojeda, 1992), these endocrinological changes could easily be missed. Furthermore, oestradiol levels did not correlate with strength measured at the same time. Phillips et al. (1996) attributed this discrepancy to a phase lag between oestrogen and muscle force changes. Petrofsky et al. (1976) also suggested that some latency exists between hormonal changes and the isometric muscular response. However, other studies which correlated strength with oestrogen found similar results to Phillips et al. (1996). Bassey et al. (1995) reported a negative association between oestrogen and handgrip strength, which has been implicated in previous studies (Wirth and Lohman, 1982; Davies et al., 1991). Rice (1988) performed a battery of strength tests during 1 to 5 days of the menstrual cycle and correlated these indices with oestradiol levels. They did not detect a significant relationship between these two variables. These results indicate that another hormone in addition to/instead of oestrogen is likely to be involved.

Muscle function can also be described from the contractility of the muscle. Sarwar et al. (1995) examined the contractile properties of the quadriceps through percutaneuos stimulation at 5 phases - early/mid-follicular, mid-cycle, mid-luteal and late-luteal. A leftward shift of the force-frequency curve as a result of slowing of relaxation was reported at the ovulatory stage, when the muscle was also more fatiguable. The mechanisms affecting these characteristics of contractile properties are probably related to metabolic changes, such as an increase in Pi or decrease in pH (Cady et al., 1989) rather than direct effects of reproductive hormones. The increase in fatigue resistance mid-luteal has also been reported in respiratory muscles (Chen and Tang, 1989), probably attributed to the progesterone-induced rise in basal body temperature, resulting in an increased blood supply to the muscle and the reduction in fatigue. These results however, are not consistent with findings of Petrofsky et al. (1976) who demonstrated lower endurance mid-luteal compared with pre-ovulatory measurements. With only two measurements, and few subjects (n=4) this latter study is flawed.

The effects of oral contraceptives on muscle strength have not been as well documented, despite the widespread use of these exogenous compounds in athletes (Lebrun, 1994). Despite few subject numbers, Petofsky et al. (1976) did not find any difference in handgrip strength between the OC and non-OC users. Isometric endurance at 40% maximal force was also stable in the OC group (n=3) but fluctuated in the non-OC users, depending on forearm temperature. Wirth and Lohman (1982) found no variation in isometric grip strength in OC-users across two phases, but reported lower endurance time and force production compared with non-OC's. The lower strength values for the quadriceps and handgrip in OC-users across 5 phases of the cycle was attributable to lower body weight of the control group (Sarwar et al., 1995). The suppressive effects of endogenous hormones by oral contraceptives stabilises force production in OC-users, and do not appear to be detrimentral to muscular performance.

2.2.8.2.2. The menopause

An examination of the time course of age-related losses in specific force of the adductor pollicis muscle have revealed that the onset of this weakness differs between males and females (Phillips et al., 1993b). A significant reduction in the force per cross-sectional area (CSA) is manifest around 50 years of age in females, whereas in males this force loss begins much later at 60 years plus. These results are shown in Fig. 2.3.7. These findings implicate an involvement of reproductive hormones influencing force production, since women of this age group are experiencing declining levels of reproductive hormones in response to ovarian failure. This has also been corroborated in animal studies. Ovariectomised mice have a lower force/CSA of the soleus muscle than young control mice (Phillips et al., 1993c). Oestrogen deficiency is the single most significant factor in post-menopausal osteoporosis (Riggs et al., 1982) and Rutherford and Jones (1992) reported a significant weakness of the quadriceps in osteoporotic women compared with an age-matched healthy group.

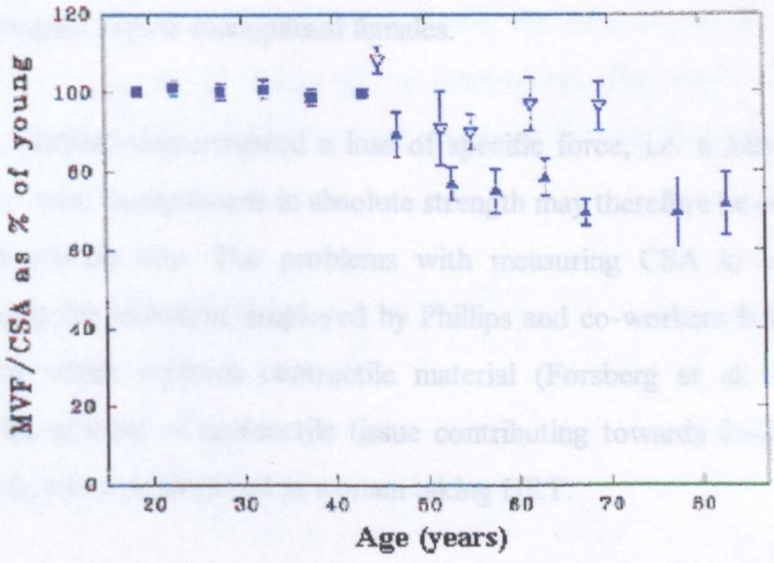


Fig. 2.2.7: *The relationship between mean (±SE) specific force and age in males and pre-menopausal females 45 years and under (■), peri- or post-menopausal women (n=67) not on HRT (▲) and peri- or post-menopausal women (n=25) on HRT (▽). Specific force is expressed as a percentage of the mean value for young subjects. From Phillips et al., 1993b.*

Whilst a reduction in reproductive hormones is associated with a loss of specific force, its effects on absolute strength are conflicting. Petrofsky et al. (1975) and Cauley et al. (1987) observed a reduction in handgrip strength in females 50 years and older. Handgrip strength in post-menopausal women was greater in those taking HRT, although when age, height and physical activity were taken into account, hormone status became less significant (Cauley et al., 1987). Bassey et al. (1996) examined data from a large representative survey and reported muscle function parameters between four groups of women. These comprised women who had regular menstrual cycles, irregular menstrual cycles, post-menopausal, amenorrhoeic women and a group taking hormone replacement therapy. There were no differences in leg extensor power or isometric strength of the quadriceps or handgrip between the four groups after correcting for fat free mass. In an older group of women (60 to 70 years), muscle performance measured through conventional isotonic exercises (e.g. 1 repetition maximum) was not significantly different between oestrogen replacement users versus hypoestrogenic females. Calmels et al. (1995) however, found that isokinetic measurements of the elbow flexors at 0.52 and 3.13 rad/s declined rapidly during the 5th and 6th decades in post-menopausal females.

Phillips et al. (1993b) demonstrated a loss of specific force, i.e. a loss of force per cross-sectional area. Comparisons in absolute strength may therefore be confounded by differences in muscle size. The problems with measuring CSA in older women, particulary using the technique employed by Phillips and co-workers is the increasing amount of fat which replaces contractile material (Forsberg et al., 1991) which exaggerates the amount of contractile tissue contributing towards force production. This tends to be more pronounced in women taking HRT.

Hormone replacement therapy is proposed to confer protection against muscle weakness associated with the menopause. Controlling for age, height and weight, Phillips et al. (1993b) reported higher force/CSA in women taking HRT compared with females who were not (Fig. 2.3.7.). In a recent large prospective randomised controlled trial, 116 post-menopausal women were tested for handgrip strength over

48 weeks and leg extensor power over 24 weeks. Hormone replacement therapy, of oestrogen only or oestrogen and progestogen preparations, was not found to increase handgrip strength or leg extensor power (Armstrong et al.,1996). These findings are consistent with those of Kohrt et al. (1995). Following the adminstration of HRT of the same preparation, 32 healthy women aged 60 to 72 years were tested over 11 months. Peak torque of the quadriceps and hamstrings at 0 and 1.05 rad/s did not change in the control or HRT groups (Kohrt et al., 1995).

The mechanisms by which reproductive hormones exert their effect is uncertain. In the human adductor pollicis muscle of elderly (Phillips et al., 1991), hypoestrogenic post-menopausal women (Phillips et al., 1993b), and in the soleus muscle of ovariectomised mice (Phillips et al., 1993b) weakness is restored by applying a rapid stretch during an isometric contraction. Lengthening the muscle forces all the cross-bridges into the high force state regardless of the force state at the onset of the stretch (Lombardi et al., 1990). It is therefore apparent that the hormones may affect the equilibrium between "high" and "low" force states at the cross-bridge. This model of varying tensions at the cross-bridge is discussed in section 2.3.2.1. Phillips et al. (1993b) proposed that this inotropic effect may be caused by oestrogen altering the sensitivity of the cross-bridges to the metabolites inorganic phosphate [Pi] or lowered pH. The precise mechanism, or the hormone responsible i.e. oestrogen or progesterone, has not been confirmed.

CHAPTER THREE

THE DEVELOPMENT OF EXPERIMENTAL METHODS

3.0. THE DEVELOPMENT OF EXPERIMENTAL METHODS

The aims of this chapter are to establish the inherent mechanical and biological variability of the equipment employed in the experimental work (Chapter 4.0). Study 3.2 was undertaken to determine the reliability of the LIDO Active® isokinetic dynamometer for measuring concentric strength across a range of slow and fast angular velocities. The results are used in the design of the experimental protocol for Study 3.3 in the assessment of reliability of muscular performance in middle-aged women. Reliability of the strain gauge system, used for measuring volitional and electrically stimulated contractions, is established in Study 3.4. These results will be important for interpreting changes in muscle function with fluctuations in endogenous hormones in Chapter 4.2. Finally, the repeatability of the hand dynamometer for measuring force production of the first dorsal interosseus (FDI) is examined in Study 3.5. It is also important to determine the reliability of stimulating this small muscle percutaneously.

3.1. Introduction

The assessment of skeletal muscle function, either pre- or post- intervention or in single measurements, necessitates reliable and reproducible measures of force output during day-to-day testing (Stokes, 1985). The degree of reliability, however, depends on the purpose for which the results are used. The measurement of maximal strength provides an index of muscle function and is a useful method to assess both ageing populations and patients with myopathic disorders (Edwards et al., 1977b). Where sequential measurements during treatment are necessary, it is important to reduce any variability which may disguise real changes in strength. This can be accomplished through carefully designed protocols, standardised application of the protocol and the use of sensitive equipment (Frontera et al., 1993).

3.1.1. Methods of assessing muscle performance

Muscular performance is commonly assessed by measuring maximal voluntary contractions in a static position. This facility is available on computerised isokinetic dynamometers, although cheaper alternatives such as the strain gauge (Edwards et al., 1977b) and torque transducer are commonly used. Many of the earlier studies measured isometric strength of small muscles such as the *adductor pollicis* (Merton, 1954; Edwards et al., 1977b), *first dorsal interosseus* (Stephens and Taylor, 1972) and *abductor digiti minimi* (Burke et al., 1974). In 1977, Edwards and Hyde introduced the hand held myometer for measuring a muscle group where the patient was required to push against the device, counteracted by the experimenter's resistance. Problems were encountered with this technique, which included the lack of precise positioning and the limitations of the strength of the tester, and thus its use was restricted to children and adults with severe weakness. Unfortunately, the repeatability of these pieces of equipment has not been reported and it is therefore difficult to compare different devices for reliability.

The *quadriceps* muscle group, which has an important role as a weight-bearing muscle, is most often examined isometrically (Young et al., 1984, 1985) using a strain gauge system with the knee flexed at 90° (Edwards et al., 1977b). The coefficient of variation (CV) for repeated measurements employing this method has been reported in young females at 7.7% (Young et al., 1984) and 8% for young males (Young et al., 1985). The use of CV as an estimate of reliability will be discussed later (section 3.1.2). Electrical stimulation is routinely used to determine maximal activation of the muscle during an isometric contraction (Merton, 1954; Belanger and McComas, 1981; Rutherford et al., 1986) in which superimposed 1 Hz impulses disappear during maximal contractions. In the quadriceps, the muscle is usually stimulated percutaneously although supramaximal stimulation via the femoral nerve has been used (Edwards et al., 1977b). This method is not advised since it is painful and there is danger of dislocating the patella (Edwards, 1978). Smaller muscles with easily accessible motor nerves e.g. adductor pollicis, can be stimulated supramaximally.

Developments in the use of hand dynamometry have provided the opportunity for measuring grip strength. This measurement is particularly useful in monitoring generalised or local disease (Anderson and Cowan, 1966) and is correlated with other performance indices (Danneskiold-Samsoe et al., 1984; Kallman et al., 1990). Repeatability studies on grip strength are limited. Kallman et al. (1990) reported a 6% CV for repeated grip tests using an adjustable hand dynamometer in subjects aged 20 to 100 years. When elderly subjects (7th decade) are considered separately, an increase in CV to 14.1 and 13.7% are reported for females and males respectively (Anderson and Cowan, 1966). It was not reported whether the CV represented repeated repetitions or day-to-day trials.

Isometric contractions do not represent habitual daily movements and isokinetic dynamometry which measures concentric strength, has therefore gained popularity (Lord et al., 1992). Isokinetic devices allow dynamic movements through a range of motion (ROM) where the velocity remains constant and the change in muscular torque is 'accommodated' by the dynamometer. The reliability of these devices have been assessed (Moffroid et al., 1969; Johnson and Siegel, 1978; Tredinnick and Duncan, 1988; Gleeson and Mercer, 1992), and CV's of 2.9 - 13.1% have been reported for peak torque of the quadriceps at different velocities over separate days (Thortensson et al., 1976; Narici et al., 1991; Gleeson and Mercer, 1992).

The movement involved in isokinetic dynamometry results in a change in muscle length. Standardisation of positioning of the subject on the device is therefore a prerequisite of testing. Reliable test protocols have been established to ensure maximal efforts are recorded. Maximum warm-up contractions are required before recording true peak torque (Johnson and Siegel, 1978) and a minimum of 3 trials is necessary to achieve stable isokinetic data for peak torque (Gleeson and Mercer, 19922). Sawhill et al. (1982) maintained that more trials are needed at higher angular velocities (6.96 rad/s). These results from young healthy subjects do not represent the performance from other populations. In the elderly, for example, it has been reported that two trials are insufficient in attaining stable peak torque (Murray et al., 1985; Harries and Bassey, 1990; Frontera et al., 1993). Furthermore, a five percent increase was found to

occur from the first to second repeated test at 1.74 rad/s in 68 year old females (Harries and Bassey, 1990), twice that observed by Frontera et al. (1993). Murray et al. (1985) also reported a greater peak torque in the second test, although only the isometric test at 45° reached statistical significance.

3.1.2. Statistical errors in repeatability studies

In many studies in which reliability has been reported for muscle strength devices, incorrect statistical techniques have been used. Results from prior studies must therefore be interpreted with caution. The analysis of variance or t-test techniques determine if there are any systematic changes or trends in the data as a result of a practice or familiarisation effect. If this is significant, subsequent tests of reliability are invalid. The correlation coefficient is often employed to measure reliability (Francis and Hoobler, 1987; Brown et al., 1992; Lord et al., 1992., Frontera et al., 1993). This technique is highly influenced by the heterogeneity of subjects, and is a measure of relationship rather than agreement (Bland and Altman, 1986). Intraclass correlation, recommended for repeatability studies (Vincent, 1994), may also be compromised by large inter-individual differences (Atkinson, 1995). The coefficient of variation (CV%) measures agreement of test-retest data, and is reported as an index of reliability (Sale, 1991). This statistical technique is also subject to error and criticism. Firstly, there are eight different methods of calculating CV which may yield different results. Secondly, the CV should only be used if the variability increases as the scores diverge i.e. the CV assumes that stronger subjects are more variable in repeated measures. This feature, known as *heteroscedasticity*, is present if the relationship between the mean scores and differences of test-retest data is significant. Finally, the CV uses the standard deviation which excludes one third of the population ie. the CV does not imply a 95% error range (Strike, 1991). To allow for this, the standard deviation should be multiplied by 1.96 (then divided by overall mean x 100). It is therefore apparent that the CV reported in studies is underestimated.

Bland and Altman (1986) advocated the use of 95% limits of agreement for expressing reliability. The agreement limits are calculated from the mean of the difference in test-

re-test scores ± 2 standard deviations and assumes that for any new subject tested, two repeated measurements would differ by ± newtons or less. Plotted on a graph, the variability can be illustrated and interpreted more easily. If the data are prone to heteroscedasticity a logarithmic transformation should be undertaken before plotting the data. Each of the following studies will be analysed using the above techniques.

3.2. DAY-TO-DAY RELIABILITY OF LEG STRENGTH MEASURED ISOKINETICALLY USING THE LIDO®ACTIVE DYNAMOMETER

Aspects of this work have been presented at the British Association of Sport and Exercise Sciences Annual Conference, Aberdeen, 1994.

3.2.1. Introduction

The reliability of the LIDO Active® dynamometer (Loredan, Davis, CA, USA) for measuring leg strength, utilising the isokinetic mode, was assessed in this study. The aims of the study were to:

1] Examine the day-to-day variability in maximal strength of the knee extensors (KE) and knee flexors (KF) using reciprocal movements.

2] Assess the reliability of slow and fast angular velocities on maximal repeated performance. These aims were fulfilled using a protocol adapted from previous studies, as reported in the literature (Johnson and Siegel, 1978; Gleeson and Mercer, 1992).

3.2.2. Methods

(i) Subjects

Ten subjects, seven males and three females, volunteered to participate in the study and gave written consent. Subjects were recruited if they were free of pain and injury to the lower extremities. Ethical approval was obtained from Liverpool John Moores University Human Ethics Committee. Table 3.2.1 summarises subject characteristics of age, height and mass.

Table 3.2.1: *Mean (± sd) of age, height and mass of young, healthy subjects*

Sex	n	Age (years)	Height (cm)	Mass (kg)
Male	7	24.1 (1.6)	1.79 (0.61)	77.7 (13.1)
Female	3	25.3 (0.6)	1.66 (0.90)	57 (8.5)

(ii) Procedure

Dynamic concentric strength was measured on an isokinetic dynamometer (LIDO Active®, Loredan, Davis, CA). Each subject was tested with the same protocol on four separate occasions, at least two days apart and at the same time of day (10.00±1 hr). The first sessions was a familiarisation with the equipment and the procedures involved. Subjects were measured for height and mass. The dominant leg was reported as the right leg for the whole group.

A standardised warm-up was initially performed on a Monark Cycle ergometer for 5 min with no resistance, at 60 rev/min. This was followed by 3 min of static stretching of the relevant muscle groups. The subjects were seated in an adjustable chair; the upper body stabilised with straps secured across the shoulders, chest and hips. A resistance pad was also positioned on the thigh, proximal to the knee joint to localise the quadriceps and hamstrings (Plate 3.2.1).

The axis of rotation of the dynamometer shaft was aligned with the axis of rotation of the knee joint, midway between the lateral condyle of the tibia and the lateral condyle of the femur. The cuff of the dynamometer's lever arm was attached to the ankle, proximal to the malleoli. These positions were recorded for individual subjects and standardised for subsequent trials. Range of motion (ROM) was preset to 0 to 90°. The gravity compensation procedure required subjects to relax, while the leg was passively extended and flexed through the entire ROM.

Subjects were instructed to grasp the handles adjacent to the chair during the tests and they then performed two submaximal knee extension and flexion contractions. Testing consisted of four reciprocal maximal voluntary isometric movements at angular velocities of 1.05 rad/s, 3.13 rad/s and 5.22 rad/s. This testing order for velocity was standardised from the slowest to the highest as recommended by Wilhite et al. (1992). Each trial was separated by one minute of passive recovery. Verbal instructions were also standardised and visual feedback was given. Gravity corrected peak torque was selected from the strength indices as a measure of muscular performance.

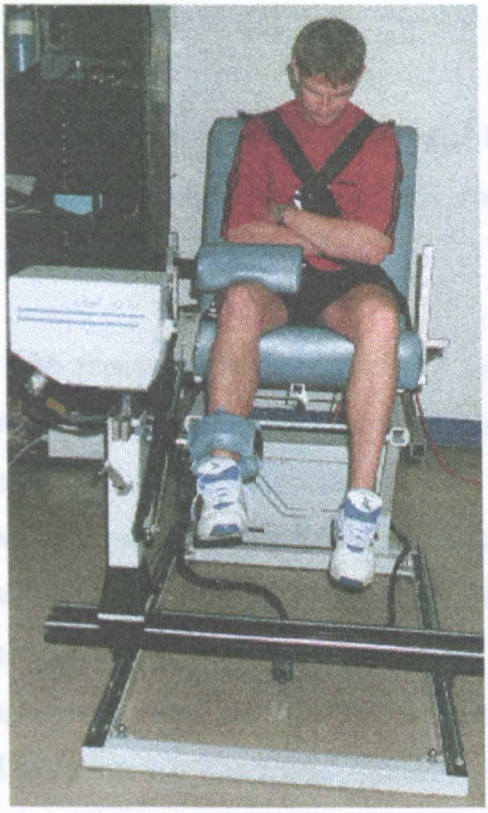


Plate 3.2.1. *The isokinetic dynamometer (Lido Active®, Loredan, Davis CA) measuring dynamic strength of the knee extensors and flexors*

(iii) Data analysis

The Statistical Package for the Social Sciences (SPSS) and Excel (Windows version 3.1) were used for data analysis. The following tests were undertaken on the data for each reliability study:

1. Analysis of variance with repeated measures (or t-test for two sets of data) was employed to detect mean differences between the test-retest trials for leg extensors and flexors across the range of angular velocities.
2. Parameters which revealed non-significant differences, indicating no trend in mean strength, were analysed for error linearity i.e. the relationship between the mean and differences of test-retest scores.
3. The 95% limits of agreement were calculated from mean difference between the two tests ± standard deviation x 2. The Bland-Altman plots illustrate the deviation from the mean across individual samples. If the error linearity was significant, demonstrating heteroscedasticity, logarithmic transformations of the data were performed. Significant differences also support the use for coefficient of variation (CV%).
4. The coefficient of variation was calculated using conventional methods (SD/ overall mean x 100).

3.2.3. Results

The results of the repeated measures ANOVA and reliability measurements for leg extensors and flexors across velocities are shown in Table 3.2.2.

Leg extensors

There were no significant differences ($p>0.05$) in peak torque between trials at each velocity for muscle performance as revealed by the ANOVA results. There was a lack of relation between mean scores and difference in test-retest data at all velocities, hence the CVs reported are not 'true' indices of reliability. Peak force decreased with

higher angular velocities (Fig. 3.2.1) and consequently limits of agreement were narrower at 3.13 and 5.22 rad/s compared with the slower velocity of 1.05 rad/s. However, at 5.22 rad/s the agreement limits were wider than at 3.13 rad/s. The reliability at fast velocities must therefore be questioned.

Leg flexors

The ANOVA results do not reveal a serial effect over the three trials for any angular velocity tested ($p>0.05$) (Table 3.2.2). At the slower velocities, the error linearity was not significant. This relationship was significant at 5.22 rad/s ($r = 0.68$; $p<0.05$). The 95% limits of agreement for flexors across all angular velocities were high, whereas at 3.13 and 5.22 rad/s they exceeded those of leg extensors. This demonstrates that the flexors have poor reliability since peak torque is much lower for flexion compared with knee extension (Fig. 3.2.1).

Table 3.2.2: Results of the repeated measures ANOVA, coefficient of variation (CV%), error linearity and 95% limits of agreement for knee extensors and flexors of the dominant leg

Muscle group Velocity (rad/s)	95% limits of agreement (Nm)	CV%	$F_{9,9}$ values (p values)	Error linearity†
Right extensors				
1.05 rad/s	-34.9 to 50.3	9.6 *18.9**	0.49 (0.62)	0.44
3.13 rad/s	-11.4 to 15.0	4.1 *7.9**	0.02 (0.98)	0.10
5.22 rad/s	-18.2 to 18.4	7.3 *14.2**	0.53 (0.62)	0.40
Right Flexors				
1.05 rad/s	-31.4 to 33.8	12.7 *24.8**	2.35 (0.12)	0.31
3.13 rad/s	-20.9 to 13.1	8.2 *16.1**	1.93 (0.17)	0.45
5.22 rad/s	-17.0 to 10.8	7.9 *15.5**	1.22 (0.32)	0.68

CV%* = standard deviation x 1.96 / overall mean x 100

† = the relationship of r is calculated from the mean of t1/t2 and the difference in scores

Mean (± SEM) peak torque (Nm) for knee extension and flexion across three test sessions are shown in Fig. 3.2.1. a) and b). It is evident that peak torque declines as the angular velocity increases.

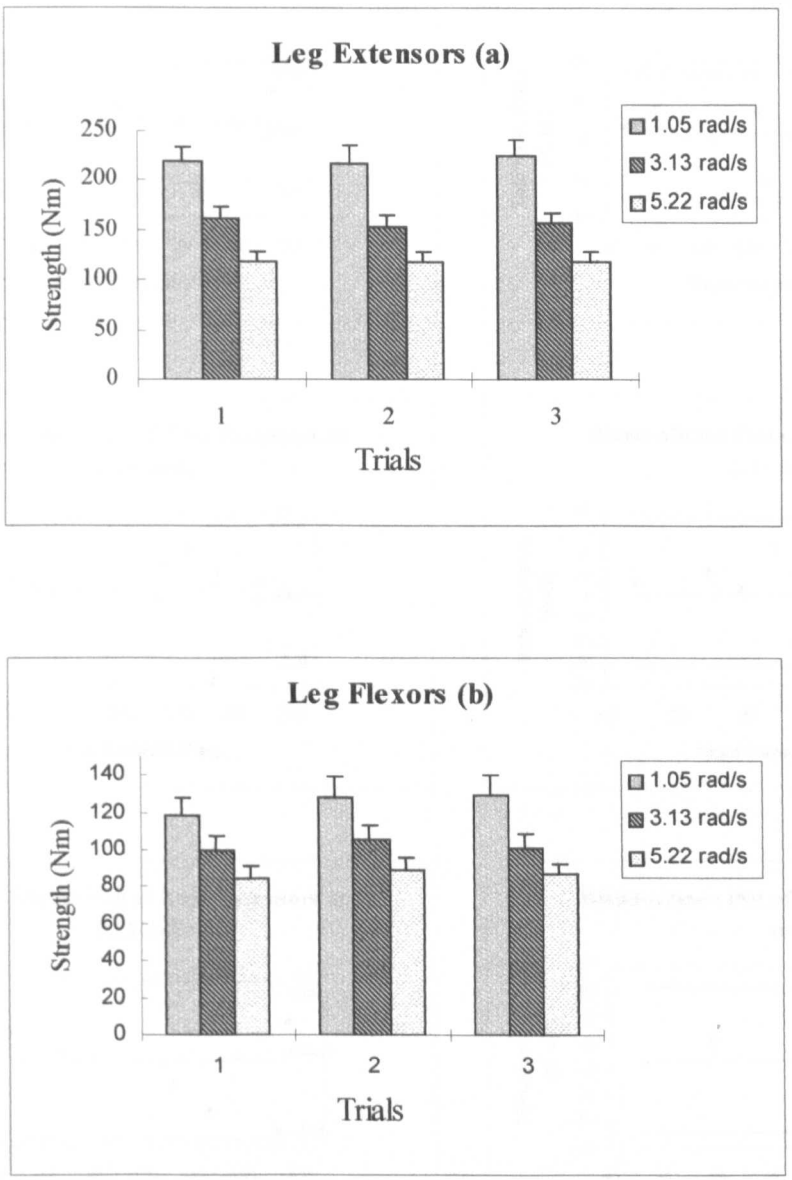
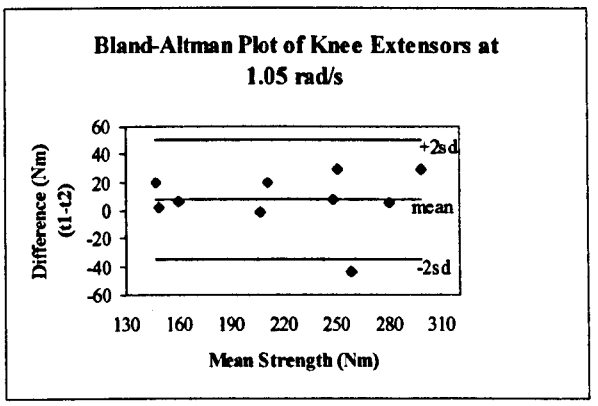
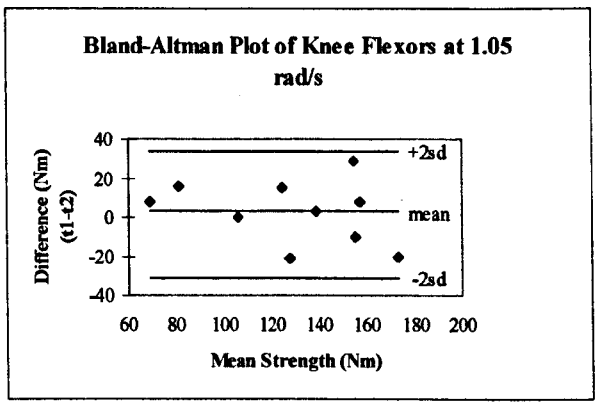
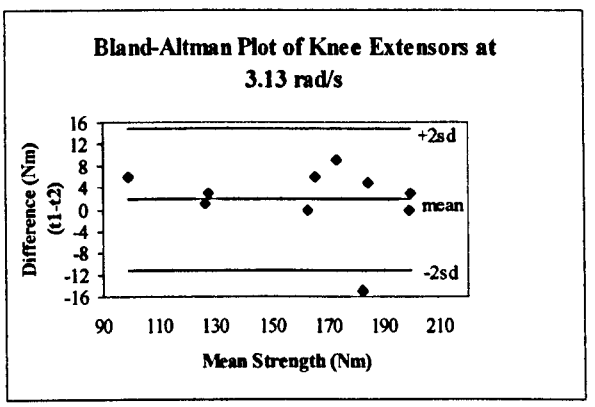


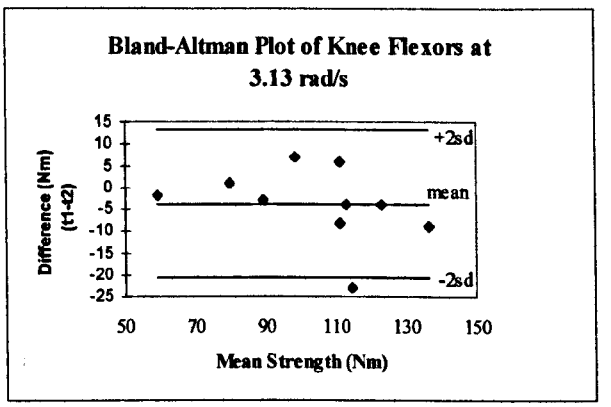
Fig. 3.2.1: *Mean (±SE) peak torque of the leg extensors (a) and flexors (b) across three trials at angular velocities of 1.05, 3.13 and 5.22 rad/s.*

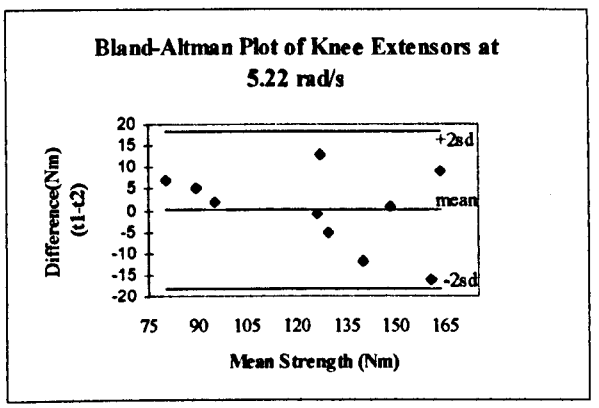
The 95% limits of agreement are plotted on graphs (Fig. 3.2.2). They illustrate the variability in strength performance under controlled conditions day-to-day.











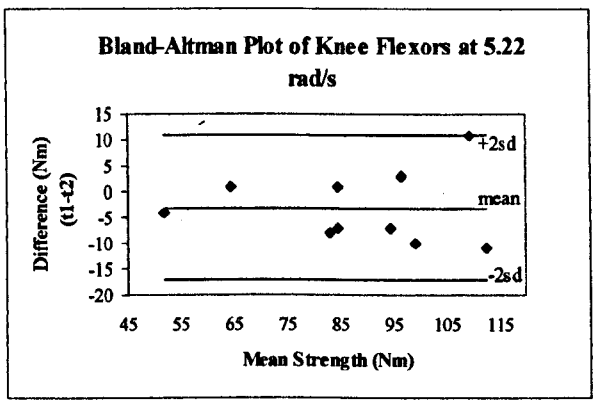


Fig. 3.2.2: shows the relationship between the mean (centre line) and difference in day-to-day scores across a range of velocities for leg extensors and flexors. The outer lines denote the 95% limits of agreement ± 2 standard deviations. Poor reliability or agreement is characterised by the deviation of data points away from the mean.

3.2.4. Discussion

Determining the inherent variability in repeated testing of muscular strength is important for subsequent interpretation of changes in performance resulting from experimental intervention, rehabilitation and/or training protocols. In the present study, there were no significant changes in peak torque between test sessions at each velocity. This indicates that performance was not affected by serial influences such as training or learning, and that random error was responsible for overall variability.

The coefficients of variation (CV) were reported for comparison purposes. The mean CV ranged between 4.1-12.7% depending on muscle group and angular velocity of movement. These results compare favourably with other studies (Thortensson et al., 1976; Gleeson and Mercer, 1992) for gravity-corrected peak torque. The CV for flexors was greater than those obtained for the extensors, although they are within the range of normal biological systems of 10-15% (Stokes, 1985). The results suggest that the test protocol is reliable and can be used to measure quadricep and hamstring strength during sequential measurements. However, there was a lack of relation between mean scores and differences in test-retest data (except for flexors at 5.22 rad/s), which contravenes the use of CV's. Coefficient of variation assumes that the test-retest variability increases with the stronger subjects (Bland, 1987). Futhermore, the CV's were much higher with the inclusion of the whole population (Table 3.2.2), and thus may be deemed less reliable.

Under these conditions, it is important to report the 95% limits of agreements. They give some indication of the variability in performance day-to-day, although the range of values considered reliable is dependent on the judgement of the experimenter. The limits of agreement of the extensors at the low velocity represent 15-23% of the mean test-retest scores. Whilst this appears to be high, the corresponding CV is 9.6%. The 95% limits of agreement at 3.13 rad/s (7.0 to 9.0% of mean test-retest) indicate that repeated measurements at this velocity are most reliable for the extensors. The negative mean scores for flexors at 3.13 rad/s and 5.22 rad/s show a tendency towards a decrease in performance from the second trial to the third trial. This reflects the

greater variability observed in flexor strength measurements (Burdett and Van Swearingen, 1987) which may be compromised by the reciprocal movements. This variability is also reflected in the agreement limits which range between 12.0 to 26.3% of the mean test-retest scores across all velocities. Therefore, greater verbal encouragement is probably required when undertaking reciprocal movements, particularly during flexion.

The results from this study have identified biological and mechanical variation as sources of error, and any changes which occur from experimental intervention must therefore exceed this variability. The risk of a type I error may otherwise confound correct interpretation of muscular performance. The application of the coefficient of variation is useful only if the same statistical methods are employed, the variability is proportional to mean scores and adjustments are made for inclusion of the whole population. If these conditions are not met, then it is necessary to use the 95% limits of agreement, although it is difficult to compare reliability with other studies as this technique is not yet widely used.

In conclusion, day-to-day testing of peak torque on the LIDO Active® dynamometer using the protocol described is recommended at slower angular velocities. Measurements at faster velocities (5.22 rad/s plus) and of flexors at all velocities are highly variable. Recommendations for future testing include the use of slower angular velocities, with four efforts following a warm-up to record true peak torque. More trials should be undertaken with the flexors, although care should be taken not to fatigue the muscle groups. This is more important with the extensors when isometric strength is also to be measured. These recommendations will be used to modify the testing protocol and this will be examined in section 3.3.

3.3. REPEATABILITY OF ISOKINETIC AND ISOMETRIC MUSCLE STRENGTH IN MIDDLE-AGED WOMEN

3.3.1. Introduction

The reliability of isokinetic muscle testing in a young heterogeneous sample using the LIDO Active® dynamometer has been established in section 3.2. These results demonstrated poorer reliability for the flexors at all velocities, and at higher angular velocities for the extensor muscles. It is important that the variability of measurements be assessed in the population in which they are to be used (Stokes, 1985). If this variability is large, erroneous interpretation of muscle strength changes may be made.

The aims of this study were to:

1] Examine the variability in leg strength using slower angular velocities of 1.05, 2.09 and 3.13 rad/s as recommended in sections 3.2.

2] Assess the reliability of isometric strength of the leg (90° knee flexion) and grip strength.

3.3.2. Methods

(i) Subjects

Twenty three middle-aged females were recruited from staff at Liverpool John Moores University after giving written consent. Eleven subjects were taking hormone replacement therapy (HRT) and 12 were not receiving treatment. A questionnaire was completed prior to participation to exclude subjects who suffered myopathic disorders, and those who were physically active. Age, height and mass of subjects are summarised in Table 3.3.1.

Table 3.3.1: Mean (± SD) for age, height and mass of post-menopausal subjects

Group	n	Age (years) Range	Height (cm)	Mass (kg)
Non-HRT	12	53.2 (4.5) 47-60	158.7 (6.0)	62.8 (9.9)
HRT	11	50.5 (4.8) 47-61	160.0 (6.2)	69.8 (13.3)

(ii) Procedure

Dynamic and isometric strength was measured on the LIDO Active® (Loredan, Davis, CA) dynamometer on three separate occasions within a two week period. Test sessions were scheduled at the same time of day for each subject. The first visit to the laboratory was used as a practice session.

Prior to testing, subjects cycled on a Monark drop-load cycle ergometer for 5 mins at a low resistance, at 60 rev/min. This warm-up was standardised. Subjects were seated on the dynamometer chair, positions were recorded for subsequent trials and muscle function of the dominant leg was measured.

3.3.2.1. Dynamic muscle strength

Standardised measurements of muscle torque for extensors and flexors were made at angular velocities of 1.05, 2.09 and 3.13 rad/s. The highest velocity measured in this study was 3.13 rad/s according to the recommendations from section 3.2. The procedure of use to measure isokinetic muscle strength have been described previously (section 3.2). Two submaximal and 2 maximal warm-up trials were undertaken prior to performing 4 maximal reciprocal movements. Subjects were encouraged to extend and flex the leg as hard as possible. Instructions were standardised.

3.3.2.2. Isometric muscle strength

Maximal voluntary contraction (MVC) of the leg extensors was measured at 90° flexion of the knee joint 5 mins after undertaking the dynamic strength testing. The quadriceps were isolated as much as possible and arms were folded throughout the

test. Subjects were instructed to push hard against the measuring device until maximal force was attained. Three maximum contractions were repeated, with 1 min rest periods between each trial. The highest value was recorded.

Grip strength was measured using a hand-held dynamometer (Takei, model 5101 Grip-D, Tokyo). Subjects were instructed to hold the dynamometer above the head with the arm extended. The arm was brought downward whilst full force was exerted onto the torque transducer. The highest grip strength of three maximal efforts was recorded.

(iii) Data analysis

The Statistical Package for the Social Sciences (SPSS) and Excel (Windows version 3.1) were used to analyse data. Mean strength between two test sessions was analysed using the dependent t-test for paired sample. The error linearity, 95% limits of agreement and coefficient of variation (CV%) were calculated according to the criteria described in section 3.2.

3.3.3. Results

3.3.3.1. Dynamic leg strength

Leg extensors - concentric

The t-test revealed a significant difference in strength at 1.05 rad/s from test 1 to test 2, implicating a serial effect of repeated testing at this velocity ($t=-2.67$; $p<0.05$). This did not occur at 2.09 or 3.13 rad/s, where the percent change in strength was much lower (Table 3.2.2). The error linearity was not significant across all velocities and thus the use of CV's is inappropiate in this instance. The 95% limit of agreement are plotted in Fig. 3.3.1.

Leg flexors

A significant difference between test 1 and test 2 data was also found at 1.05 rad/s ($t_{22}= -3.49$; $p<0.01$) and 2.09 rad/s ($t_{22} = 2.28$; $p<0.05$). This is reflected by an 8.6 % increase in mean strength at 1.05 rad/s. There were no significant changes at the faster

velocity (3.13 rad/s). The variability of flexors are shown in Fig. 3.2.1, where the 95% limits of agreement are illustrated.

3.3.3.2. Isometric strength

The results of the t-test did not show any significant differences in strength over the two test sessions for isometric leg strength (t_{22} = -0.43; p>0.05) or grip strength (t_{22} = -0.37; p>0.05). The r value from the error linearity test were also not significant (Table 3.2.2), hence the CV reported are not representative of its reliability. Bland-Altman plots of all parameters, displaying the 95% agreement limits and the mean of test-retest differences are plotted in Fig. 3.3.1.

Table 3.3.2: *Mean (±SD), percent change and t values for test one and two.*

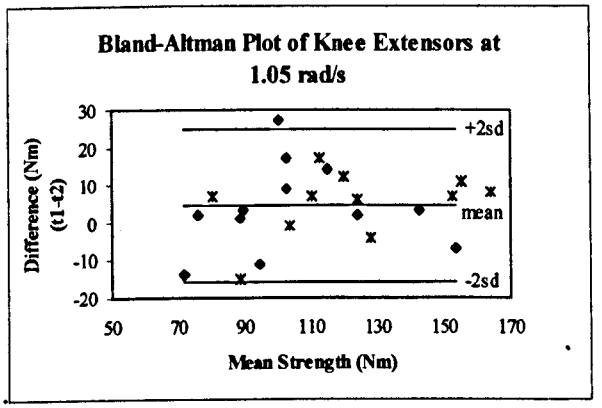
Variable	Test 1 (Nm)	Test 2 (Nm)	% change	t_{22} (p)
Knee extensors				
1.05 rad/s	110.7 (25.8)	115.1 (27.8)	4.0	-2.67 (0.05)†
2.09 rad/s	83.6 (16.5)	84.7 (16.6)	1.2	-0.91 (0.38)
3.13 rad/s	65.0 (17.0)	65.2 (16.3)	0.3	-0.22 (0.83)
Knee flexors				
1.05 rad/s	58.7 (12.7)	63.7 (15.5)	8.6	-3.49 (0.002)*
2.09 rad/s	48.5 (10.2)	51.0 (12.1)	2.2	2.28 (0.03)†
3.13 rad/s	40.4 (9.8)	40.4 (9.2)	-0.1	0.05 (0.96)
Isometric				
Leg 90°	103.9 (22.7)	104.9 (21.7)	0.96	-0.43 (0.669)
Grip	27.4 (4.58) (ft lbs)	27.57 (3.97) (ft lbs)	0.66	-0.369 (0.716)

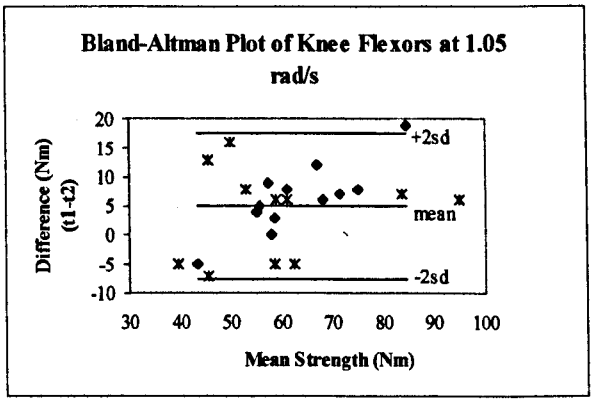
†Significant at p<0.05 levels for two-tailed t-test
*Significant at p<0.01 level for two-tailed t-test

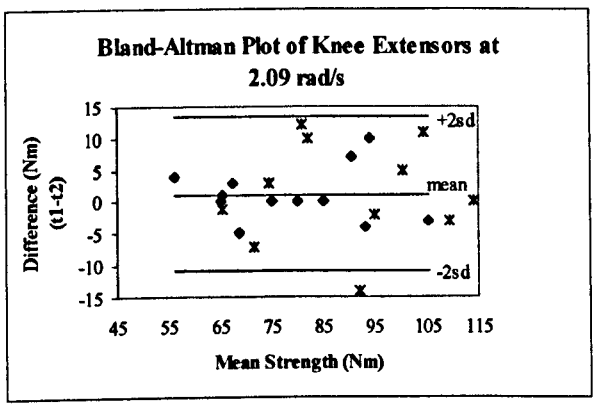
Table 3.3.3: *Tests of agreement of the test-retest data - error linearity (r,) 95% Limits of agreement and coefficient of variation (%) in middle-aged females.*

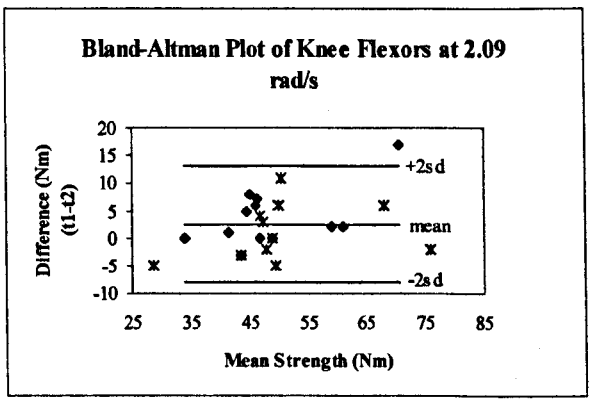
Muscle group	Error linearity (r)	95% Limits of agreement (Nm)	Coefficient of Variation (%)	*
Knee extensors				
1.05 rad/s	-0.11	-16.0 to 24.8	9.0	**17.7**
2.09 rad/s	0.20	-11.0 to 13.3	7.4	**14.5**
3.13 rad/s	-0.04	-7.6 to 8.0	6.1	**11.6**
Knee flexors				
1.05 rad/s	0.17	-7.6 to 17.7	11.3	**22.2**
2.09 rad/s	0.29	-8.1 to 13.1	10.7	**20.9**
3.13 rad/s	-0.17	-8.9 to 8.8	10.9	**21.4**
Isometric				
Leg 90°	0.37	-20.2 to 22.1	10.1	**19.5**
Grip	0.10	-4.02 to 4.36 (ft lbs)	7.6	**14.9**

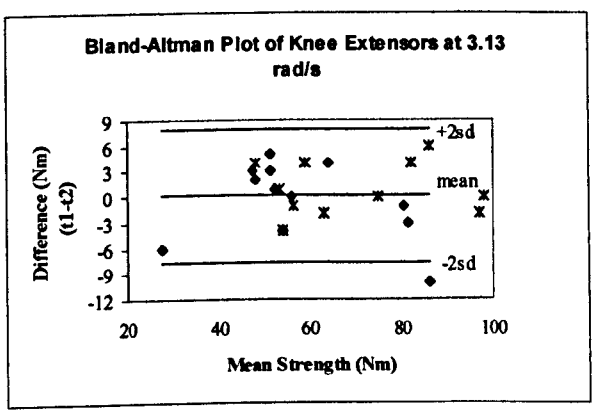
*** Coefficient of variation (%)** = standard deviation x 1.96 / overall mean x 100

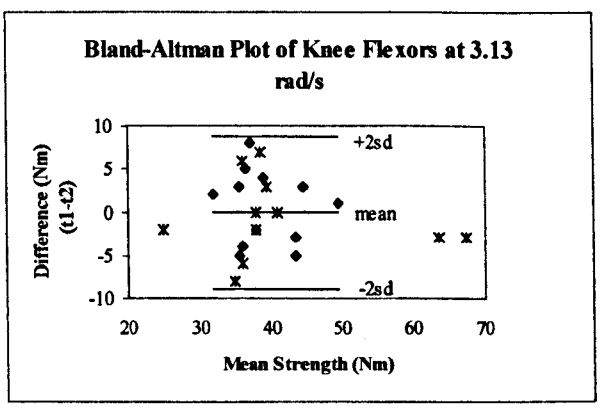


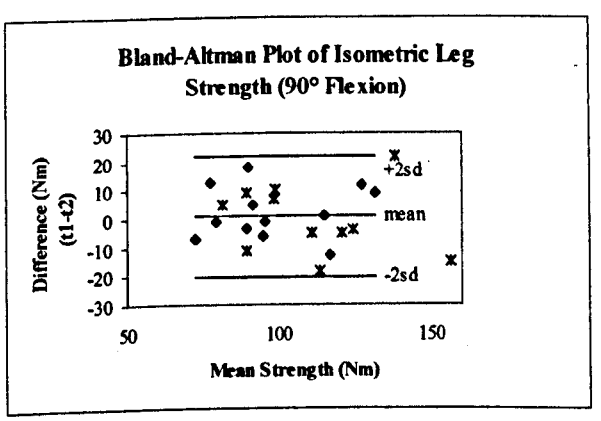












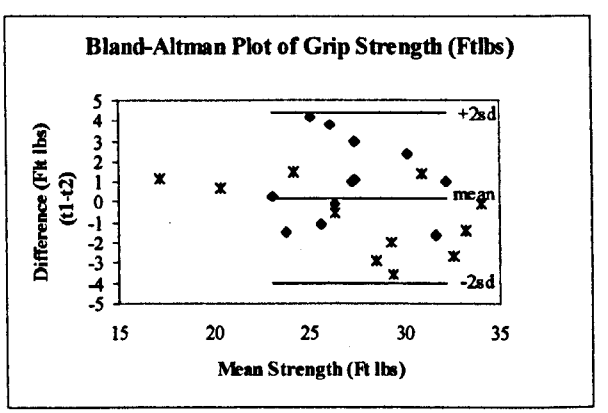


*Fig. 3.3.1: The relationship of the mean and difference in strength scores between day 1 and day 2 for different muscle groups and across varying velocities. The closer the mean (centre line) is to zero, and central tendency of data points towards the mean, the better the agreement. The 95% agreement limits (outer lines) represent both non-HRT and HRT subjects combined. The data points of both groups have been plotted separately to show the variability ◆ = Non-HRT; * = HRT group.*

3.3.4. Discussion

Sequential measurements of muscle strength are often required to evaluate responses to treatment, monitor effects of training programmes and muscle weakness due to disease, immobilisation and so on. It is therefore important that dynamic and isometric strength are adequately reproducible for the sample population tested. In this study, repeatability was assessed in middle-aged hypoestrogenic females and age-matched subjects taking hormone replacement therapy (HRT), sample groups matched to those required in study 4.1.

The results show that a systematic increase in strength of the knee extensors at 1.05 rad/s occurred between test 1 and test 2 ($p>0.05$), an increase of 4%. These results agree with previous studies (Harries and Bassey, 1990), where a five percent increase in strength measured at 1.74 rad/s was reported between the first two test sessions. An initial practice session, given to mitigate this tendency, was not sufficient to familiarise subjects with movement at this velocity. Strength of the flexors also increased significantly by 8.6% ($p<0.01$) suggesting that reciprocal movements are not effective for attaining peak torque of both muscle groups. The use of reciprocal, maximal contractions have been criticised (Rothstein et al., 1987), although the reliability of these measurements have been reported (Levine et al., 1991). The 95% agreement limits, calculated for strength of the KE's at 1.05 rad/s (14 to 22% of mean test-retest scores) were in the same range as those observed in young subjects. Increasing the number of practice trials rather than sessions, may compensate for this learning effect. This will be considered in future testing of the post-menopausal women of the longitudinal study.

In agreement with the findings from young subjects, the flexors demonstrate poor reliability. The coefficients of variation for this muscle group across all velocities were high (Table 3.3.3), although the lack of linearity contravenes the use of this reliability index. The variability of this muscle group has been documented previously for females (Burdett and Van Swearingen, 1987), males (Gleeson and Mercer, 1992) and both sexes (study 3.2). Even though a physiological explanation cannot be proffered, all studies employed reciprocal movements during extension and flexion.

Maximal isometric strength of the knee extensors measured at 90° of knee flexion was lower than concentric strength assessed at 1.05 rad/s. These results do not conform with normal force-velocity relations determined *in vivo* where torque decreases as velocity increases (Thortensson et al.,1976), or corroborate previous findings in elderly females (Harries and Bassey, 1990; Murray et al., 1985) and in males (Osternig, 1975; Thortensson et al., 1976). In theory, greater strength is generated during static contractions since more myosin cross-bridges have time to attach (Edman, 1992). During dynamic contractions, there is less time for the formation of cross-bridges as the fibres shorten. Less tension is therefore elicited. It is presumed that force generation at 90° of flexion is lower due a mechanical disadvantage. At this angle, fibres lengthen and less cross-bridges attach. The employment of twitch interpolation through electrically stimulated contractions is important to confirm maximal activation of the muscle (Rutherford et al., 1986). This technique was not available for this study, but will be utilised in study 4.1, where it will be important to preclude central inhibition/motivation as factors in muscle weakness.

So far, there has been no indication of heteroscedasticity in the data. It appears from Fig. 3.2.1 however, that stronger subjects yielded greater between-test variability for isometric grip strength than weaker subjects. Surprisingly, the correlation coefficient was not significant, indicating a lack of relation between mean scores and difference in test-retest data. The coefficient of variation (7.6%) compares favourably with 6% reported for grip dynamometry of repeated measurements (Kallman et al., 1990). However, as suggested by the r value, the 95% limits of agreement are more appropriate to report the variability of this data. There is good consistency between day-to-day testing, where the limits of agreement are within 15 to 16.0% of the mean test-retest scores. The disadvantage with grip dynamometry is the difficulty in standardising hand position in subsequent tests. This did not appear to affect differences in the mean day-to-day scores in this study.

There was evidence of a learning effect at the slower angular velocity (1.05 rad/s). This could be multifactorial as a result of too few familiarisation sessions, warm-up/practice trials and/or greater effort involved in reciprocal movements. Extension and flexion

should, therefore, either be performed separately or more contractions undertaken to achieve stable peak torque values. The variability of the flexors across all velocities in middle-aged women is consistent with the findings in young subjects. Isometric grip and leg strength appear to be reliable using the methods employed, although twitch interpolation should be used during maximal isometric contraction of the knee extensors.

In conclusion, based on these findings the protocol needs to be modified before being employed in the longitudinal study (section 4.1) so that a greater number of practice/warm up trials are allowed prior to testing. This will ensure the attainment of peak torque at 1.05 rad/s. The flexors were highly variable when measured in post-menpausal women, as reported in younger subjects in study 3.2. The interpretation of changes in strength of this muscle group are therefore made with caution. Superimposed electrical impulses to the knee extensors during a maximal voluntary isometric contraction is recommended to confirm maximal activation of the muscle.

3.4. DAY-TO-DAY VARIATION IN MUSCLE FUNCTION OF THE QUADRICEPS ASSESSED FROM MAXIMAL VOLUNTARY CONTRACTION AND PERCUTANEOUS ELECTRICAL STIMULATION.

3.4.1. Introduction

In the previous study, the reliability of isometric leg strength measurements was assessed using the computerised LIDO® isokinetic dynamometer. This system is not sensitive enough to analyse force production from electrically stimulated contractions using the isometric mode. It was therefore necessary to utilise a strain gauge system similar to that described by Edwards et al. (1977b) to measure maximal voluntary contractions (MVC) and force generated from electrically stimulated contractions of the quadriceps. These included responses to a train of electrical impulses of increasing frequencies, referred to as the programmed stimulation myogram (PSM), and the fatigue index (FI%) calculated from the force loss induced by repeated electrical stimulation. The reliability of the protocol designed to measure these indices of muscle function will be examined in this section. Depending on the results of this study, the protocol will be implemented and/or modified for the use in study 4.2, to assess the temporal changes in reproductive hormones on muscle function.

The aims of the study were to:

1] Investigate the reliability of MVC of the quadriceps utilising the strain gauge system.

2] Assess the reliability and repeatability of forces generated from electrically stimulated contractions of increasing frequencies.

3] Compare, and examine the reliability of two fatigue protocols of different stimulation patterns.

3.4.2. Methods

(i) Subjects

Twelve young males volunteered to participate in the study with mean (±Sd) characteristics: age 27.1 (±3.1); mass 82.8 (±17.4); height 1.76 (±0.06). Written informed consent was obtained after details of the experiment were explained. All subjects were free of injury to the lower limb and were required to undertake the same activities 24 hours prior to testing.

(ii) Procedure

Voluntary and electrically stimulated contractions were measured with the subject seated in an adjustable chair with the leg suspended at a 90° angle. To prevent extraneous bodily movements, the hips and shoulders were restrained with straps. Force of the quadriceps was measured from the ankle, where the attachment was connected to a strain gauge by a metal force transducer (Plate 3.4.1). The quadriceps were electrically stimulated using surface electrodes (7.6 by 12.7 cm, Chattanooga, Bicester, UK) positioned on the proximal and distal anteriolateral side of the thigh of the dominant leg. Electrical impulses were delivered through the electrodes at 250 volts with a pulse width of 200 μs using a computer driven stimulator (Model DS7, Digitimer Ltd, Welyn Garden City, UK). Force output was channelled through an amplifier, interfaced with a data acquistion system (Biopac MP100WS, Santa Barbara, CA).

(iii) Experimental protocol

3.4.2.1. Maximal voluntary contraction

Maximal voluntary contraction (MVC) of the quadriceps was measured three times with a 1 min rest between each effort. Maximal activation of the quadriceps was confirmed using percutaneous electrical stimulation of 1 Hz impulses, delivered to the

muscle during each contraction (Rutherford et al., 1986). The disappearance of the impulses indicates maximal effort as shown in Fig. 3.4.1. Subjects were required to fold their arms during each contraction, and to 'kick out' as hard as possible and as fast on instruction from the experimenter.

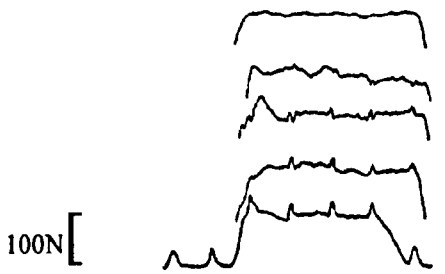


Fig. 3.4.1. A voluntary contraction at differing levels of effort, showing the extra force generated by superimposed twitches. At maximum voluntary contraction, the superimposed twitches disappear. From: Rutherford et al., 1986.

3.4.2.2. Electrical stimulation

3.4.2.2.1. Contractile properties

A train of electrical impulses was delivered to the quadriceps at increasing frequencies of 1, 10, 20, 50 and 100 Hz. Each tetanic stimuli lasted 3 s and a 5 s rest was programmed between each frequency (Fig. 3.4.2). The current was estimated from the maximal 1 Hz impulses tolerated. For subjects who did not respond favourably to the voltage selected, particularly at the higher frequencies, the current was reduced until a tolerable level was attained. Although the aim was to stimulate over 20% of the muscle, the force generated at 100 Hz ranged 10 to 46 % of maximal voluntary contraction across the sample. The force-frequency relationship, plotted as an index of the contractile properties of muscle, is normalised so the lower frequences are expressed as a percentage of the force generated at 100 Hz. The response of quadriceps muscle group to this pattern of frequencies, termed the programmed stimulation myogram (PSM), was examined in fresh and fatigued muscle and the

reliability of this test assessed under both conditions. The ratio between low, unfused tetani and high, fused tetanic contractions (20/50% or 10/100%), as an index to the shape of the force-frequency curve, was calculated from the forces generated during the PSM.

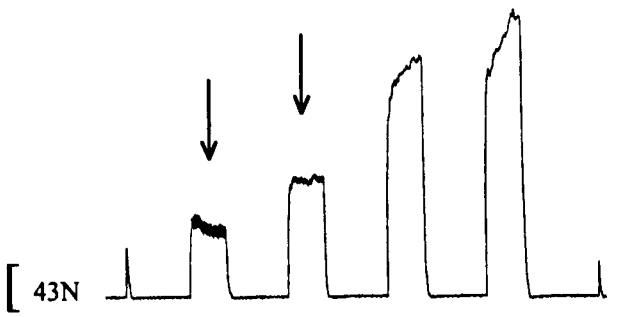


Fig. 3.4.2: A train of electrical impulses (1, 10, 20, 50, 100, 1Hz) of three seconds duration, with 5 seconds rest, delivered 5 minutes before, and immediately after the fatigue test. The unfused tetani at 10 and 20 Hz are evident in this myogram (as shown by arrows).

3.4.2.2.2. Fatiguability

Fatigue of the quadriceps was induced using electrically stimulated contractions, modified from the protocol of Burke et al. (1973). Two different fatigue exercises were employed to determine the most reliable and effective protocol for inducing fatigue. Frequency of 40 Hz impulses were delivered over 3 min in both tests, with differences in the duration of stimulation and on/off times of contractions. In the first fatigue test, impulses lasted 3 s with a 5 s rest interval. In the second protocol, 1 second impulses were separated by a 1 s rest interval. The force generated during the last 5 twitches of the test was expressed as a percentage of the initial 5 twitches and called the fatigue index %.

The tests of muscle function of the quadriceps were assessed 6 times over a 3-month period. The two fatigue protocols were assessed across 3 trials each, whereas other indices of muscle function (MVC, contractile properties) were examined during the initial three sessions. A standardised 5 min warm-up on a cycle ergometer (Monark) at 70 rev/min preceded the strength tests which were conducted at the same time of day (± 1 hour) on each occasion.

The experimental protocol was as follows:

- 5 min warm-up on cycle ergometer
- 1 Hz impulses - to determine maximal tolerable current for MVC
- Stimulation at 1, 10, 20, 50, 100 Hz - to determine maximal tolerable current

◊ 3 min rest:

- Maximal voluntary contractions x 3

◊ 5 min rest

- Stimulation at 1, 10, 20, 50 and 100 Hz

◊ 5 min rest

- Fatigue test
- Stimulation at 1, 10, 20, 50 and 100 Hz

(iv) Data analysis

The Statistical Package for the Social Sciences (SPSS) and Excel were used for data analysis. Repeated measures ANOVA was initially performed on the data. If no serial trends occurred, the error linearity test, 95% limits of agreement and coefficient of variation (CV%) were calculated between data of test 2 and 3. These methods have been detailed in section 3.1.

3.4.3. Results and Discussion

3.4.3.1. Maximal voluntary strength

There were no significant differences in MVC across the first three test sessions ($F_{2,22} = 0.11$; $p>0.05$). This suggests that there were no learning influences during the initial tests. This is not an indicator of agreement and further analyses were subsequently undertaken. The linearity between mean scores and difference of test and retest data was non-significant ($r = -0.36$). This lack of relation violates the use of the coefficient of variation as discussed earlier (section 3.1). For comparison purposes, however, CV was calculated across all parameters. The CV's of both methods are shown in Table 3.4.1. The CV (%) for isometric strength of the quadriceps was 6.8%, compared with 9.6% for dynamic leg strength at 1.05 rad/s (see section 3.2). Other authors who have

used a similar device reported CV between 2 tests at 4.0% and 4.4% for young and elderly females respectively (Young et al., 1984). The present results compare favourably with those obtained in previous research. However, in addition to the characteristics of the data which do not uphold the use of CV (ie. lack of linearity), the high CV of 13.4% calculated here is the result of using the adjusted method. The 95% limits of agreement (-90.7 and 89.73N) are plotted in Fig. 3.4.5.

3.4.3.2. Contractile properties

Electrical stimulation is used to assess muscle function and reveal the characteristics of contractile properties beyond that of maximal voluntary contractions. The pattern of force generation with increasing frequencies of electrical impulses enables the force-frequency relationship to be established and the identification of the nature of fatigue. The shape of the force-frequency curve can be revealed by examining the force generated at low frequencies as a percentage of force at high frequencies. The most reliable index - 10/100 or 20/50%, in fresh and fatigued muscle will be reported.

3.4.3.2.1. 10/100% ratio

Fresh muscle

The results of the repeated measures ANOVA found that the mean ratio of 10/100 % in fresh muscle were not significantly different across the three test sesions ($F_{2,22}$ = 0.72; $p>0.05$). The coefficient of variation was large (22.1% and 44.3% after accounting for 95% of the population), indicating poor reliability. Even though the lack of linearity contravenes the use of CV, the 95% limits of agreement concur with the poor reliability of this ratio, with a range of ± 44.0% of the mean test-retest scores (Fig. 3.4.6).

Fatigued muscle

There were no sequential effects of the 10/100 % across three test sessions following the first ($F_{2,22}$ = 2.27; $p>0.05$) and second ($F_{2,22}$ = 1.86; $p>0.05$) fatigue protocols. The reliability indices are shown in Table 3.4.1. The 95% limits of agreement were

narrower after the second protocol (ranging -29.5 to 44.8% of mean test-retest scores) compared to the first protocol (-36.1 to 50.44% of mean test-retest scores) although neither test demonstrated good repeatability. This is also reflected by the high CV's (unadjusted values of 21.6 and 18.6% for the first and second protocols respectively). The error linearity was non-significant and therefore the limits of agreement are important for interpreting the reliability of this data.

3.4.3.2.2. 20/50%

Fresh muscle

The 20/50 % ratio is used more often as a characteristic of the force-frequency curve compared to the 10/100% ratio. Over the first three trials of the test-retest data, there were no significant differences in the 20/50% ($F_{2,22}$ = 2.24; $p>0.05$). The mean difference between test 2 and 3 was high (3.28 N) although the CV (9.8 or 19.2% for adjusted population) was much lower compared to the 10/100%. The 20/50% is therefore more reliable than the lower ratio, as indicated by the 95% limits of agreement (ranged 14 to 25% of mean test-retest scores) (Fig. 3.4.6). The CV's reported here are considerably higher than that documented for the quadriceps (5.5%) (Edwards and Newham, 1984) and sternomastoid muscle (4.5%) (Edwards et al., 1984) measured in a single person. However, the detail of calculating CV by these authors was not reported. The overall mean of the 20/50% was lower (66.5%) compared with 80.7% reported by Edwards and Newham (1984), which could be attributable to the differences in force generated in the two studies. The percutaneous stimulation of the quadriceps, activating only a portion of the muscle, has been questioned in previous work (Davies et al., 1982), with claims that the relationship between forces produced at low- and high- frequencies are voltage dependent. Edwards and Newham (1984) reported forces >10% MVC with 50 Hz stimulation compared with 10-46% MVC at 100 Hz for the sample in this study. Even though the authors claimed this ratio to be reliable, they were cautious about its reliability at very low levels of voltage. The present results suggest that the 20/50% ratio is more reliable than 10/100% in fresh muscle.

Fatigued muscle

The 20/50% ratio, calculated across three test sessions following two different fatigue protocols, were not significant different the first ($F_{2,22}$ = 0.14; p>0.05) or second fatigue protocols ($F_{2,22}$ = 0.38; p>0.05). The unadjusted CV increased from 9.8% in fresh muscle to 13.6% and 16.6% following the first and second fatigue test respectively. Edwards et al. (1984) also reported an increase from 4.5% in fresh muscle to 7.4% in the fatigued state for the sternomastoid muscle. The 95% limits of agreement are shown in Table 3.4.1.

3.4.3.2.3. Force-frequency relationship

The response of the quadriceps across a range of stimulation frequencies is illustrated in Fig. 3.4.3. Mean forces generated at each frequency did not vary across the three trials. The ratios described above give an indication of the characteristics of the force-frequency curve.

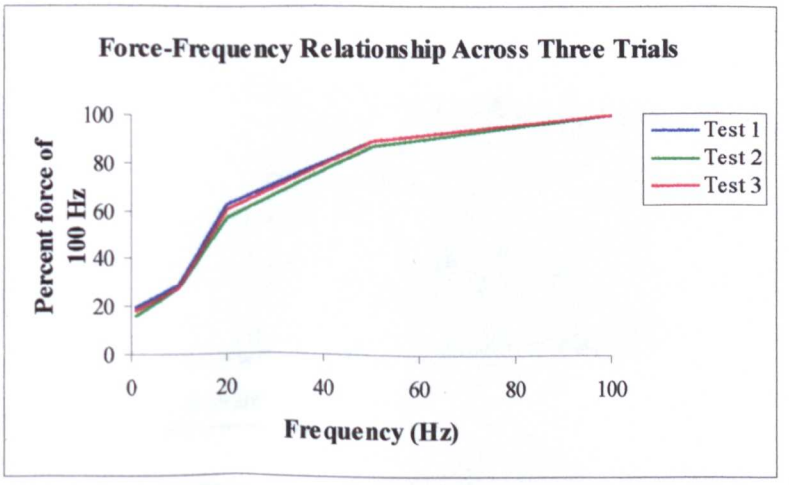


Fig. 3.4.3: *The force-frequency curve over a range of stimulation frequencies of 1, 10, 20, 50 and 100 Hz. The comparison between three controlled test sessions.*

3.4.3.3. Fatiguability

Force generation at the end of the fatigue test was expressed as a percentage of the initial value and termed the fatigue index (FI%). Two fatigue tests were performed to i] determine which test is most effective inducing fatigue and ii] assess the reproducibility of both protocols. The first test involved stimulating the muscle for 3 s, with a 5 s rest period. The quadriceps in the second test were electrically stimulated for 1 s, with 1 a s rest. The frequency of stimulation of both tests was 40 Hz, and the duration of stimulation lasted 3 min. The peak and mean tension of the impulse were recorded and analysed separately.

Test 1 - ANOVA did not reveal any significant changes in FI% for peak tension ($F_{2,22}$ = 0.11; $p<0.05$) or mean tension ($F_{2,22}$ = 0.16; $p<0.05$) over three test sessions. Thus, any day-to-day fatigue or learning effects were controlled. The unadjusted CV (9.9 and 11.0 for PT and MT respectively) were within the 'acceptable' range for biological systems (Stokes,1985), although the lack of linearity and high adjusted values do not render the use of this reliability index. The limits of agreement are reported in Table 3.4.2. The fatigue trace in Fig. 3.4.4 illustrates the force loss over 3 min.

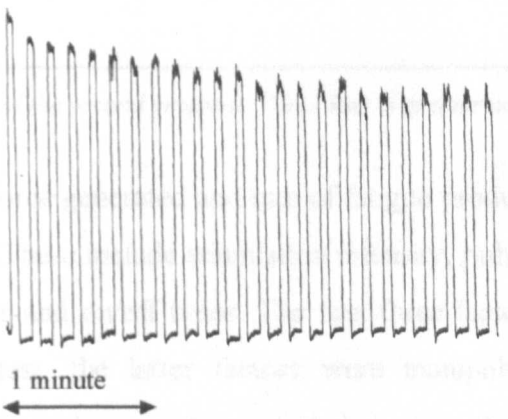


Fig. 3.4.4. *The fatigue trace of the first protocol showing the force loss across time.*

Test 2 - There were no significant differences in the test-retest data of the second protocol for peak tension ($F_{2,22}$ = 0.9; $p>0.05$) and mean tension ($F_{2,22}$ = 0.25; $p>0.05$), excluding a serial effect of repeated testing. There was also a lack of linearity between the mean scores and difference between test 2 and 3 in this method (r values in Table

3.4.2.) which contravenes the use of CVs. Despite this, the CV (%) did not differ widely between the two protocols. The second protocol was more comparable to the fatigue test employed in other studies than the test of 3 s stimulation. The coefficient of variation of the fatigue index documented for the adductor pollicis (16%) and the first dorsal interosseus muscle (15%) (Rutherford and Jones, 1988), where the muscle is stimulated for 250 ms every second for 3 min, are higher than those reported for the quadriceps in this study. However, because of the different statistical methods of calculating CV, it is misleading to make comparisons of different muscles across studies, particularly if the error linearity is not known. Fig. 3.4.5 illustrates the fatigue loss across this test.

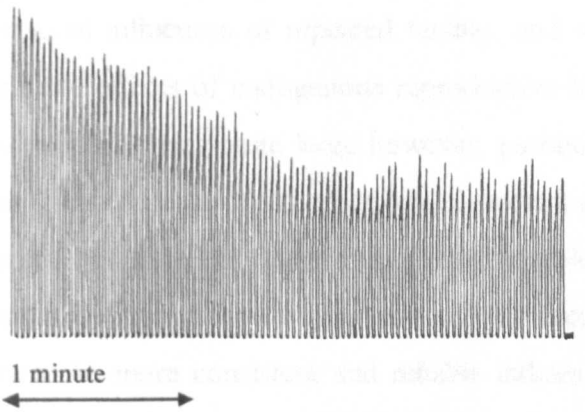


Fig. 3.4.5: *The fatigue trace of the second protocol. Force loss is greater across the test.*

Many factors affect the force generated and rate of fatigue produced during electrically stimulated contractions. These include stimulation intensity, pulse frequency, length of test, duration of stimulus and on/off times. The first three factors were controlled or standardised for each test, the latter factors were manipulated in two different protocols. Any discrepancies between test-retest data were therefore due to random variation. The 95% limits of agreement were slightly narrower for the first protocol which may suggest better repeatability, although these differences were very small (Table 3.4.2). Magnitude of force loss was significantly greater in the second protocol for peak tension (PT) (36.7%) and mean tension (MT) (40.9%) (Fig.3.4.5) compared with PT (21.7%) and MT (23.2%) of the first protocol (Fig.3.3.4). Significant differences were found between these tests for the two parameters (PT - t=6.616; MT

- t=6.635;p<0.01). The second protocol was considered a more effective method of inducing fatigue.

In summary, ANOVA has shown that there were no learning effects across the three trials for any parameter measured. The variability between test-retest trials were therefore due to random biological and mechanical variation. The lack of linearity between the difference in test-retest data and mean scores excluded the use of CV, although this is the most frequently reported index of reliability across previous studies and has therefore been calculated for comparison here. The 95% limits of agreement are important despite a less defined interpretation of reliability; the degree of reliability is judged by the experimenter. In conclusion, the protocol used in this study was not subject to learning or serial influences of repeated testing, and will be employed in study 4.2 to investigate the effects of endogenous reproductive hormones on muscle function. The random variability was quite large however, particularly for electrically stimulated contractions. This could be due to electrode placement and level of voltage. Extra care will therefore be taken to standardise these variables. Following close examination of the results, the use of the 20/50% ratio and the second fatigue protocol (1 s intervals) appears to be more consistent and reliable indices of muscle function than the 10/100% and first fatigue protocol (3 s stimulation) respectively.

Table 3.4.1. Test-retest reliability indices for maximum voluntary contraction (MVC), 10/100 and 20/50 Hz ratios (%) in fresh and fatigued muscle.

		FRESH		FATIGUED			
	MVC	10/100 (%)	20/50 (%)	10/100 (%)		20/50 (%)	
	(N)			Test 1	Test 2	Test 1	Test 2
Mean Difference	-0.492	-0.025	3.283	2.27	2.42	0.79	1.81
SD	(45.11)	(6.01)	(6.51)	(6.85)	(5.90)	(8.02)	(9.43)
Overall mean	660.64	27.19	66.51	31.66	31.76	58.8	56.73
Linearity (r)	-0.36	0.18	0.21	0.07	0.17	0.01	0.06
CV%							
†	6.8	22.1	9.8	21.6	18.6	13.6	16.6
‡	13.4	43.3	19.2	42.4	36.4	26.7	32.6
95%	-90.7 to	-12.1 to	-9.7 to	-11.43 to	-9.38 to	-15.2 to	-17.1 to
LA	89.7	12.0	16.3	15.97	14.22	16.8	20.7

† *SD of differences divided by overall mean x 100*
‡ *SD x 1.96 divided by overall mean x 1*

Table 3.4.2: Test-retest reliability for fatigue index (FI%) of peak tension and mean tension (expressed as the end force as a percentage of initial force). Test1 involved stimulating the muscle for 3 s with a 5 s rest. One second impulses were delivered in test 2 with a 1 second rest.

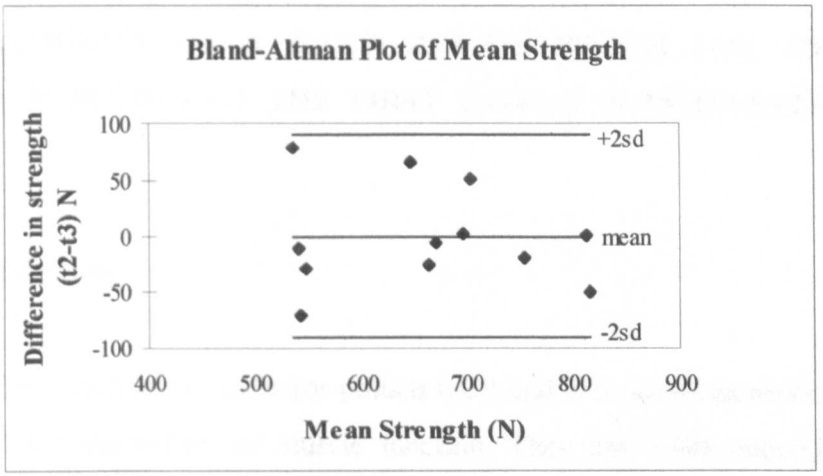
METHOD	FATIGUE TEST			
	Test 1		Test 2	
	Peak tension	Mean tension	Peak tension	Mean tension
Mean of Difference	-0.56	0.32	42.21	1.03
(± SD)	(7.79)	(8.45)	(8.20)	(6.66)
Overall mean *	78.3	76.8	63.3	59.0
Error linearity (r)	-0.46	-0.1	0.14	-0.06
CV%				
Unadjusted †	9.9	11.0	12.9	11.3
Adjusted ‡	19.5	21.6	25.4	22.1
95% Limits of Agreement	-16.1 to 15.0	-17.2 to 16.5	-14.2 to 18.6	-12.3 to 14.4

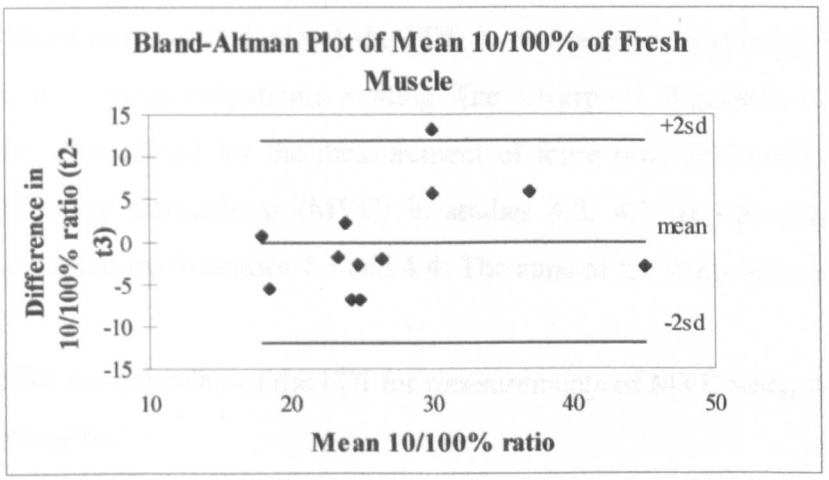
* *Test + retest*

† *SD of differences divided by overall mean x 100*

‡ *SD x 1.96 divided by overall mean x 100*

95% LA are the 95% limits of agreement





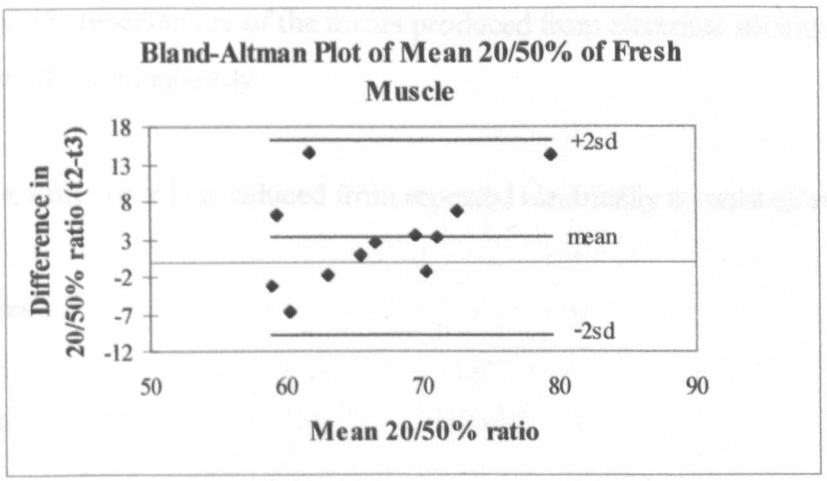


Fig. 3.4.6: The relationship between the mean scores and difference in test-retest data for MVC, 10/100% and 20/50% in fresh muscle.The outer lines represent the 95% limits of agreement where sd denotes ± standard deviation. The broken line is the overall mean of the test-retest data.

3.5. RELIABILITY OF A HAND DYNAMOMETER FOR MEASURING MUSCLE FUNCTION OF THE FIRST DORSAL INTEROSSEUS MUSCLE (FDI)

3.5.1. Introduction

Small muscles, such as the adductor pollicis (AP) and first dorsal interosseus (FDI) are often used for assessment of muscle function. They are easily accessible and less complex than larger, multiple groups and can be stimulated painlessly via their respective motor nerve. The hand dynamometer used in this study and later chapters was constructed to measure force of the FDI. It was portable and could therefore be transported for testing outpatients visiting The Liverpool Women's Hospital. The dynamometer was utilised for the measurement of force production of the FDI from maximal voluntary contractions (MVC) in studies 4.2, 4.3 nd 4.4, and electrically stimulated contractions in studies 4.3 and 4.4. The aims of the study were to:

1] Establish the reliability of the FDI for measurements of MVC using the hand dynamometer

2] Assess the repeatability of the forces produced from electrical stimulations delivered percutaneously.

3] To measure force loss induced from repeated electrically stimulated contractions.

3.5.2. Methods

(i) Subjects

Young males subjects (n=14) gave written consent to participate in the study. Mean (±SD) characteristics are age:26.9 (4.37), range 22-39 years; mass: 77.6 (8.45) kg; height: 175.7 (6.97) metres. Subjects were excluded if they suffered pain or discomfort of the hand, or were taking any medication likely to affect performance.

(ii) Experimental procedure

Maximal voluntary contraction, contractile properties and fatigue resistance were assessed on 3 occasions, separated by 3-5 days. Force production was measured using a custom-built dynamometer designed to isolate the FDI muscle. The forearm, which rested on the diagonal slope of the platform, was secured 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, UK). The strain gauge was calibrated with known weights. The thumb was fully abducted and secured with a strap around the proximal phalange. The remaining fingers were strapped together and restrained by velcro webbing to prevent force production from other muscles (Plate 3.4.1.). Upward movement of the index finger was prevented by a clamp tightened at the base of the phalange. The position of the hand was standardised for each session to ensure the muscle length was consistent between trials. The hand and forearm were initially immersed in warm water at 44°c for 10 min to increase blood flow and throughout the experiment a reading lamp was positioned at a standard distance over the muscle. Whilst muscle temperature was not measured, this procedure was standardised and repeated on both occasions in an attempt to standardise muscle temperature.

The FDI was stimulated percutaneously with self-adhesive surface electrodes (3S healthcare, London, UK). 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 Hz and a 40 Hz tetani to confirm accurate location of the electrodes. Electrical impulses were applied at 150 volts at a pulse width of 100 μs duration with a computer driven Digitimer stimulator (Model DS7, Digitimer Ltd, England). The force output was amplified and visually displayed on an Apple Macintosh computer, interfaced with a data acquisition system (Biopac MP100WS, Santa Barbara, CA).

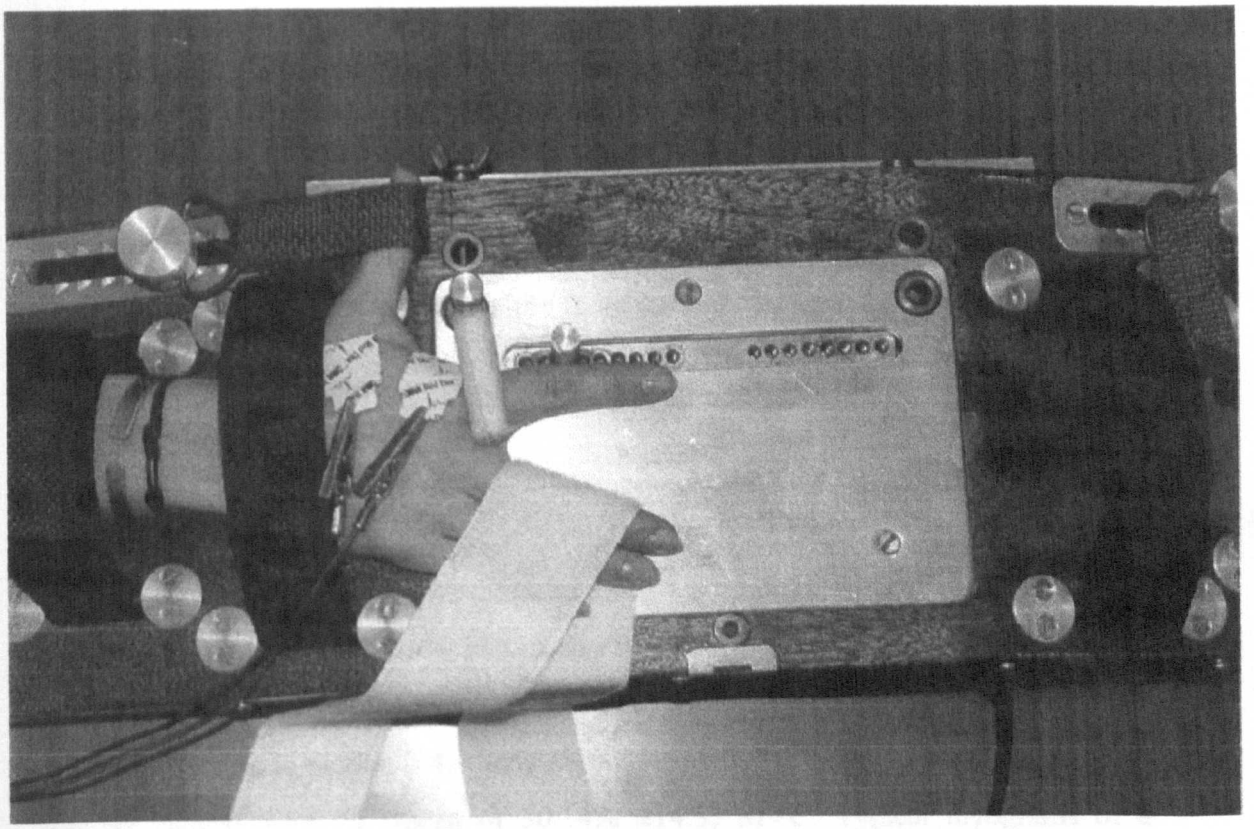


Plate 3.5.1. *Dynamometer showing the index finger in relation to 1] force transducer 2] electrodes and 3] thumb*

3.5.2.1. Maximal voluntary contraction

Maximal voluntary contraction of the FDI was measured whilst fully abducting the index finger. This is the only muscle involved in producing this movement. Superimposed percutaneous 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 seconds rest interval. The highest of three trials was recorded.

3.5.2.2. Contractile properties

A train of electrical impulses were delivered percutaneously to the FDI using the same protocol as the leg of 1, 10, 20, 50 and 100 Hz of 3 s duration, with a 5 s recovery. The frequency at low velocities was expressed as a percentage of high frequencies (10/100 and 20/50%) to characterise the force-frequency curve. This method has been described in section 3.4.

3.5.2.3. Fatigue characteristics

Fatigue resistance of the FDI was assessed using a protocol modified from Burke et al. (1973). This involved repeatedly stimulating the muscle for 3 min at 40 Hz with a 1 s interval between each tetanic contraction. This protocol was adapted, based on the recommendations in section 3.4. Using percutaneous stimulation, forces generated at 100 Hz ranged 5-53.7% (mean of 30.14% ±14.3) MVC. Typical myograms of a tetanic contration in a fresh and fatigued state are shown in Fig. 3.5.1.a] and b]. The fatigue index (FI%) was calculated as for the leg (end force as a percentage of initial force). Speed of relaxation was measured as the time taken for peak force to reach half-peak force. A 5 min rest was allocated between the train of increasing frequencies and before commencing the fatigue test.

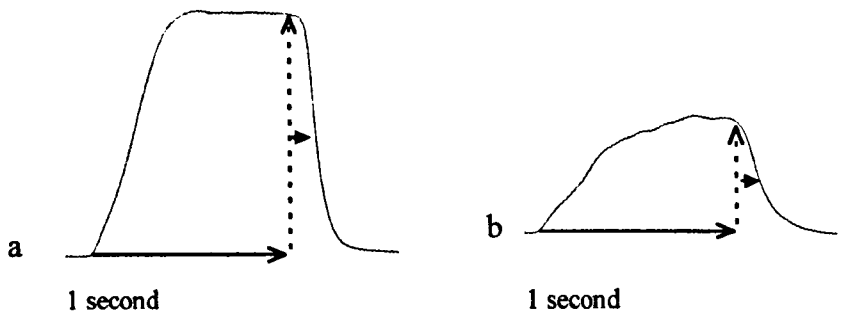


Fig. 3.5.1: A 40 Hz tetanic twitch of fresh [a] and fatigued [b] muscle. The horizontal arrow represents the half-relaxation time (time taken for muscle to reach half-relaxation).

(iii) Data analysis

The statistical methods of analysing the data have been described previously (section 3.2). A repeated measures ANOVA was initially used to identify any trends over the test sessions. If a serial effect did not occur, the error linearity and 95% limits of agreement were calculated between the first two tests. The coefficient of variation was calculated irrespetive of r, for comparison purposes.

3.5.3. Results and discussion

3.5.3.1. Maximal voluntary contraction

There were no significant differences in maximal voluntary contraction (MVC) over the three trials ($F_{2,24}$ = 0.51; $p>0.05$). There was a lack of relation between mean strength scores and test-retest difference and thus the 95% limits of agreement were used to interpret the reliability. The overall mean was 44.18N with -8.54 to 8.43 N 95% limits of agreement (ranging ±19% of mean test-retest scores) (Fig 3.5.2.). Hence, for any new young subject tested, two repeated measurements (including the first visit) would differ by 8 N or less. Changes in strength due to hormonal influences would have to exceed 8 N or otherwise they would be disguised by the inherent

variability. Unadjusted CV was 9.6% compared with 12% for the FDI in other studies (Rutherford and Jones, 1988).

3.5.3.2. Contractile properties

The repeatability of day-to-day measurements of contractile properities of the FDI were assessed by comparing the forces generated at low frequencies with those at high frequencies (10/100 and 20/50% ratios).

3.5.3.2.1. 10/100% ratio

There was no significant difference in the 10/100% ratio over the three trials ($F_{2,24}$ = 1.10; $p>0.05$). There was a lack of linearity between mean scores and differences in test-retest data ($r= 0.46$), and CV's were very high (64.1% and 125.6% for unadjusted and adjusted data respectively). This compares with 15.9 and 32.3% found for 20/50% ratio, respectively. The 95% limits of agreement are also very wide (Fig 3.5.3) (-131.0 to 125.3 % of mean test-retest scores) which further suggests poor reliability of this ratio as a feature of the force-frequency curve. This ratio will be excluded from analysis in the experimental studies.

3.5.3.2.2. 20/50% ratio

The repeated measures ANOVA was not significant for the 20/50% ratio over the three trials ($F_{2,24}$ = 1.43; $p>0.05$). The overall mean of the two trials was high (-3.95) denoting a decrease in the percentage of this ratio from the first to the second test. This was not statistically significant. The overall mean of 72.2% compares with 80.7% for the quadriceps (Edwards and Newham, 1984) and 79.0% for the sternocleidomastoid muscle (Edwards et al., 1984). Results are shown in Table 3.5.1. The limits of agreement demonstrates that this ratio is more reliable than the lower ratio (10/100%) and will be used in the study 4.4.

3.5.3.3. Fatiguability

There were no significant differences in the ANOVA for peak tension ($F_{2,20}$ = 0.08; $p>0.05$); mean tension ($F_{2,20}$ = 0.35; $p>0.05$) or relaxation time ($F_{2,20}$ = 0.31; $p>0.05$) across the three trials. The differences in test-retest data are shown in Table 3.5.2. Overall mean of peak tension (49.2 N) was higher compared with mean tension (44.1 N), although the reliability does not differ. Relaxation time however, has poor reliability, with a high mean difference and wide 95% limits of agreement. This is not demonstrated by the CV and it was therefore not surprising that error linearity was not significant. Table 3.5.2. lists the results of these parameters.

The reliability of the dynamometer for measuring muscle function of the FDI has been assessed over three day-to-day sessions. The ANOVA results were not significantly different for all variables measured, precluding a learning or familiarisation effect of repeated measures. This is important since a practice session is not possible in the experiments where this protocol will be employed. The variability is quite large, possibly due to the inter-individual differences in stimulation intensity. Intra-individual voltages were kept constant..

In conclusion, maximal volitional and electrically stimulated contractions are not prone to a serial or learning effect measured day-to-day, and can therefore be used in studies 4.3 and 4.4, where a familiarisation session will not be given. The 95% limits of agreement indicate that the measurement of maximal voluntary contraction of the first dorsal interosseus muscle, using the hand dynamometer, is reliable. There is greater variability with forces generated from electrically stimulated contractions. This is more pronounced for the 10/100% ratio, and therefore this index of force/frequency will be excluded. This poor reliability was also reported for the quadriceps. Relaxation rate was highly variable, but will be reported as a measure of the speed of muscle. There is a concomitant increase of the CV with the limits of agreement for electrical stimulation compared with those reported for maximal force production. It is anticipated that the repeatability of these variables will be enhanced if increased voltages are used.

However, not all subjects were tolerant of higher voltages. This may be overcome with supramaximal stimulation of the ulnar nerve. This was not undertaken due to problems forseen with patients recruited and tested at the hospital, unfamiliar with the sensations and procedure.

Table 3.5.1: *Test-retest reliability for maximum voluntary contraction (MVC), 10/100 and 20/50 Hz ratios (%).*

METHOD	MVC (N)	10/100%	20/50%
Mean Difference	-0.05	-0.54	-3.95
(±SD)	(4.24)	(11.99)	(11.54)
Overall Mean	44.18	18.71	72.23
Error Linearity (r)	0.31	0.46	-0.52
CV%			
Unadjusted†	9.6	64.1	15.9
Adjusted‡	18.8	125.6	31.3
95% Limits of Agreement	-8.5 to 8.34	-24.54 to 23.45	-27.03 to 19.13

† Unadjusted = SD / overall mean x 100

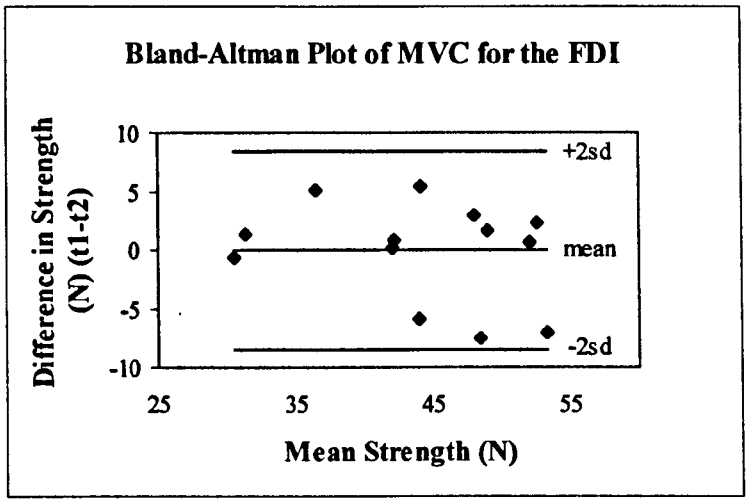
‡ Adjusted = SD x 1.96 / overall mean x 100

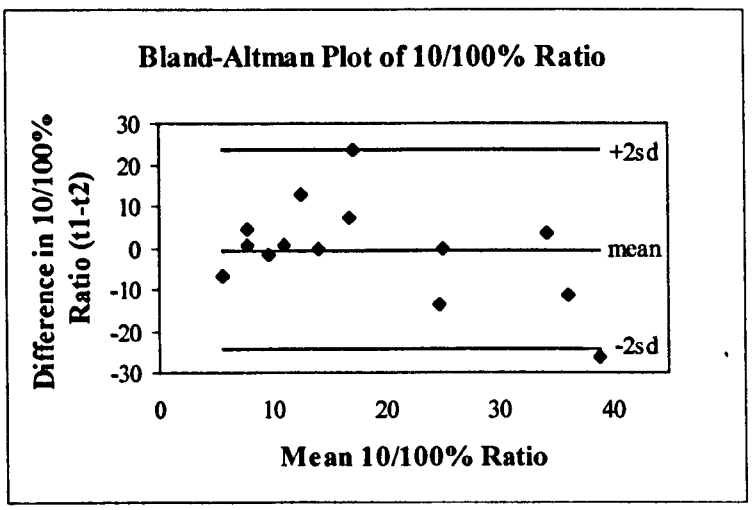
Table 3.5.2: *Test-retest reliability for fatigue parameters - fatigue index (FI%) of peak tension (PT), mean tension (MT) and relaxation rate. These indices are expressed as the end force as a percentage of the initial force.*

METHOD	Peak Tension	Mean Tension	Relaxation Rate
Mean Difference	-1.04	-2.71	-15.88
(±SD)	(9.08)	(9.08)	(25.87)
Overall Mean (%)*	49.24	44.05	182.46
Error Linearity (r)	0.27	0.42	0.34
CV%			
Unadjusted†	18.4	19.1	14.2
Adjusted‡	36.1	37.3	27.8
95% Limits of Agreement	-19.2 to 17.1	-19.2 to 13.7	-67.6 to 35.9

* End force as a percentage of initial for† Unadjusted = SD / overall mean x 100

‡ Adjusted = SD x 1.96 / overall mean x 100





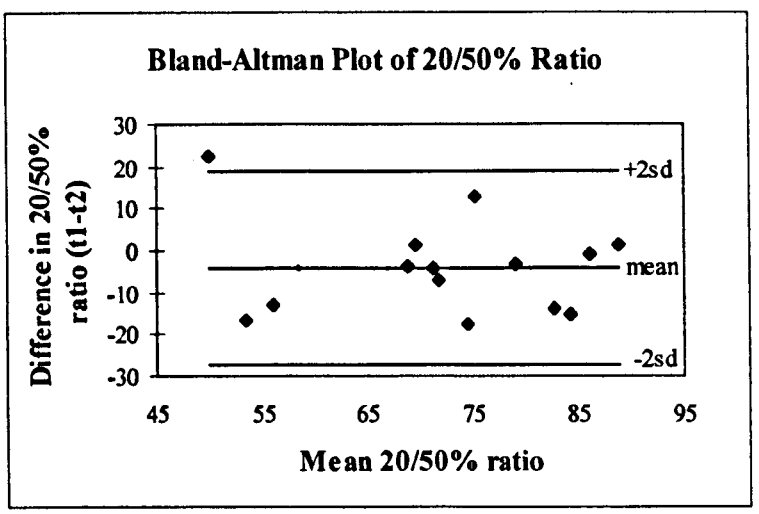


Fig. 3.5.2: The relationship between mean scores and test-retest differences for maximal voluntary contraction (MVC), 10/100% ratio and 20/50% ratio. The centre line represents the mean of test 1 and test 2, and the outer lines are the 95% limits of agreement (mean ± 2SD).

3.6. Summary

1) The reliability of the LIDO® Active dynamometer for measuring concentric strength of the knee extensors was established in a sample of young heterogenous subjects. Whilst the equipment was deemed reliable at 1.05 and 3.13 rad/s, the variability was greater at higher angular velocities (5.22 rad/s).

2) The reliability of the knee flexors was compromised across all angular velocities. This was attributed to the requirement to perform recriprocal movements.

3) The assessment of the variability in muscle performance of middle-aged women revealed a systematic increase of peak torque of the knee extensors at 1.05rad/s. Whilst the effort involved in undertaking reciprocal extensor/flexor actions could contribute to this learning effect, it is anticipated that extra warm-up trials will stabilise peak torque. Strength of the leg flexors were also highly variable, and are not considered a reliable muscle group for assessing hormonal influences.

4) Day-to-day measurements of maximal isometric handgrip and leg strength have proven reliable, although it is recommended that the twitch interpolation technique be employed to confirm the maximal activation of the quadriceps.

5) The strain gauge assembly, utilised for measuring the force-generating capacity of the quadriceps, was reported to be a reliable system. Forces generated from electrically stimulated contractions were prone to greater variability in performance. This was particularly noted for the ratio of forces at 10/100 Hz. The variabilty was also greater in fatigued, compared to fresh muscle.

6) The hand dynamometer was deemed reliable for measurements of maximal force production of the first dorsal interosseus muscle. Forces generated from electrically stimulated contractions were highly variable, particularly for the 10/100 Hz ratio.

CHAPTER FOUR

THE EXPERIMENTAL STUDIES

4.0. THE EXPERIMENTAL CHAPTER

In the previous chapter the reliability of the equipment to be employed for assessing hormonal influences on muscle function was established. These results have been used to determine the most reliable and effective methods for measuring aspects of muscle function. Those which have exhibited great variability day-to-day will not be used.

The experimental studies described within this chapter aimed to establish the influence of reproductive hormones on muscle function. Four separate studies were undertaken, measuring different muscles in young and middle-aged women, and assessing muscle function through volitional and electrically stimulated contractions. In study 4.1, the rate of force loss in hypoestrogenic post-menopausal women was assessed over 12 months, a model which represents a chronic loss of hormones.. Performance was compared with a perimenopausal group and a sample of women taking hormone replacement therapy (HRT). Additionally, the role of HRT was investigated. Temporal changes in hormones on maximal force of a large muscle group (quadriceps) and a small single muscle, the first dorsal interosseus (FDI) was examined during the menstrual cycle (study 4.2). Responses to electrically stimulated contractions were also examined in the quadriceps to gain further insight into the mechanisms of hormonal action.

These studies will determine the effects of chronic and acute changes in reproductive hormones on muscle function. The main objective of study 4.3 was to elucidate the role of oestrogen on muscle strength, where acute changes in oestrogen were examined in the FDI while progesterone remained relatively stable. Finally, study 4.4 investigated changes in muscle function of the FDI and assessed the effects of HRT in post-menopausal women. The influence on muscle function during changes from a hypoestrogenic to a hormonally replenished state was determined, with a focus on the differences of an oestrogen and oestrogen/progestogen primed muscle during the phases of HRT.

4.1. A LONGITUDINAL ANALYSIS OF MUSCLE STRENGTH IN MIDDLE-AGED FEMALES OF DIFFERENT HORMONAL STATUS

*Aspects of this work have been presented at the First European Congress of Sports Science, Nice, May, 1996 and at the Neurobiology of Ageing Conference, Dublin, March, 1997. An abstract has been published in the Journal of Physiology, **501**.P. 170P, 1997.*

4.1.1. Introduction

An age-related decline in muscle strength of the order of 30-40% (Larsson et al., 1979) is concomitant with a reduction in muscle mass (Grimby and Saltin, 1983). Recently, a decline in the force-generating capacity of the adductor pollicis muscle (AP), expressed as force per cross-sectional area (force/CSA), was reported in the elderly at 27% compared with younger controls (Bruce et al., 1989). Given the difficulty in measuring physiological cross-sectional area in humans, even in a parallel-fibred muscle such as the AP, isolated whole muscle in rodents have been examined. A reduction (20%) in specific force of hindlimb muscle has also indicated an age-related deficit in strength independent of atrophy (Brooks and Faulkner, 1988; Phillips et al., 1991). The onset of this weakness, investigated in males and females aged 17 to 90 years (Phillips et al., 1993b), is most rapid in peri-postmenopausal women. Since women taking hormone replacement therapy (HRT) do not exhibit this weakness, a hormonal component is strongly implicated.

Problems arise when reporting specific force in a multiple muscle group such as the quadriceps. Due to its architectural complexity, the CSA is difficult to measure accurately and, as a result, there have been conflicting reports of the changes in specific force with ageing. A loss of strength in the quadriceps, unexplained by atrophy has been documented in elderly men (Young et al., 1985) but not in women (Young et al., 1984). Muscle mass in both studies was measured using ultrasound scanning. Muscle mass, estimated from urinary creatinine excretion, was related to strength of lower, proximal, and upper distal limbs (Frontera et al., 1991). However, using computed tomography Rutherford and Jones (1992) found a decline in force/CSA

from the 5th decade in women and a total loss of specific force between the 2nd and 8th decade of 27%.

Whilst Phillips et al. (1993b) have reported the onset and time-course of muscle weakness of the AP in a cross-sectional study, the rate of individual force loss has not been examined. The object of this study was to monitor maximal strength of the quadriceps over 12 months in hypoestrogenic women (1-3 years post-menopausal). Muscle mass was not measured because of the difficulties associated with this muscle group, although an aged-matched control group taking HRT was assessed. The quadriceps was selected due to its role as a weight-bearing 'functional' muscle group; hand grip strength was also examined as an upper limb comparison. The effects of hypoestrogenia/progestogenia on shortening velocity of the quadriceps was also examined. The following hypotheses were devised:

Hypothesis 1. Maximal strength declines in women within 1 to 3 years post-menopause over 12 months.

Hypothesis 2. There is no change in maximal strength in females taking hormone replacement therapy (HRT).

Hypothesis 3. Strength loss is of the same proportion with increasing angular velocities.

The aims of this study were to:

1] Determine if strength loss occurs over a 12 month period.

2] Assess the role of HRT in preserving muscle strength

3] Examine the effect of hormonal status on shortening velocity of the quadriceps

4] Investigate the response of different muscle groups to hypoestrogenia.

4.1.2 Methods

(i) Subjects

Thirty middle-aged female subjects volunteered to participate in the study. Subjects were recruited from the menopause clinic at Liverpool Women's Hospital and by advertisements in a local newspaper. Subjects were subdivided into three groups; eleven women were taking hormone replacement therapy (HRT) either shortly before or soon after baseline measurements (HRT preparations are shown in Table 4.1.2). Nine females were perimenopausal and experiencing symptoms of the climacteric ie. vasomotor disturbances, irregular periods. Finally, ten postmenopausal women were recruited according to the inclusion criteria listed below. A venous blood sample was taken to confirm high follicle stimulating hormone (FSH) and luteinising hormone (LH) levels (>20 U/L). Baseline characteristics of subjects are shown in Table 4.1.1. There were no significant differences in mass ($p=0.79$) or height ($p=0.86$) between the three groups. These parameters were used to calculate body mass index (BMI) (kg/m^2) (Table 4.1.1.).

***Table 4.1.1**: Mean (±SD) baseline characteristics of subjects in the peri-, post- and HRT groups.*

Variables	Peri-menopause	Post-menopause	HRT
Age (yrs - decimal)	49.96 (3.11)	51.97 (3.08)	50.05 (3.84)
Height (m)	1.59 (3.52)	1.62 (6.85)	1.59 (5.45)
Mass (kg)	73.58 (9.11)	72.33 (14.57)	67.50 (10.31)
BMI *	28.88 (2.83)	27.36 (4.67)	26.56 (2.03)
LTPA (kJ/wk) ‡	5168 (2539)	10409 (9741)	6181 (6149)

* Body mass index

‡ Leisure time physical activity

A questionnaire was administered to all volunteers for inclusion/exclusion into the study. Medical and gynaecological profiles and history of HRT were reported. Physical activity was assessed using the Leisure Time Activity Questionnaire (Lamb and Brodie, 1991) to ascertain fitness levels of subjects. This survey requires the recollection of leisure time physical activity over a 'typical' two week period (Appendix 1.0). Energy expenditure was calculated from the weekly activities recalled. Differences in activity levels are presented in Table 4.1.1. Large inter-individual variations exist, although

energy expenditure was mainly attributed to garden/household activities. Ten percent of subjects suffered from thyroid disease, which was treated with exogenous thyroxine replacement.

Inclusion criteria	*Exclusion criteria*
a) Sedentary, but physically healthy	Myopathic/neuropathic/skeletal disorders likely to affect performance
b) Aged 45-55 years	Medication possibly affect motivation
c) Normotensive	Hypertensive (>140/90)
<u>Postmenopausal group</u>	
d) Amenorrhoea at least 12 months	Amenorrhoea > 5 years
e) High FSH/LH levels (>20 U/l)	Premature menopause (<40 years)
<u>Perimenopausal group</u>	
f) Vasomotor symptoms	Amenorrhoeic
g) Irregular periods (cycle length >35 days)	Younger 45 years
<u>HRT</u>	
h) Beginning HRT/taking HRT 1-2 months	> 55 years

Table 4.1.2. Hormone replacement therapy preparations taken by subjects (n=11)

Route of administration	Preparation	Oestrogen	Progestogen
Oral	Climagest (x1)	Oestradiol valerate	Norethisterone
Oral	Trisquens (x1)	Oestradiol/oestriol	Norethisterone acetate
Oral	Prempak-C (x2)	Oestradiol/oestriol	Norgestrel
Transdermal	Estracombi (x1)	Oestradiol	Norethisterone acetate
Oral	Livial * (3)	-	-
Oral	Premarin** (x2)	Conjugated oestrogens	-
Transdermal	Estracombi** (x1)	Oestradiol	-

* Livial contains tribolone - a gonadomimetic.
**These preparations are unopposed oestrogens taken by hysterectomised women

Drop outs

Forty subjects were initially recruited into the study. Fourteen females were taking hormone replacement therapy, thirteen subjects were assigned to the peri-menopausal group and thirteen subjects were post-menopausal. Ten subjects (HRT = 3; peri-menoapausal = 4; Post-menopausal = 3) dropped out between baseline and the second

testing session due to medical and/or personal problems. Following the penultimate testing session, three subjects in the postmenopausal group were withdrawn from the study due to illness (x2) and death (x1). Analysis of data was therefore undertaken over 9 and 12 months.

(ii) Procedure

On arrival at the laboratory, body mass of subjects was recorded and, following 5 min of seated rest, blood pressure was measured using an automatic sphygmomanometer (Model 8111, Dynamar, Critikom, Bracknell, UK). Subjects with values greater than 150/100 were diagnosed a hypertensive and were requested to seek medical advice before continuing with the study. Leg and grip strength was then measured following a 5 min, self-paced warm-up on a cycle ergometer (Monark).

Strength measurements

Dynamic strength of the quadriceps and hamstrings was assessed using an isokinetic dynamometer (LIDO Active®, Davies CA) according to the protocol described in Chapter 3.3. Due to the systematic increase in peak torque between test 1 and 2 at 1.05 rad/s in section 3.2, this parameter was stablised with 2 submaximal and 4 maximal practice trials. Maximal voluntary isometric contraction (MVIC) of the quadriceps was measured with the leg flexed at the knee at an angle of 90°. The maximum force-generating capacity of the muscle group was monitored with percutaneous stimulation using surface eletrodes. One Hz twitches were delivered at a tolerable current for 10 s. The MVIC was recorded when the superimposed twitches diminished. One min rest was given between each contraction. Following a 5 min recovery, the highest of 5 trials was recorded.

Intraindividual tests were undertaken at the same time of day (± 1 hour). A familiarisation session preceded the baseline trial. Subjects were required to visit the laboratory every ten weeks for 12 months, arranged with the experimenter one to two weeks beforehand. A total of 5 sessions, excluding the familiarisation test, were attended.

(iii) Data Analysis

The Statistical Package for Social Sciences (SPSS) was used for data analysis. Mean (± standard deviation) and percent changes were calculated. A two-way analysis of variance (group x3) with a repeated measures factor of visit (x5), was employed to calculate strength changes between groups over the 12 months. Analysis was also undertaken across 4 visits (up to 9 months) of all the subjects (n=30) so that the performances of the post-menopausal subjects who dropped out before the final test session were analysed. A two-way ANOVA with a repeated measures factor of velocity (x4) and visit (x2) was also used to calculated the differences in strength of the three groups across all angular velocities, and standardised peak torque . To avoid the occcurrence of a Type I error (when the assumption of sphericity, or homogeneity of variance is not true), the Huynh-Feldt correction factor was used. Post-hoc tests (Scheffé) were carried out to determine differences between variables from the ANOVA. Significance was set at a 5% level.

Allometric modelling

Physiological variables are often dependent on body size. To normalize for differences in body size allometric modelling was used to 'remove' these individual influences from the analysis using the equation:

$$Y = ah^{b1}.m^{b2}\varepsilon \text{ (equation 1)}$$

where Y represents the physiological variable, h = height, m = body mass and ε the multiplicative error ratio term. The model naturally overcomes the presence of heteroscedasticity (when the error diverges with an increase in mean scores) and, after log transformation, the model parameters can be fitted using the linear regression methods. Since strength varies with body size (Edwards et al., 1977b), force was adjusted for height and mass according to the method of Nevill (1994).

4.1.3. Results

Mean force values ± standard deviation (SD) of different muscle groups across all angular velocities are shown in Tables A.1, A.2 and A.3 (Appendix 2.0) for HRT, peri- and post-menopausal groups respectively. Measurements in three post-menopausal women were not recorded at the last test due to reasons described in the methods, and thus were excluded from analysis over 12 months.

4.1.3.1. Longitudinal changes in strength between peri-, post-menopausal and hormonally replenished women over 12 months

Maximal force, measured across a range of angular velocities, was assessed over 12 months between three treatment groups. Force was log transformed and corrected for covariates log of height and weight. The two-way ANOVA with repeated measures did not reveal any significant differences between (group x 3) or within (visit x 5) subjects ($p>0.05$). There was no significant group/visit interactions at any velocity for the muscle groups tested. The interaction plots for these factors are shown in Fig 4.1.1 to 4.1.8. The F values (significance of F), with b exponent of height and weight, for all variables are shown in Table A.4 (Appendix 2.0).

The log of height and weight were incorporated into all analyses as covariates, and were omitted if they were not significant. Height was not included as a covariate for isometric strength, which affected the significance of the b exponent of weight. Height and weight were included for concentric contractions (1.05 to 3.13 rad/s) and hand grip strength.

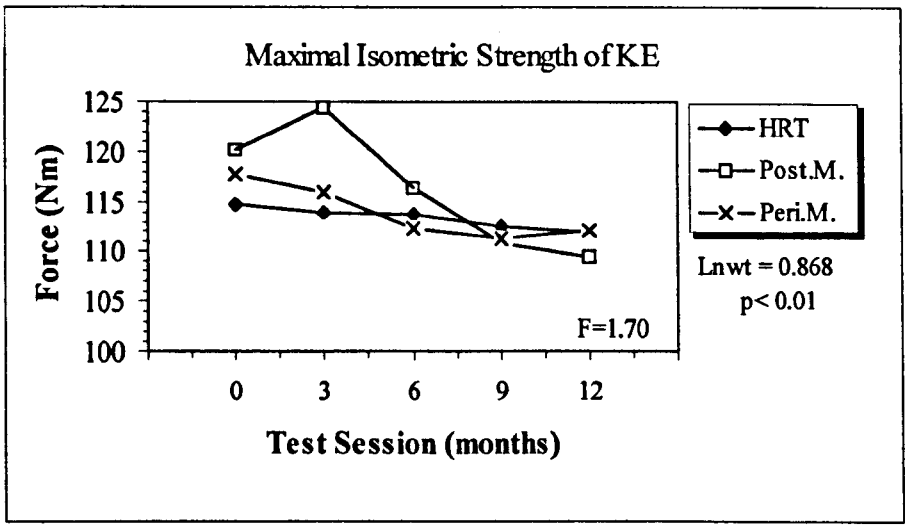


Fig. 4.1.1. Mean force values for isometric contraction (90° flexion) of the knee extensors between the HRT, Post-menopausal (Post.M.) and Peri-menopausal (Peri.M.) groups over 12 months. HRT N=11, Post.M. N=7. Peri.M. N=11. The F value is presented for Group v Visit interaction. Lnwt indicate log of covariate weight and its b value.

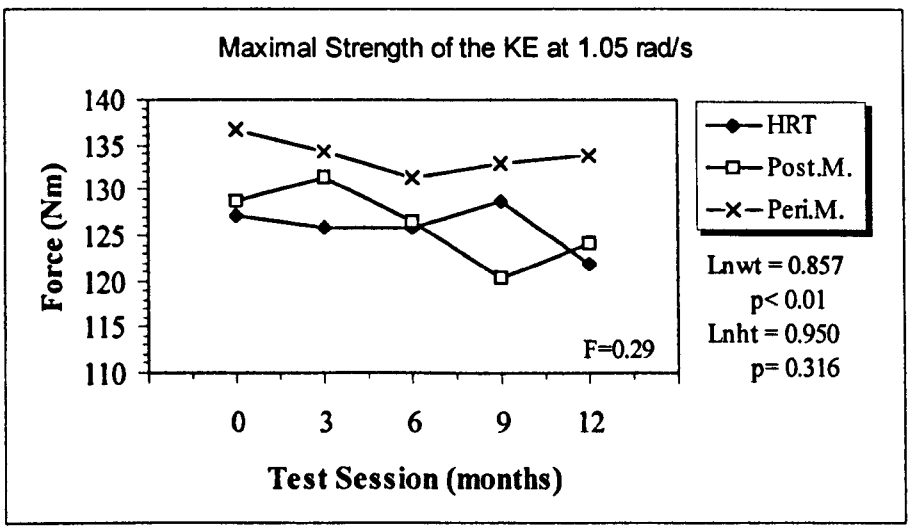


Fig. 4.1.2. Mean force values for concentric contraction of the knee extensors (KE) at 1.05 rad/s between the HRT, Post-menopausal (Post.M.) and Peri-menopausal (Peri.M.) groups over 12 months. HRT N=11, Post.M. N=7. Peri.M. N=11. The F value is presented for Group v Visit interaction. Lnwt and Lnht indicate log of covariates weight and height respectively - and their b values.

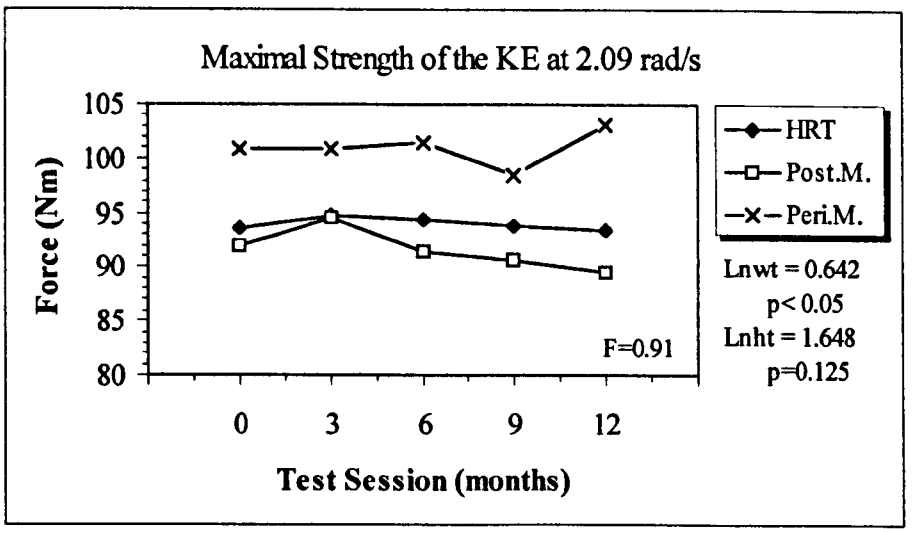


Fig. 4.1.3. Mean force values for concentric contraction of the knee extensors (KE) at 2.09 rad/s between the HRT, Post-menopausal (Post.M.) and Peri-menopausal (Peri.M.) groups over 12 months. HRT N=11, Post.M. N=7. Peri.M. N=11. The F value is presented for Group v Visit interaction. Lnwt and Lnht indicate log of covariates weight and height, respectively - and their b values.

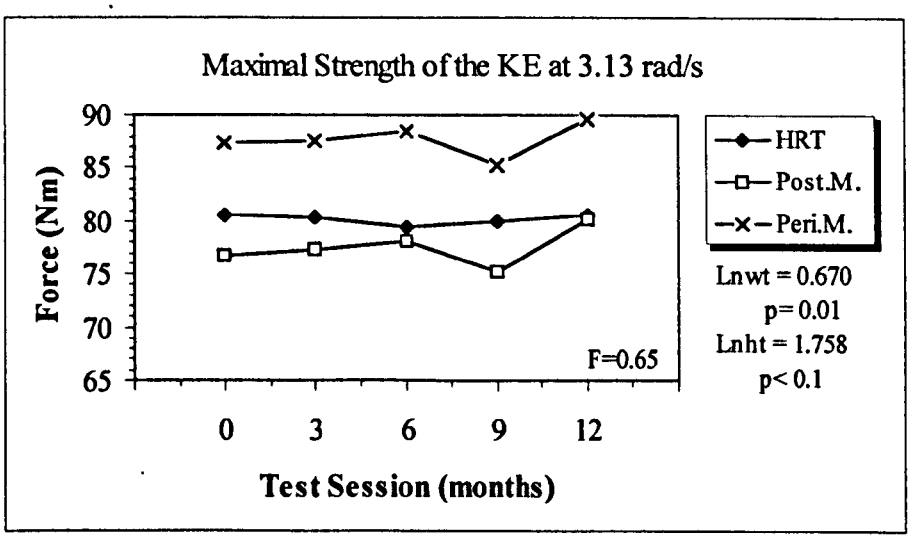


Fig. 4.1.4. Mean force values for concentric contraction of the knee extensors (KE) at 3.13 rad/s between the HRT, Post-menopausal (Post.M.) and Peri-menopausal (Peri.M.) groups over 12 months. HRT N=11, Post.M. N=7. Peri.M. N=11. The F value is presented for Group v Visit interaction. Lnwt and Lnht indicate log of covariates weight and height, respectively - and their b values.

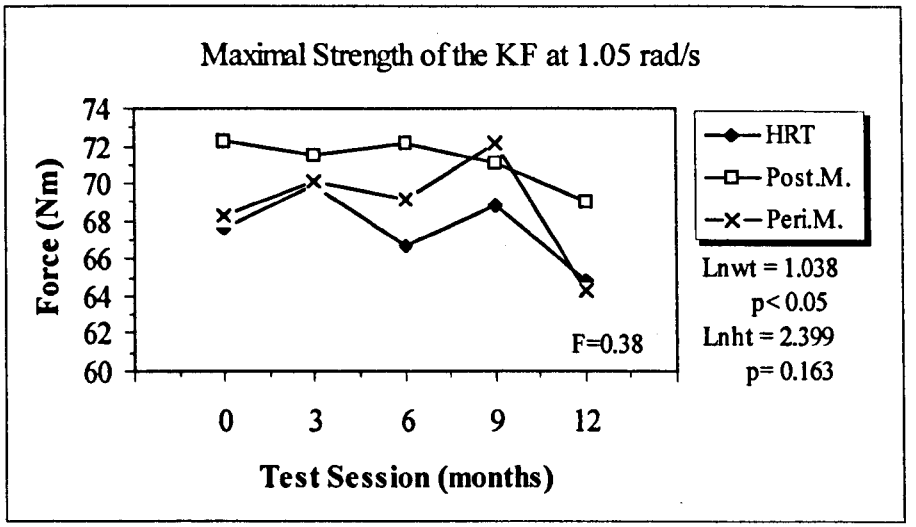


Fig. 4.1.5. Mean force values for concentric contraction of the knee flexors (KF) at 1.05 rad/s between the HRT, Post-menopausal (Post.M.) and Peri-menopausal (Peri.M.) groups over 12 months. HRT N=11, Post.M. N=7. Peri.M. N=11. The F value is presented for Group v Visit interaction. Lnwt and Lnht indicate log of covariates weight and height, respectively - and their b values.

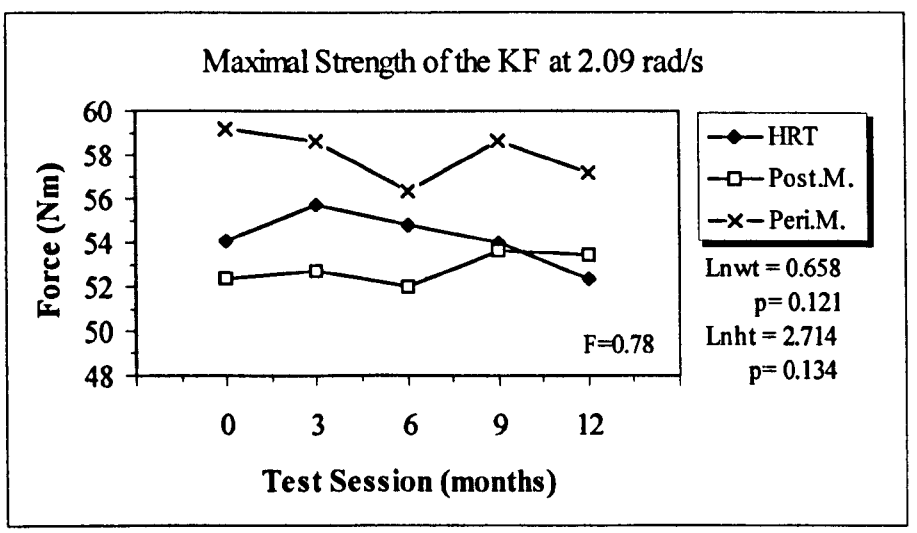


Fig. 4.1.6. Mean force values for concentric contraction of the knee flexors (KF) at 2.09 rad/s between the HRT, Post-menopausal (Post.M.) and Peri-menopausal (Peri.M.) groups over 12 months. HRT N=11, Post.M. N=7. Peri.M. N=11. The F value is presented for Group v Visit interaction. Lnwt and Lnht indicate log of covariates weight and height, respectively - and their b values.

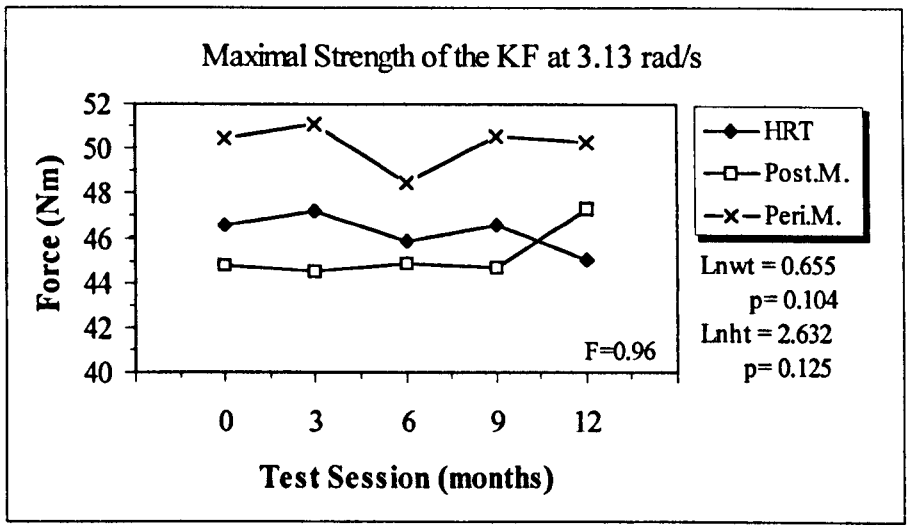


Fig. 4.1.7. Mean force values for concentric contraction of the knee flexors (KF) at 3.13 rad/s between the HRT, Post-menopausal (Post.M.) and Peri-menopausal (Peri.M.) groups over 12 months. HRT N=11, Post.M. N=7. Peri.M. N=11. The F value is presented for Group v Visit interaction. Lnwt and Lnht indicate log of covariates weight and height, respectively - and their b values.

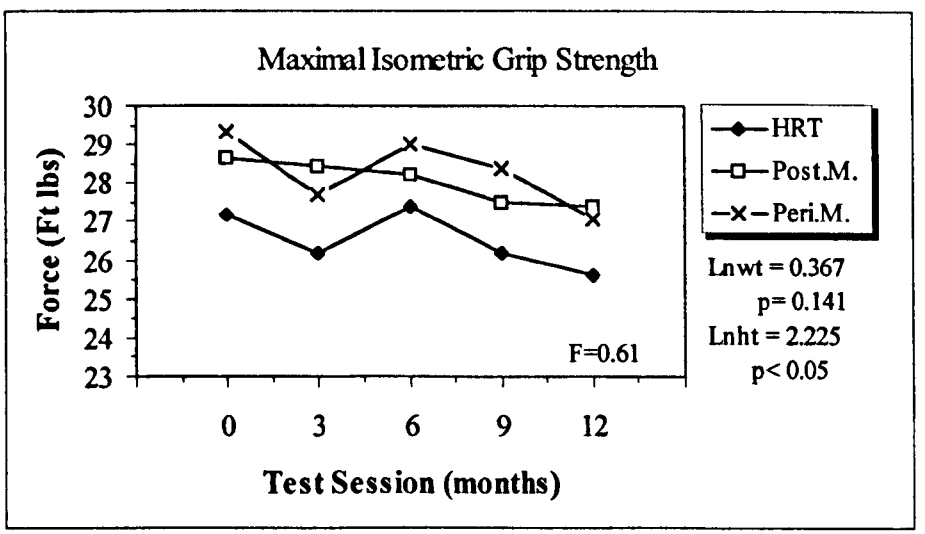


Fig. 4.1.8. Mean force values of isometric grip strength between the HRT, Post-menopausal (Post.M.) and Peri-menopausal (Peri.M.) groups over 12 months. HRT N=11, Post.M. N=7. Peri.M. N=11. The F value is presented for Group v Visit interaction. Lnwt and Lnht indicate log of covariates weight and height, respectively - and their b values.

4.1.3.2 Comparison of post-menopausal women and females taking HRT over 9 months

There were no significant differences in strength between the three treatment groups over 12 months. The peri-menopausal group was removed, and the differences in performance of hypoestrogenic post-menopausal females compared with a group taking hormone replacement therapy (HRT). The role of HRT on muscle strength was also examined. Strength measurements up to 9 months were analysed so that all ten post-menopausal subjects were included in the analysis. At the slow, or zero, angular velocities of the knee extensors there was significant group x visit interaction for isometric ($F_{3,57} = 4.45$; $p<0.05$) and concentric strength at 1.05 rad/s ($F_{3,57} = 4.04$; $p<0.05$).

There were also significant differences over time (the 'within' subjects factor) for both variables. Post-hoc tests located significant differences in isometric strength for the post-menopausal group between T_1 and T_4, T_1 and T_3, T_2 and T_4 and T_1 and T_3 ($p<0.01$), whilst force remained stable for the HRT group (Fig 4.1.9). The deficit in force measured isokinetically at 1.05 rad/s for the post-menopausal group was significant between T_1 and T_4; T_3 and T_4 ($p<0.05$); T_2 and T_3; T_2 and T_4 ($p<0.01$). A slight increase in strength at the last session was observed for the HRT group, although this was not significant (4.1.10). There were no sigificant changes in strength at the faster angular velocities for the knee extensors (2.09 and 3.13 rad/s), although the HRT group were were able to generate greater force compared with the post-menopausal females at all time points (Fig. 4.1.11 to 4.1.12). Strength of the knee flexors are not reported here because of the variability in force of this muscle group (demonstrated in section 3.3), but are shown in Fig. 4.1.5 to 4.1.7 for all groups.

The interaction plot of mean force for grip strength (Fig. 4.1.13) vary over the duration of the testing for both groups. Significant differences between visits were found (HRT group = T_2 and T_3; T_3 and T_4 ($p<0.05$): Post-menopausal group = T_1 and T_2 ($p<0.05$); T_1 and T_4 ($P<0.01$)) although these changes were variable.

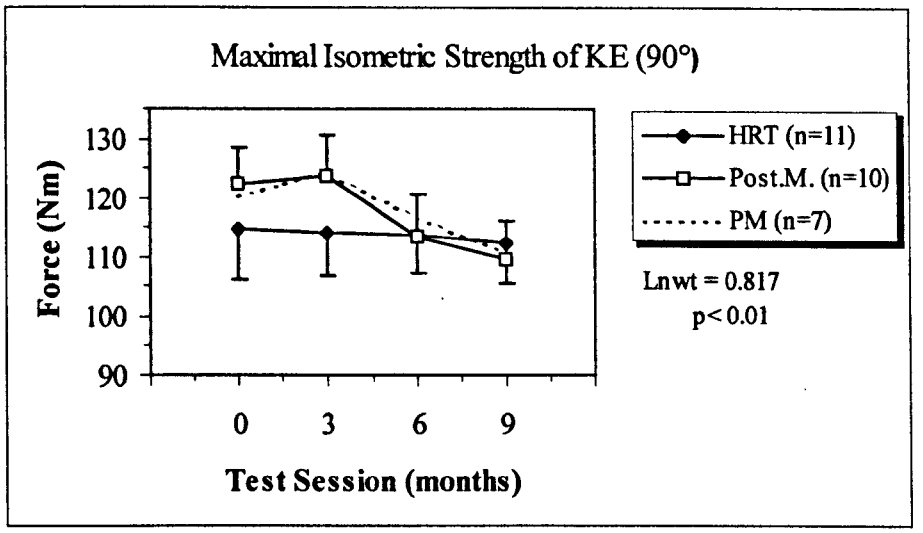


Fig. 4.1.9. The relationship betweeen mean (±SE) isometric force (90° knee flexion) of the knee extensors (KE) between hypoestrogenic and hormonally replaced females over 9 months. The broken line represents the mean force of the number of females who completed all 5 tests (n=7). Lnwt is the abbreviation of log of covariate weight and its b value. SE = standard error.

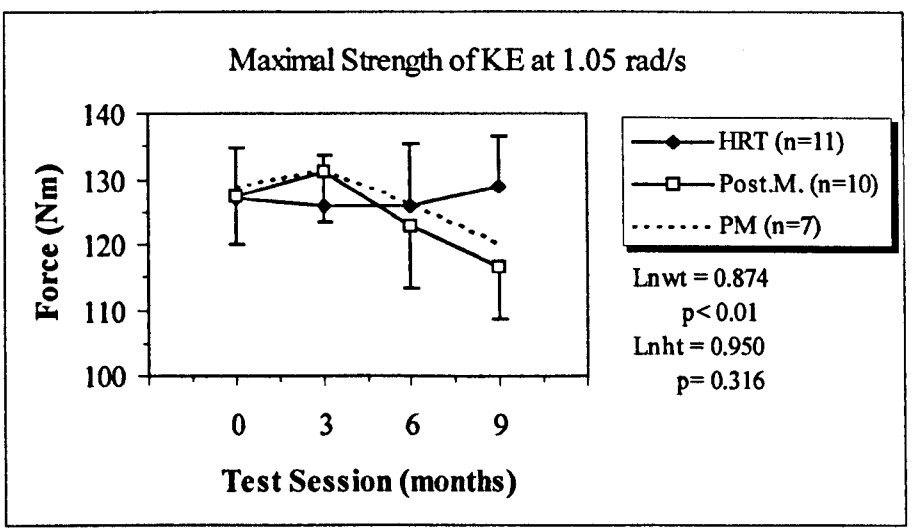


Fig. 4.1.10. The relationship betweeen mean concentric force (±SE) of the knee extensors (KE) at 1.05 rad/s between hypoestrogenic and hormonally replaced females over 9 months. The broken line represents the mean force of the number of females who completed all 5 tests (n=7). Lnwt and Lnht are abbreviations of log of covariates weight and height respectively, and their b values. SE = standard error.

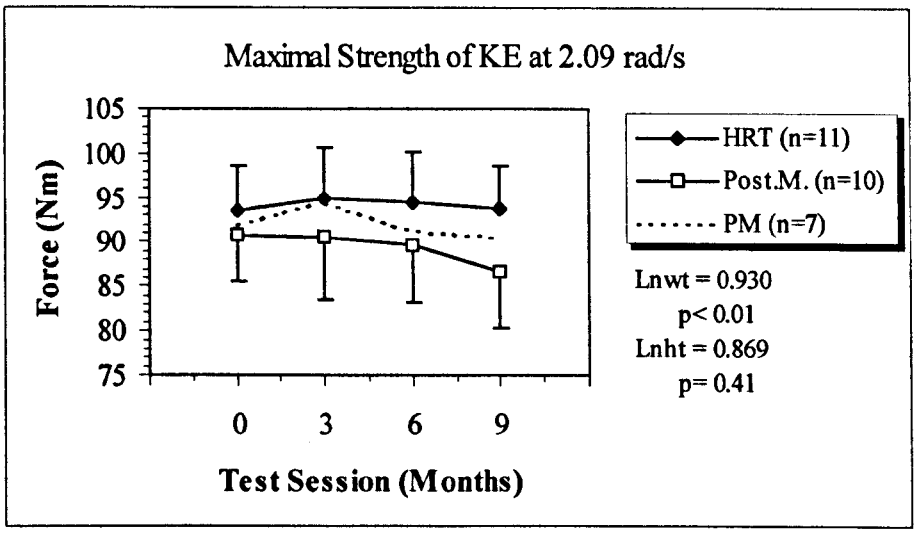


Fig. 4.1.11. The relationship betweeen mean concentric force (±SE) of the knee extensors (KE) at 2.09 rad/s between hypoestrogenic and hormonally replaced females over 9 months. The broken line represents the mean force of the number of females who completed all 5 tests (n=7). Lnwt and Lnht are abbreviations of log of covariates weight and height respectively, and their b values. SE = standard error.

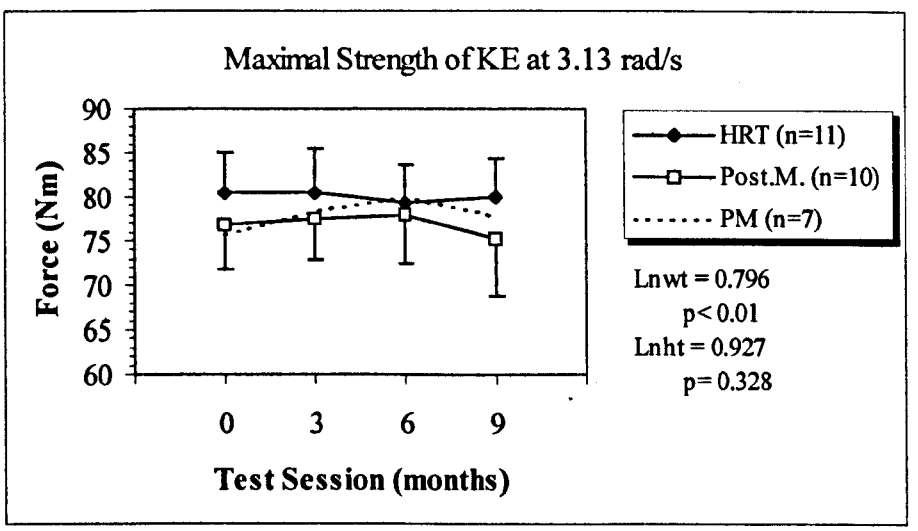


Fig. 4.1.12. The relationship betweeen mean concentric force (±SE) of the knee extensors (KE) at 3.13 rad/s between hypoestrogenic and hormonally replaced females over 9 months. The broken line represents the mean force of the number of females who completed all 5 tests (n=7). Lnwt and Lnht are abbreviations of log of covariates weight and height respectively, and their b values. SE = standard error.

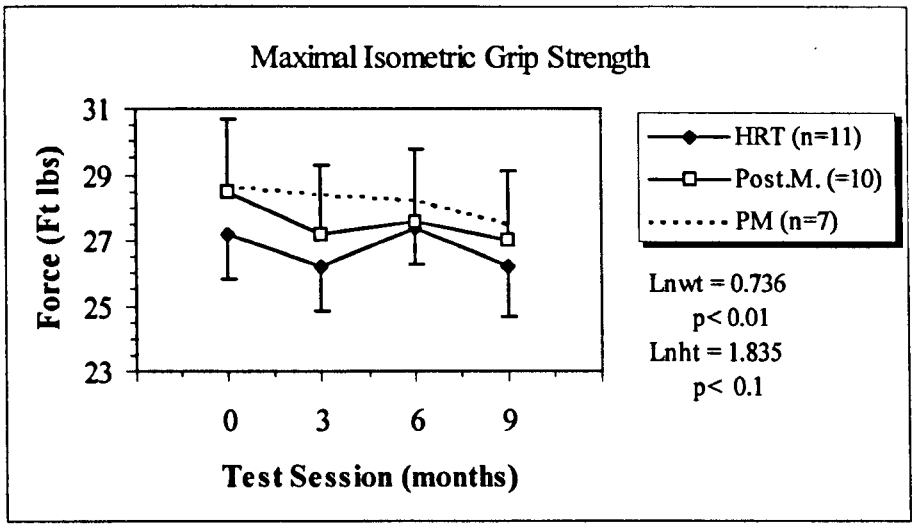


Fig. 4.1.13. The relationship betweeen mean force (±SE) of isometric grip strength between hypoestrogenic and hormonally replaced females over 9 months. The broken line represents the mean force of the number of females who completed all 5 tests (n=7). Lnwt and Lnht are abbreviations of log of covariates weight and height respectively, and their b values. SE = standard error.

4.1.3.3. Percent change over 12 months between three group

Differences in the percent change of strength of the knee extensors and grip strength between baseline (T_1) and 9 (T_4) and 12 (T_5) months are illustrated in Fig. 4.1.14 to 4.1.18. A one-way ANOVA calculated differences in the magnitude of change in force deficit (%). No significant changes were found between the three groups across 9 months or 12 months ($p>0.05$).

4.1.3.4. Percent change over 9 months - HRT versus post-menopausal women

Comparisons in the percent change in strength for baseline and 9 months were made between the HRT and post-menopausal groups. Significant differences were found for isometric ($t_{19} = 2.43; p<0.05$) and dynamic strength measurements at 1.05 rad/s ($t_{19} = 2.29; p<0.05$).

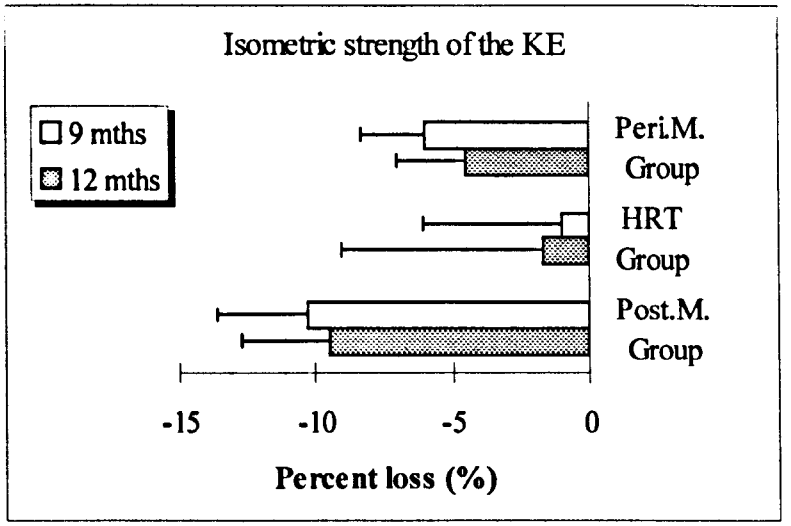


Fig. 4.1.14. Percent change (± SE) in force(Nm) from baseline (T_1) to 9 (T_4) and 12 (T_5) months for isometric contraction of the knee extensors (KE) measured at 90° of knee flexion. SE = standard error; Post.M. = post-menopausal women; HRT = hormone replacement therapy; Peri.M. = peri-menopausal females.

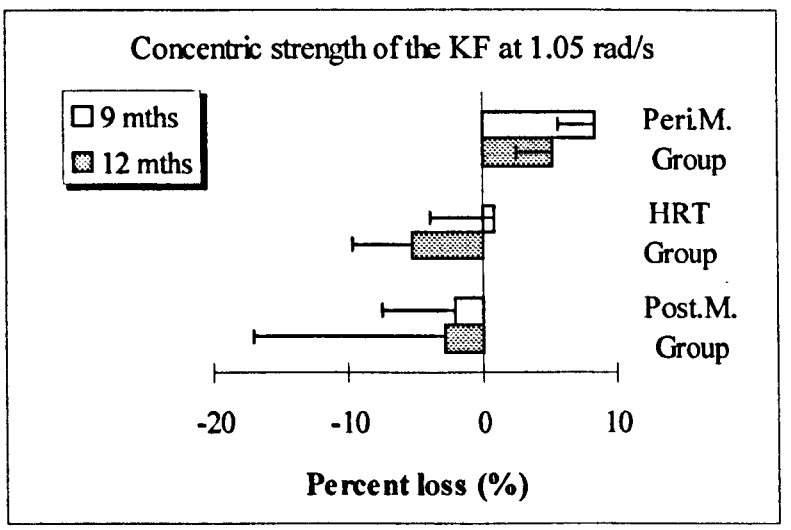


Fig. 4.1.15. Percent change (± SE) in force(Nm) from baseline (T_1) to 9 (T_4) and 12 (T_5) months for concentric contraction of the knee extensors (KE) measured at a velocity of 1.05 rad/s. SE = standard error; Post.M. = post-menopausal women; HRT = hormone replacement therapy; Peri.M. = peri-menopausal females.

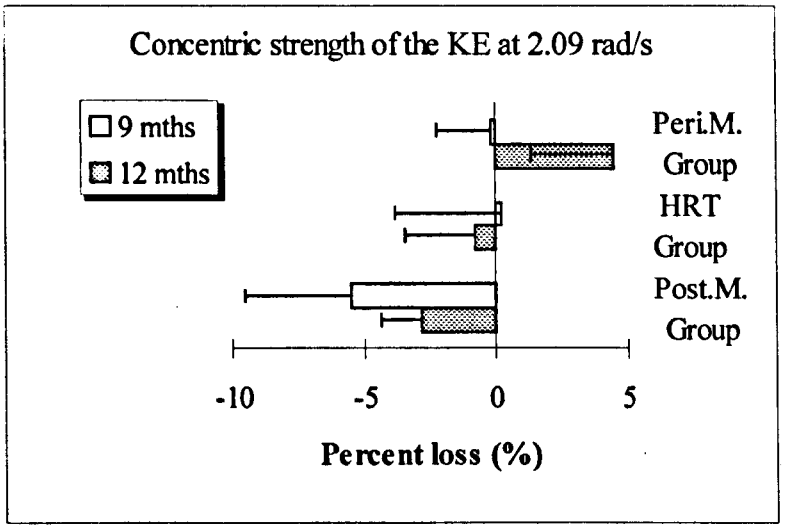


Fig. 4.1.16. Percent change (± SE) in force (Nm) from baseline (T_1) to 9 (T_4) and 12 (T_5) months for concentric contraction of the knee extensors (KE) measured at a velocity of 2.09 rad/s. SE = standard error; Post.M. = post-menopausal women; HRT = hormone replacement therapy; Peri.M. = peri-menopausal females.

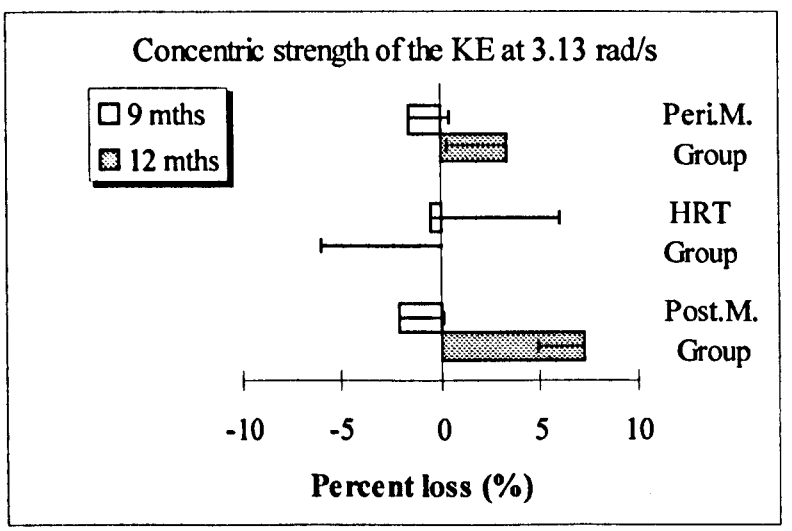


Fig. 4.1.17. Percent change (± SE) in force (Nm) from baseline (T_1) to 9 (T_4) and 12 (T_5) months for concentric contraction of the knee extensors (KE) measured at a velocity of 3.13 rad/s. SE = standard error; Post.M. = post-menopausal women; HRT = hormone replacement therapy; Peri.M. = peri-menopausal females.

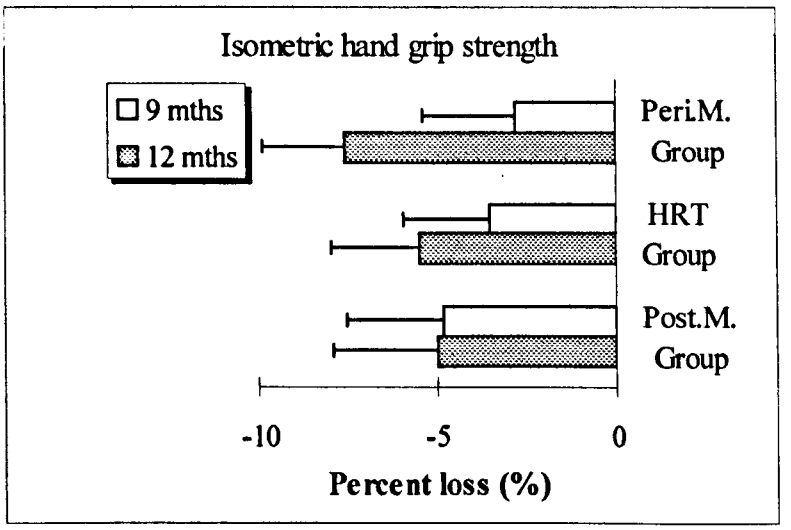


Fig. 4.1.18. Percent change (± SE) in force (Ft lbs) from baseline (T_1) to 9 (T_4) and 12 (T_5) months for grip strength measured during an isometric contraction. SE = standard error; Post.M. = post-menopausal women; HRT = hormone replacement therapy; Peri.M. = peri-menopausal females.

4.1.3.5. Force-angular velocity relationship

The force-velocity curves were examined between treatment groups at baseline and at 12 months. The relationship between absolute strength and angular velocity at both time points are shown in Fig. 4.1.19 and 4.1.20. The log transformation of strength was calculated statistically with the log of height and weight as covariates to correct for body stature (Fig. 4.1.23 and 4.1.24). A two-way ANOVA with repeated measures of velocity (x4) and visit (x2) showed that there was no affect of group on force by visit ($F_{2,22}$ =0.68; p>0.05) or by velocity ($F_{6,66}$ = 1.93; p>0.05). Further, there was no interaction between group by velocity or visit ($F_{6,66}$ = 1.80; p>0.05).

The force produced isometrically at 90° of flexion was lower than at slower angular velocities of 1.05 rad/s. This is not typical of the force-velocity curve in which force declines with increasing angular velocity. This trend was not affected when strength was normalised for body size. Force was greatest isometrically at 60° of flexion when measured on the final visit. Further measurements at a faster angular velocity of 5.22

rad/s resulted in lower force production. This pattern occurred for all treatment groups and when scaled for body size (Fig. 4.1.20 to 4.1.22).

4.1.3.6. Standardised force-velocity relationship

The relative rate of force loss with angular velocity was examined by expressing force as a percentage of isometric force. Force is relative to maximal isometric force, although in this study maximal force was attained at 1.05 rad/s. Since relative force is presented, scaled results were not used.

A comparison of standardised force between baseline and 9 months are shown for the HRT group (Fig. 4.1.23) and post-menopausal females (Fig. 4.1.24). There was no significant affect of forces over time and across velocities for either group ($p>0.05$). This is evident in the HRT group (Fig. 4.1.23), although higher relative force with increasing velocities in the post-menopausal group indicate that greater relative force is generated at 9 months. This is probably due to a significant decline in isometric force at 9 months and the maintenance of forces at higher angular velocities. Forces at 1.05 rad/s had decreased in proportion to isometric force.

A comparison between groups at baseline (Fig. 4.1.25) and at 9 months (Fig. 4.1.26) shows that the HRT group generates higher forces as a percentage of their isometric force. There were no significant differences between groups x velocity ($F_{3,57} = 2.11$; $p>0.05$) or by visit ($F_{1,19} = 0.51$; $p>0.05$).

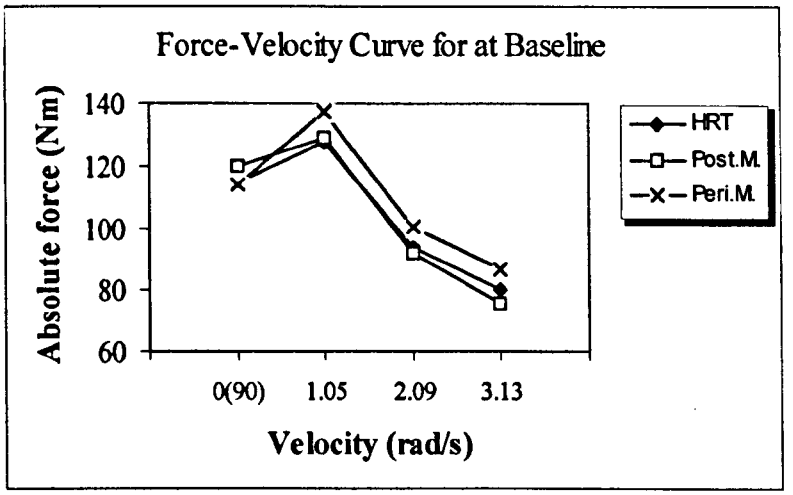


Fig. 4.1.19. Force (Nm) - angular velocity relationship for absolute strength at baseline between the three treatment groups. HRT =hormone replacement therapy; Post.M. =Post-menopausal group; Peri.M.=Peri-menopausal group.

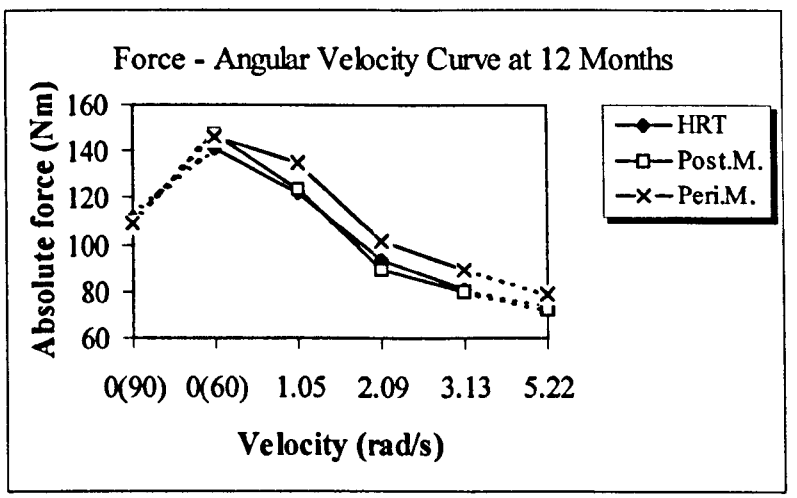


Fig. 4.1.20. Force (Nm) - angular velocity relationship for absolute strength at 12 months between the three treatment groups. HRT =hormone replacement therapy; Post.M. =Post-menopausal group; Peri.M.=Peri-menopausal group.

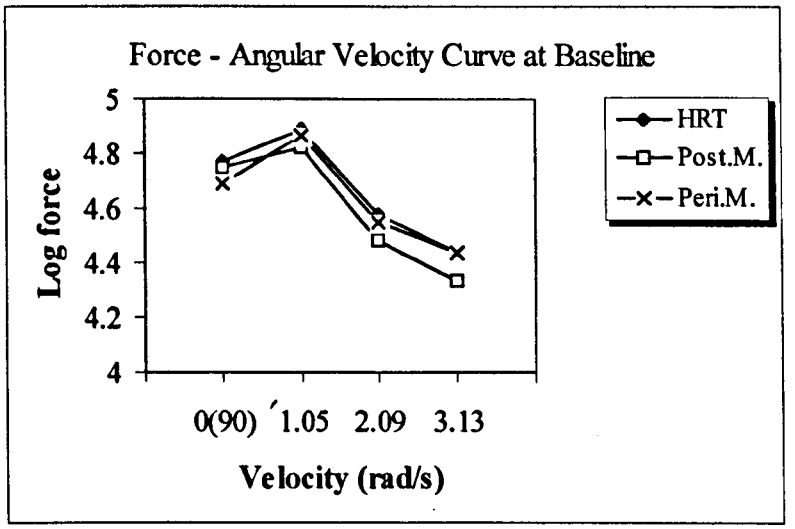


Fig. 4.1.21. Log of force (Nm) — angular velocity relationship for strength at baseline between the three treatment groups. Mean values adjusted from the log of strength and covariates height and weight. HRT =hormone replacement therapy; Post.M. =Post-menopausal group; Peri.M.=Peri-menopausal group.

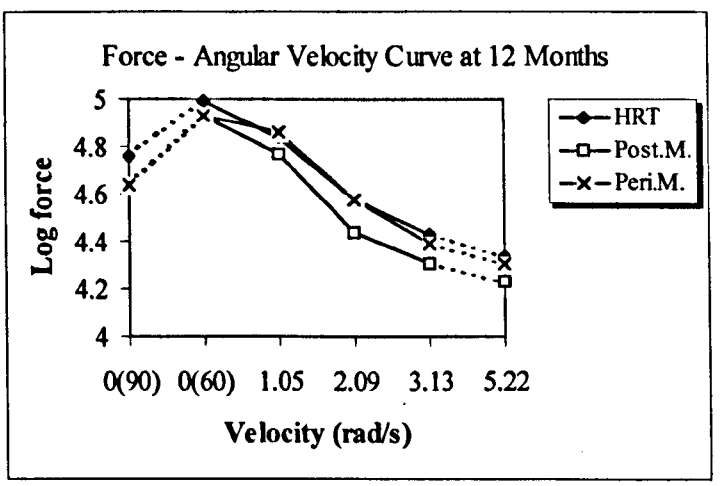


Fig. 4.1.22. Log of force (Nm) - angular velocity relationship for strength at 12 months between the three treatment groups. Mean values adjusted from the log of strength and covariates height and weight. HRT =hormone replacement therapy; Post.M. =Post-menopausal group; Peri.M.=Peri-menopausal group.

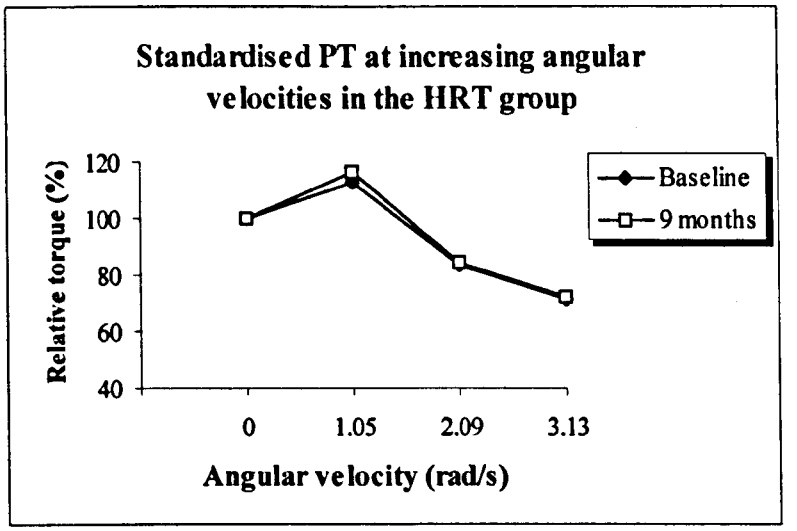


Fig. 4.1.23. Mean standardised peak torque against angular velocity of knee extension of females in the HRT group at baseline and 9 months (T4). Concentric force is expressed as a percentage of isometric force measured at 90° of knee flexion.

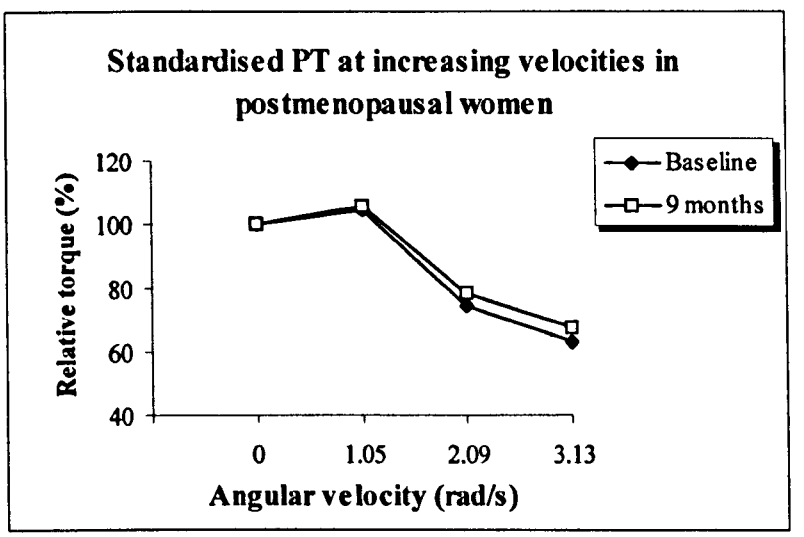


Fig. 4.1.24. Mean standardised peak torque against angular velocity of knee extension of post-menopausal females at baseline and 9 months (T4). Concentric force is expressed as a percentage of isometric force measured at 90° of knee flexion.

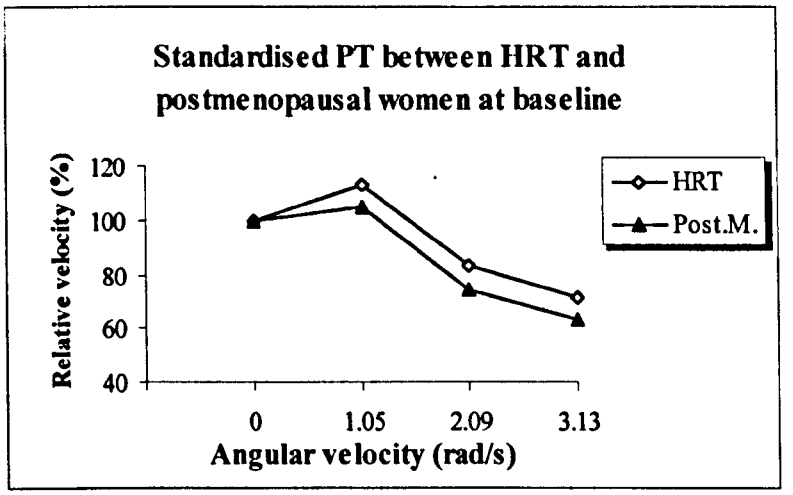


Fig. 4.1.25. A comparison of standardised peak torque against angular velocity between females in the HRT and post-menopausal groups at baseline. HRT = Hormone replacement therapy; Post.M. = Post-menopausal group.

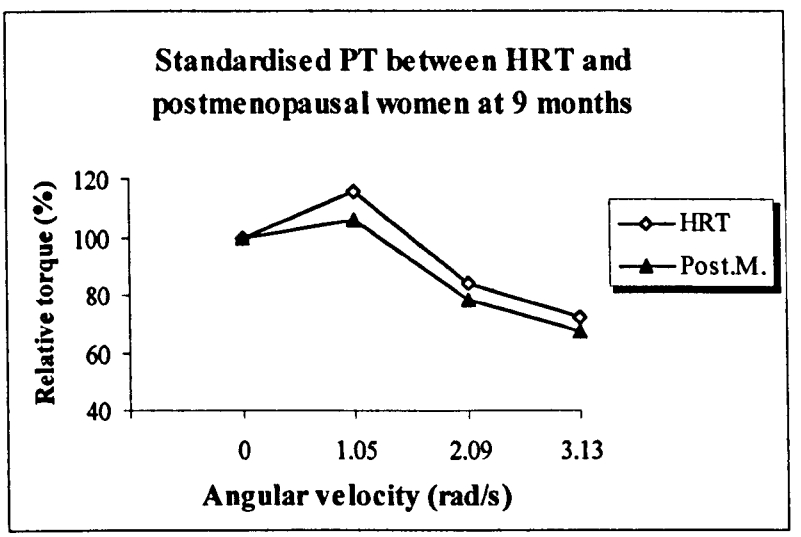


Fig. 4.1.26. A comparison of standardised peak torque against angular velocity between females in the HRT and post-menopausal groups at 9 months. HRT = Hormone replacement therapy; Post.M. = Post-menopausal group.

4.1.4. Discussion

The force per cross-sectional area of the adductor pollicis (AP) muscle is significantly weaker in peri-post-menopausal women compared with age-matched females taking hormone replacement therapy (HRT) (Phillips et al., 1993b). These findings have provoked much interest, particularly with the clinical implications of an increased risk of falling associated with muscle weakness (Wickham et al., 1989). Muscle groups of significant functional capacities, the quadriceps and handgrip, were assessed over 12 months in post-menopausal women, peri-menopausal women experiencing eratic changes in the menstrual cycle, and a group of females taking HRT.

Maximal strength between the three groups did not significantly change over 12 months for any muscle group tested or at any angular velocity. However, a reduction in mean strength at both zero and slow angular velocities was observed in the post-menopausal group when all three groups were compared (Fig. 4.1.1 and 4.1.2). These strength changes were not manifest in the peri-menopausal or the HRT groups where strength remained relatively constant over the duration of testing. Mean strength was higher in the peri-menopausal group than the females of the post-menopausal and HRT groups. Differences in bodyweight may contribute to this, although it is possible that a strength deficit resulting from oestrogen deficiency had not yet affected this group of women who, despite elevated gonadotropin levels, still menstruated. The lower strength scores found during isometric contractions cannot be explained, although differences in strength were scaled in the statistical analysis.

The peri-menopausal females represent a group with unstable hormonal patterns and consequently irregular cycles. Given the reported changes in strength during the menstual cycle (Phillips et al., 1996; Sarwar et al., 1995), these subjects should be tested at standardised times for reliable assessment of muscle function. This group were therefore excluded, and subsequent analyses undertaken between the hypoestrogenic/ hypoprogestogenic post-menopausal women and the age-matched HRT group. Since 3/10 post-menopausal subjects dropped out between 9 and 12 months, the final test session was not analysed. A significant loss of force of the

quadriceps (knee extensors) for isometric and concentric contractions at slow angular velocities (1.05 rad/s) (10.0 and 9.1% respectively) was reported in the postmenopausal group. There were no differences at higher angular velocities (2.09 or 3.13 rad/s), or for the knee flexors and handgrip strength. Maximal strength was stable in the HRT group for all parameters measured.

These findings demonstrate a hormonally-related loss of strength of the quadriceps, at a rate of 9-10% per annum, for zero or slow shortening contractions in women within 1-3 years post-menopause. Recent longitudinal studies assessing muscular performance and hormone status have failed to corroborate these results. In a randomised controlled study, leg strength was measured at 0 and 1.05 rad/s over 11 months in post-menopausal women aged 60 to 72 years. There were no changes in peak torque for either the control group or a HRT group taking the same preparation from the onset of the study (Kohrt et al., 1995). Leg strength was measured at velocities employed in the current study but the age range differed. The subjects in Kohrt's study were considerably older, whereas the target age of females in this study was 51 to 55 years, depending on the age at menopause. The subjects were recruited 1 to 3 years post-menopause since this is the vulnerable age of rapid bone loss which attain a plateau after 5 years (Krolner and Nielson, 1982). The rate of force loss observed in this study is hypothesised to follow the same pattern as the response of bone loss (Krolner and Nielson, 1982). Strength deficits related to the loss of reproductive hormones may have already taken place in 60 to 72 year old subjects. At this age, age-related atrophy would be responsible for further reductions in strength (Grimby and Saltin, 1983).

In a study examining the effects of HRT on muscular performance and balance, Armstrong et al. (1996) failed to detect a loss of leg and handgrip strength over 24 and 48 weeks respectively in post-menopausal women not taking HRT. This age group of 45 to 70 years ranged from middle-aged menopausal women to elderly females. These results were consistent for women taking HRT. Whilst the current findings indicates the time course and rate of strength loss associated with the menopause, supporting the cross-sectional observations, other longitudinal studies do not support these suggestions. The changes in strength following the menopause may be attributable to

muscle atrophy. Armstrong et al. (1996) suggested that declining activity levels are more accountable for strength losses at the menopause rather than hormonal deficiency. If this is the case, there would have been a significant loss of force at higher velocities, particularly since type II fibres are lost more quickly in disuse atrophy. Furthermore, this weakness was not manifest in the HRT or perimenopausal women.

The time-course for muscle weakness has been reported in cross-sectional studies in which a dramatic decline in force/CSA of the AP muscle occurred following the menopause (Phillips et al., 1993b). These findings are supported by Calmels et al. (1995), who demonstrated an accelerated loss in muscle strength of the elbow flexors measured isokinetically at 0.52 and 3.13 rad/s between the 5th and 6th decades. This weakness was associated with a reduction in bone mineral density of the lumbar and femoral regions. The authors also proposed that the strength deficit of the lower limb was not manifest until the 7th and 8th decade conflicting the current findings. Rutherford and Jones (1992) reported a significant decrease in the force/CSA of the quadriceps, where CSA was measured using computed tomography, between the 5th and 6th decades which concur with the results from the longitudinal data.

Consistent findings of an effect of reproductive hormones on muscle strength are mainly derived from studies measuring upper limb, distal muscles. Measurements from lower limb proximal muscles are more conflicting. Bassey et al. (1996) compared muscular performance of the quadriceps and handgrip strength between four representative age-matched group of females with different hormonal status. In this cross-sectional study, strength corrected for covariates age and fat-free mass did not differ between any group. Taafe et al. (1995) used conventional isotonic exercises (e.g. 1 repetition maximum) to assess muscle strength in post-menopausal women and females taking oestrogen replacement therapy (ORT). There were no differences in performance when corrected for body mass between the ORT and non-ORT groups. These subjects were older (7th decade) than those reported previously.

Handgrip is often measured to assess muscle function in upper limbs. The time course of changes in grip strength of males and females over a wide age range has been demonstrated to peak in the third decade, declining linearly thereafter (Kallman et al., 1990). A marked reduction in grip strength has been reported in women after 50 years, around the time of the menopause (Petrofsky et al., 1975; Cauley et al., 1987). Cauley et al. (1987) proposed that exogenous oestrogens delay the age-related loss of grip strength. However, multiple linear regression revealed that height, age and activity levels independently predict handgrip strength. There were no significant differences in handgrip normalised for differences in body size when either two or three groups were considered ($p>0.05$). The inability to standardise hand position may account for the variation in strength over the 12 months (Fig. 4.1.8), although the results from section 3.3 found good day-to-day repeatability in maximal strength of this muscle group.

The mechanism by which reproductive hormones exert their effect is still uncertain, although increasing evidence suggests an inotropic effect through increased force at the cross-bridge. This is supported from work where a rapid stretch is applied to muscle during an isometric contraction. In muscle from aged (Phillips et al., 1991) and ovariectomised mice exhibiting weakness (Phillips et al., 1993b), an increase in the ratio of stretch to isometric force occurs. This has also been demonstrated in the human adductor pollicis muscle of post-menopausal women (Phillips et al., 1993b). During eccentric actions weakness in the elderly was less pronounced than in concentric contractions (Vandervoort et al., 1990). Stretching the muscle is proposed to force all the cross-bridges into a high force state (Lombardi and Piazzesi, 1990). Reproductive hormones are thought to alter the equilibrium of the cross-bridges to the low force state, similarly to the effects of an increase in inorganic phosphate (P_i) (Phillips et al., 1992).

Muscle weakness is absent during lengthening, although force/CSA during isometric and shortening contractions of the soleus and extensor digitorum in aged mice are reduced at the same proportion, independent of shortening velocity (Brooks and Faulkner, 1988). If the effects of ageing and hormonal are of the same aetiology, then strength loss should be manifest in post-menopausal women irrespective of angular

velocity. Concentric contractions of the quadriceps were measured isokinetically at increasing angular velocities of 0, 1.05, 2.09 and 3.13 rad/s. The only significant changes in maximal strength were found for isometric and concentric contractions at 1.05 rad/s. Force declines with increasing angular velocity as a feature of the force-velocity curve. At faster velocities, power is important which is additionally affected by fibre composition. Bassey et al. (1996) compared leg power in women of varying hormonal status and did not report any difference between regularly menstruating, females, those with irregular cycles, women taking HRT and post-menopausal women not taking hormonal supplementation. The authors also failed to detect a difference in absolute strength of the quadriceps and handgrip. Leg extensor power did not change over 24 weeks in hypoestrogenic or hormonally replenished females (Armstrong et al., 1996).

The force-velocity curves were analysed further between treatment groups at baseline and at the last test session. With the exception of isometric contraction, strength declined with increasing velocities for all treatment groups, characteristic of the hyperbolic curve of force-velocity relations described by Hill (Gülch, 1994). There were no significant differences between groups across angular velocity at baseline or at 12 months for absolute or log transformation of strength corrected for body size ($p>0.05$). Isometric strength was measured at 90° of knee flexion. This is not the optimal angle for generating maximal force which is affected by muscle length (Osternig, 1986). Murray et al. (1985) reported peak torque at 60° knee flexion. At the final test, the force-velocity curve was investigated further with the additional variables of isometric (60° of knee flexion) and concentric contractions at 5.22 rad/s. This modified angle for isometric tests generated a greater force than at slower angular velocities. There were no significant differences however, between the three groups across velocities for absolute or normalised srength (Fig. 4.1.20 and 4.1.22).

Angle of peak torque was not measured in this study, although the decreasing strength with higher angular velocities has been postulated to be the result of a larger acceleration period at faster velocities in which it is necessary for the limb to 'catch up' to the predetermined speed of the dynamometer (Osternig, 1986). At high velocities of

muscle shortening, the joint passes the optimal angle for maximal force before contractile component attain maximal tension. The proportion of fast twitch fibres also accounts for ability to develop maximal force at faster velocities (Thortensson et al., 1976). Mean force across velocities decreases for post-menopausal women and increases for HRT females when strength is corrected for differences in body size at baseline and at 12 months (Fig. 4.1.20 and 4.1.22). These differences are not significant, but are marked at the end of the study in concert with the loss of strength across all angular velocities.

Standardised force-velocity relations were calculated to compare the relative rate of force loss with angular velocity across time and between groups. Force was expressed as a percentage of isometric force. Speed-dependent loss of force is reported to accelerate in elderly women (Harries and Bassey, 1990) and in both sexes (Aniansson et al., 1983; Laforest et al., 1990) across increasing angular velocities. There were no significant effects of hormonal changes on the standardised force-velocity relationship. However, in post-menopausal women (Fig 4.1.24) the relative force at 9 months was greater than at baseline at higher angular velocities probably because force was maintained at higher angular velocities while isometric force and concentric force at 1.05 rad/s decreased over time.

There were no significant differences in standardised force-velocity relations between the HRT and post-menopausal group, although it appears that the HRT group were able to generate greater force at increasing angular velocities relative to isometric force. This suggests that force was reduced at a greater rate with speed in the post-menopausal groups at both time points. Since this did not reach statistical significance, it appears that forces at higher angular velocities were not affected by changes in hormonal status.

Maximal strength of the quadriceps at slow angular velocities reduced significantly over 12 months in hypoestrogenic post-menopausal women. The deficiency in reproductive hormones at the menopause is associated with this strength loss. The stability of force production in females taking hormone replacement therapy

demonstrated the efficacy of this treatment as a prophylaxis to muscle weakness. Thus, in women where HRT is contraindicated consideration should be given to alternative treatment, such as the implementation of exercise protocols.

4.2. THE RELATIONSHIP BETWEEN REPRODUCTIVE HORMONES AND MUSCLE FUNCTION OF THE QUADRICEPS AND FIRST DORSAL INTEROSSEUS (FDI) DURING THE MENSTRUAL CYCLE IN YOUNG, HEALTHY FEMALES

4.2.1. Introduction

The results from study 4.1 revealed a significant loss in force of the quadriceps over 1 year in hypoestrogenic post-menopausal women. The reduction in maximal strength for isometric and slow velocities of shortening (1.05 rad/s) at 9 to 10% per annum was not evident at faster angular velocities. These findings suggest that hormonal losses resulting in the menopause are implicated in this reduction in force, supported by the observations that females taking HRT do not exhibit this weakness.

It is apparent therefore that the quadriceps are responsive to the chronic reduction in endogenous oestrogen and/or progesterone. The loss of reproductive hormones at the menopause does not occur rapidly, but can take up to 10 years from the onset of the climacteric to attain baseline post-menopausal hormonal levels. It is unknown if this response to oestrogen and progesterone deficiency is rapid, or is a prolonged adjustment. The ~10% loss in force production was found within 3 years postmenopause. To assess the sensitivity of muscle to changes in oestrogen and the loss of progesterone, acute changes in these hormones across the menstrual cycle will be examined in young females. Subjects will be monitored repeatedly across the cycle thus controlling for differences in muscle size.

The menstrual cycle is a ciracmensal rhythm characterised by fluctuations in reproductive hormones, regulated by pituitary gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH). A comparison of the sensitivity of a large muscle group, the quadriceps to a small muscle, the first dorsal interosseus will be undertaken. The hormonally-related force loss found in study 4.1 was not evident at increasing velocities of shortening in post-menopausal women. Previous studies examining strength across the menstrual cycle have failed to detect changes in dynamic

contractions at 2.09 and 3.13 rad/s (Dibrezzo et al., 1991; Richardson and George, 1993). Muscle function - maximal voluntary contraction (MVC), contractility of the quadriceps through delivery of increasing frequencies of electrically stimulated contractions, and fatigue resistance will therefore be examined isometrically.

The changes in maximal voluntary contraction (MVC) during the menstrual cycle has been investigated in the adductor pollicis (AP) (Phillips et al., 1996) and the quadriceps muscle group (Sarwar et al., 1995). Sarwar et al. (1995) reported peak strength of the quadriceps at mid-cycle which was attributed to the high oestrogen levels prior to the LH surge. Without hormone samples and frequent strength measurements, these conclusions are based on conjecture. Phillips et al. (1996) fulfilled these recommendations and found that the peak in force of the AP muscle was followed by a rapid loss observed around mid-cycle. These strength changes were not correlated with circulating oestrogen levels, although this hormone was attributed to the loss of force observed. In this study, oestrogen, progesterone, FSH and LH levels will be measured and related to muscle function. These hormones will also be used as a marker for accurate comparisons in muscle strength between subjects. The assessment of muscle function of the leg will be performed over seven separate measurements, and ten measures of maximal strength of the FDI will be made.

The aims of the study were to:

1] Examine changes in maximal voluntary contraction of the quadriceps and FDI across the cycle where frequent measurements were made.

2] Investigate contractile properties of the quadriceps during the menstrual cycle.

3] Assess the fatiguability of the quadriceps across different phases.

These aims will be fulfilled through the following hypotheses:

Hypothesis 4: Maximal strength of the quadriceps and FDI is greater when oestrogen concentrations are at their highest.

Hypothesis 5: Changes in hormone levels result in a shift of the force-frequency curve.

Hypothesis 6: The quadriceps are least fatiguable when progesterone levels are high.

4.2.2 Methods

(i) Subjects

Young, healthy active females were recruited for the study. They were subdivided into two groups of ten subjects; the first group consisted of 10 women (age - 26.6 [5.48] range 19-34 years: weight - 59.9 [9.13] kgs; height - 1.62 [0.07] metres) (mean ± standard deviation) who were not taking any form of hormonal treatment. This non-oral contraceptive (non-OC) group had cycle lengths of 28-35 days as determined from self-report and temperature charts recorded from the preceding cycle (see Appendix 3.0). The control group (n=10) (age - 26.5 [4.77]; weight - 61.1 [9.94] kgs; height - 1.66 [0.06] metres) (mean ± sd) were taking combined oral contraceptive (n=7) or triphasic preparations (n=3). Details of the oral contraceptive (OC) preparations are given below (Table 4.2.1). Subjects gave written informed consent and were excluded if they experienced pain or injury to the lower limb or hand.

Table 4.2.1: *Oral contraceptive preparations of the control (OC) group.*

Preparation	Oestrogen (mcg)	Progestogen (mg)
Combined		
Loestrin	Ethinyloestradiol 20	Noresthisterone acetate* 1
Cilest (x2)	Ethinyloestradiol 35	Norgestimate 0.25
Femodene (x2)	Ethinyloestradiol 30	Gestodene 0.075
Marvelon	Ethinyloestradiol 30	Desogestel 0.15
Brevinor	Ethinyloestradiol 35	Norethisterone 0.5
Triphasic†		
Trinordiol	Ethinyloestradiol 30 (6 days)	Levonorgestrel (6 days) 0.05
Logynon (x2)	40 (5 days)	(5 days) 0.075
	30 (10 days)	(10 days) 0.125

** Converted (>90%) to norethisterone as the active metabolite*

† Both triphasic pills have the same formulation

(ii) Procedure

4.2.2.1. Blood measurements

Blood samples from the antecubital vein were taken on five visits and sampled for progesterone, oestrogen, follicle stimulating hormone (FSH), luteinizing hormone (LH). These hormones were assayed at The Royal Liverpool Hospital. These results were used to confirm cycle phase. All samples were taken at the same time of day for each subjects. According to a 28 day cycle, the phases were divided into: menses (days 1-3); mid-follicular (MF-days 7-9); pre-LH peak (PreLH-day 12, or 3 hours prior to ovulation); LH peak (LH-day 14); post LH peak (PoLH-days 16-17); mid-luteal (ML-days 21-23) and late-luteal (LL-days 25-28). Blood samples were taken at menses, preLH, LH peak, post-LH peak and mid-luteal. The oral contraceptive (OC) users were tested on corresponding days throughout their cycle. A blood sample was taken at midfollicular instead of menses which represented the seventh pill-free day. Menses corresponded to the first day off the pill, and mid-follicular as day 7, the last pill-free day.

Leg measurements - Seven strength measurements were made across the cycle. Cycle phases were estimated from the midcycle LH peak, assisted with oral temperature charts recorded from the preceding cycle, and estimated from ovulation - 14 days prior to menstruation.

First dorsal interosseus - Strength measurements of the FDI were undertaken at the same time as the quadriceps. An extra four sessions were also performed on day 4 (between menses and mid-follicular), day 10 (before pre-ovulation), day 19/20 (prior to mid-luteal) and day 26.

4.2.2.2. Maximal voluntary contraction

Maximal voluntary contractions (MVC) of the quadriceps and FDI were measured using a strain gauge. A 5 min warm-up on a cycle ergometer (Monark) at 55 rev/min preceded strength measurements of the leg, and 10 minutes immersion in hot water increased blood flow to the FDI. The best of three MVCs were recorded. The protocol for the leg and FDI are detailed in Chapter 3.3 and 3.4 respectively.

4.2.2.3. Contractile properties

The contractile properties of the leg in fresh and fatigued muscle were measured from electrically stimulated contractions delivered at 1, 10, 20, 50 and 100 Hz. The ratio between 20 and 50 Hz were used as an index of the force-frequency curve.

4.2.2.4. Fatigue resistance

Fatiguability of the quadriceps was assessed using the protocol, adapted from Burke (1973), described in Chapter 3.3. The muscle was stimulated with 40 Hz impulses for 1s intervals over 3 min. This method proved more effective inducing fatigue compared with the protocol of 3 s stimulation.

(iii) Data analysis

A repeated measures ANOVA (repeated factor of visit) was employed to detect differences between phases of all varaiables for the experimental non-OC and oral contraceptive (OC) users. Missing data post-LH peak were interpolated when analysis was undertaken across all phases. Analysis was also performed across six phases (minus postovulation). A post-hoc test (Tukey's) was used to identify differences found from the ANOVA. Measurements of the OC group were also analysed in this way, with combined and triphasic pill users pooled. They were also analysed separately to compare the different preparations on muscle function.

4.2.3 Results

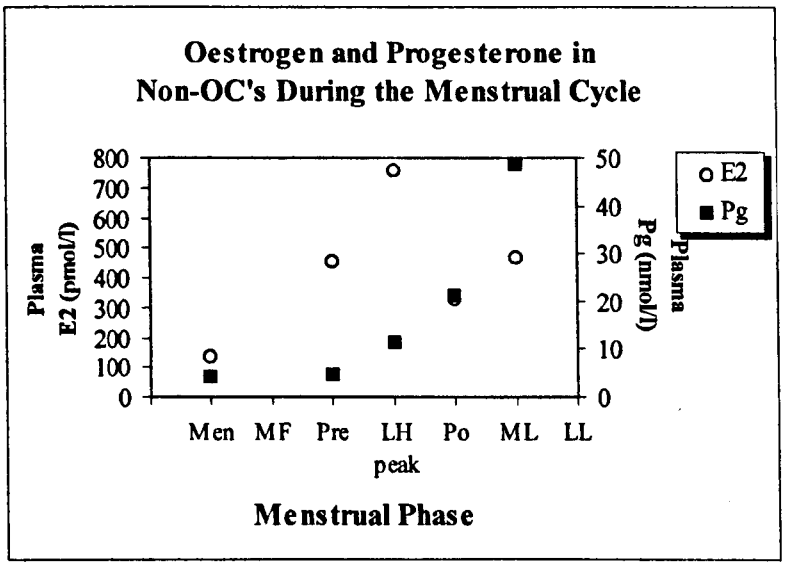
4.2.3.1. Hormone levels across the menstrual cycle

The reproductive hormones oestrogen and progesterone, and gonadotropins LH and FSH were measured at 5 time points across the menstrual cycle. The characteristics of these hormones are described below.

4.2.3.1.1. Reproductive hormones

Oestrogen (17β-oestradiol) and progesterone levels in non-OC users are shown in Fig. 4.2.1. Values are missing at phases where samples were not taken. Oestrogen increased during the follicular phase and peaked mid-cycle (marked as the LH peak) which is consistent with high oestrogen concentrations inducing the LH surge. Subsequently, oestrogen decreased rapidly (within 1-2 days) in concert with ovulation. Oestrogen then increased mid-luteal but does not return to the same levels of the follicular phase. Progesterone levels were low during the early and late follicular phase. A gradual increase was then observed with the LH peak which sharply increased thereafter. The peak in progesterone occurred mid-luteal, which is consistent with ovulation (levels >35 nmol/l).

In the OC group, hormone levels were measured on the seventh pill-free day rather than day 1 as with the non-OC group. Oestrogen and progesterone levels remained low due to the suppression of the hypothalamic-pituitary-ovarian axis. Oestrogen levels were slightly higher in the first phase in response to the relief of the inhibitory effects of the exogenous hormones and subsequent increases in FSH. (Fig. 4.2.1). The ratios of oestrogen to progesterone were calculated for individual values and are plotted in Fig. 4.2.3.



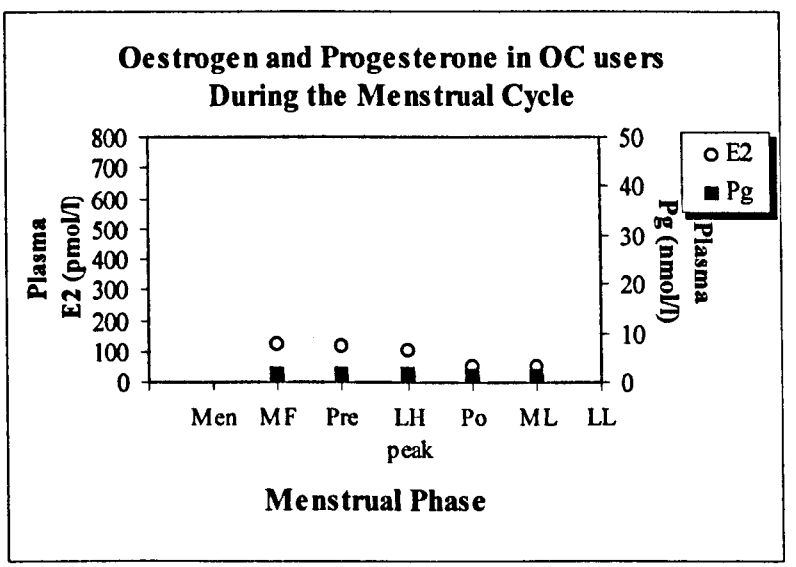
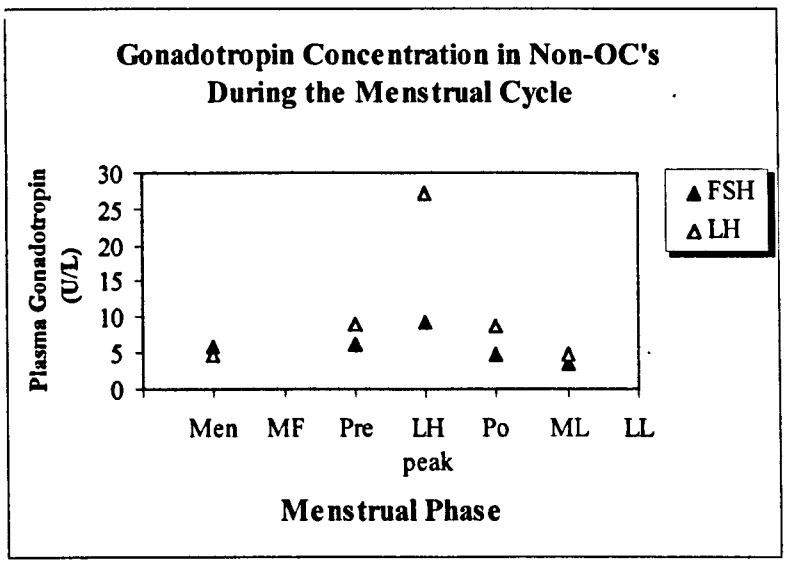


Fig. 4.2.1: Relationship between oestrogen and progesterone during the reproductive cycle, comparing non-OC and OC-users. Men = menses; MF = mid-follicular; Pre = pre-luteinizing hormone (LH) peak; LH peak; Po = post-LH peak; ML = mid-luteal; LL = late-luteal.

4.2.3.1.2. Gonadotropins

The gonadotropin concentrations in non-OC and OC users are shown in Fig. 4.2.2. The cyclical changes in FSH and LH in ovulating females are indicated by the rise in these hormones midcycle. Follicle stimulating hormone increases steadily during the follicular phase, with a modest peak in concert with the surge in LH. This LH surge induces ovulation. Both gonadotropins decreased thereafter.



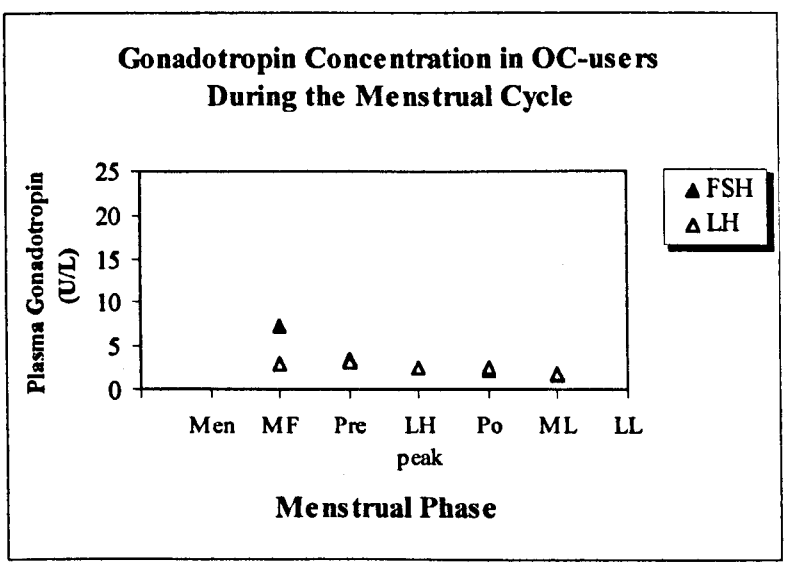


Fig. 4.2.2: Relationship between follicle stimulating hormone (FSH) and luteinizing hormone (LH) in non oral contraceptive (non-OC) and OC-users during the menstrual cycle. Men = menses; MF = mid-follicular; Pre = pre-luteinizing hormone (LH) peak; LH peak; Po = post-LH peak; ML = mid-luteal; LL = late-luteal.

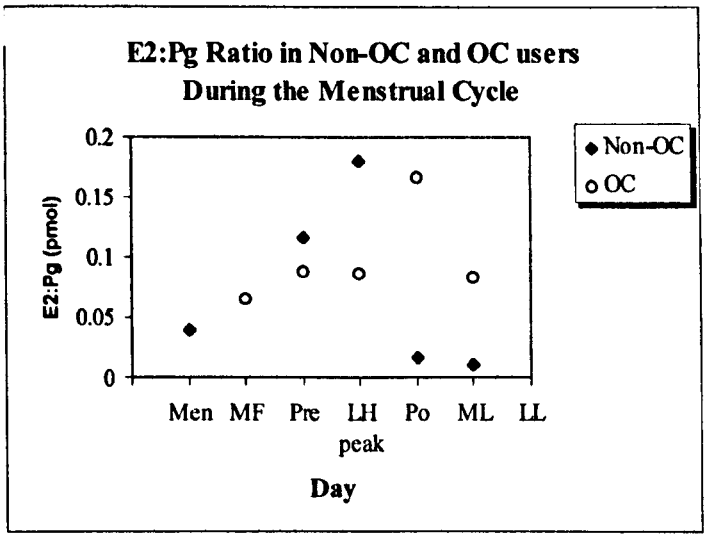


Fig. 4.2.3. The ratio of oestrogen to progesterone in non-OC and OC users. Men = menses; MF = mid-follicular; Pre = pre-luteinizing hormone (LH) peak; LH peak; Po = post-LH peak; ML = mid-luteal; LL = late-luteal.

4.2.3.2. Maximal voluntary strength of the quadriceps

4.2.3.2.1. Absolute force

In the non-OC group, significant changes in maximal voluntary force of the quadriceps were found across the cycle for all phases (x7) ($F_{6,48}$=3.48: $p<0.05$) and across 6 phases ($F_{5,40}$= 4.20: $p<0.05$); the post-LH peak phase was excluded due to a greater number of missing values which were interpolated. Muscle strength was lowest pre-LH peak and increased by 12% at midluteal (Fig.4.2.4). The post-hoc test revealed that menses, mid-follicular, mid-luteal and late-luteal phases were significantly different from pre-LH peak ($p<0.01$). There were also significant changes in strength measured at the LH peak from mid-follicular, late-luteal ($p<0.05$), and mid-luteal ($p<0.01$).

Force production of the quadriceps did not change significantly across the cycle for the OC group ($p>0.05$). Results of the OC group were also analysed separately according to contraceptive preparation (Fig. 4.2.5). There were no significant changes in strength of the combined OC group ($F_{9,54}$=0.6: $p>0.05$). In the triphasic group there was a low number of subjects (n=3), hence too few degrees of freedom in the residual error calculation. The results from both groups were therefore pooled for each variable analysed.

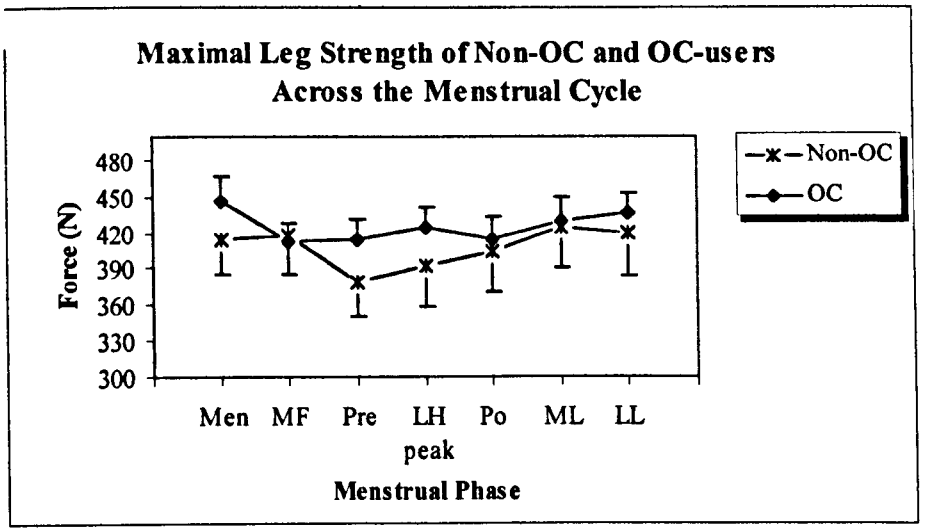


Fig. 4.2.4: Comparison of mean (±SE) maximal leg strength across the menstrual cycle of non-OC and OC-users. Men = menses; MF = mid-follicular; Pre = pre-LH peak; Ov = ovulation; Po = post-LH peak; ML = mid-luteal; LL = late-luteal.

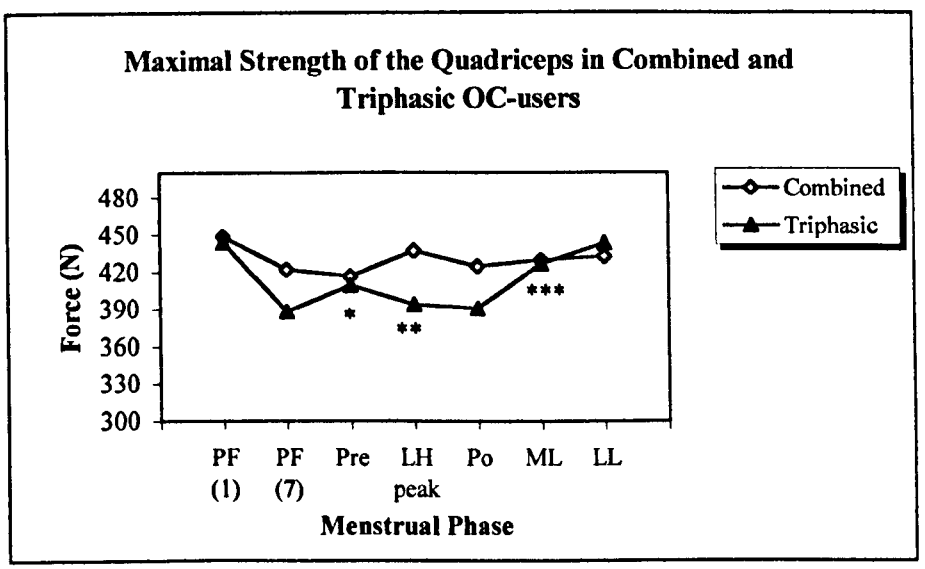


*Fig. 4.2.5: Comparison of maximal leg strength between combined and triphasic OC-users. The two first data points represent pill free days (PF). Pre = Pre-LH peak; LH peak; Po = post-LH peak; ML = mid-luteal; LL = late-luteal - phases which correspond to the reproductive events in non-OC subjects. * = low oestrogen/progestogen; ** = high oestrogen/medium progestogen; *** = low dose oestrogen/high progestogen components of triphasic preparations (Table 4.2.1).*

4.2.3.2.2. Relative force

Muscle force was expressed relative to the mean of all measurements for each subject so that proportional changes in strength could be examined (Phillips et al., 1996) (Fig. 4.2.6). The changes in relative force were significantly different over the 7 time points in the non-C group ($F_{6,48}$ = 2.06; $p<0.05$), but not in the OC subjects ($F_{5,54}$ = 1.35; $p>0.05$).

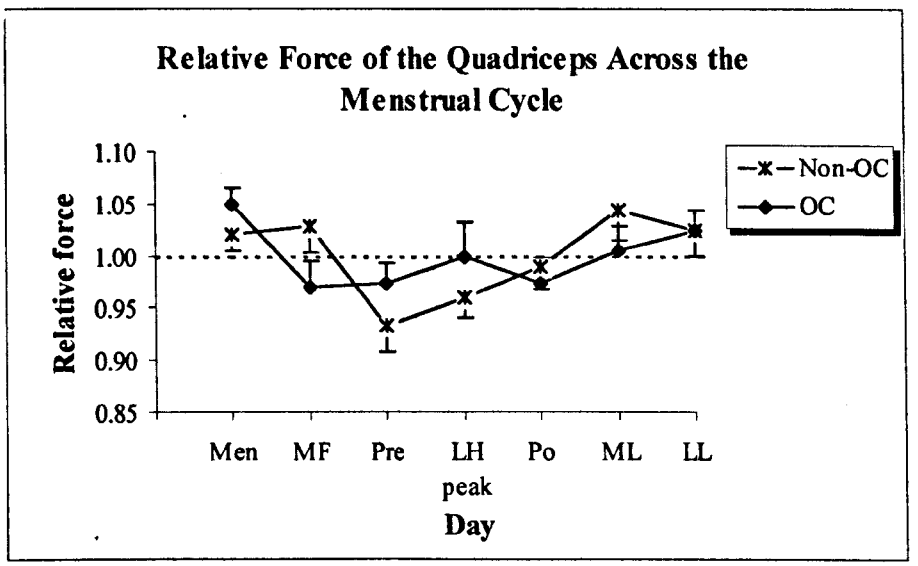


Fig. 4.2.6: Mean (±SE) relative muscle strength of the quadriceps in non-OC and OC users across the menstrual cycle. Force is expressed relative to the mean of all measurements (dashed line) for that subject.

4.2.3.3. The relationship between relative muscle force and hormonal patterns

The relationship between the relative force of the quadriceps and hormones governing the menstrual cycle were explored to determine if hormonal concentrations are related to the changes in strength. Correlations were performed between strength and hormone levels measured on the same occasion for individual subjects across the cycle. There were no significant correlations between force and oestrogen (r = -0.264) (Fig. 4.2.7), LH (r = -0.255) or FSH (r = -0.125). There was a significant positive correlation between relative force and progesterone (r = 0.330; $p<0.05$) (Fig. 4.2.8) and a negative correlation with the ratio of oestrogen:progesterone (r = -0.416; $p<0.05$) (Fig. 4.2.9).

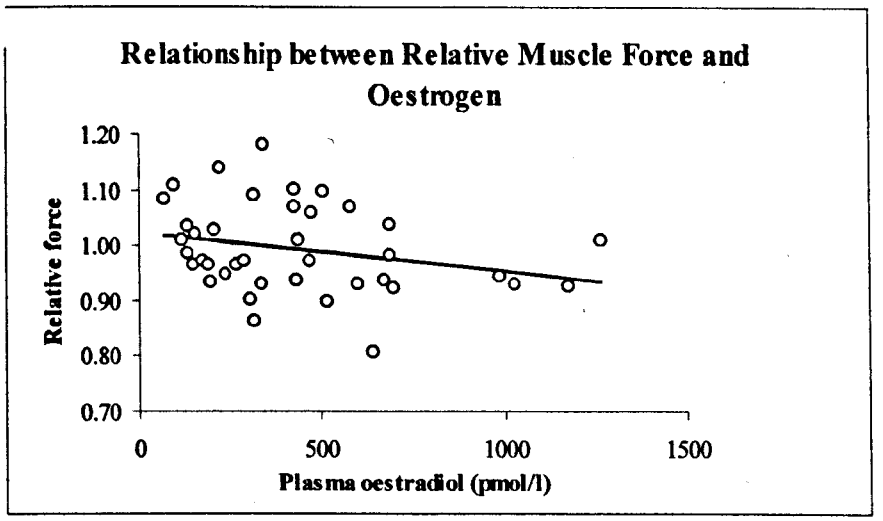


Fig. 4.2.7: The relation between relative muscle force and oestrogen concentrations for individual subjects across the menstrual cycle when measured at the same time. The solid black line represents the regression line.

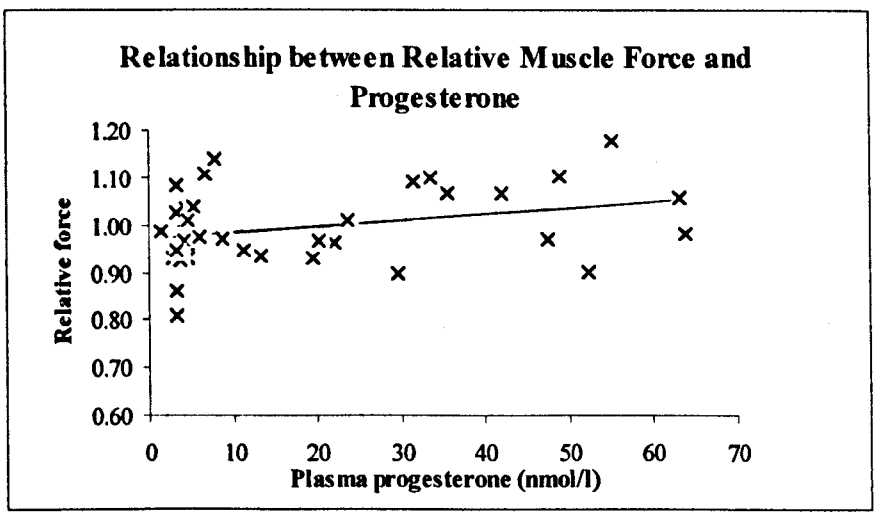


Fig. 4.2.8: The relation between relative muscle force and progesterone concentration for individual subjects across the menstrual cycle when measured at the same time. The solid black line represents the regression line.

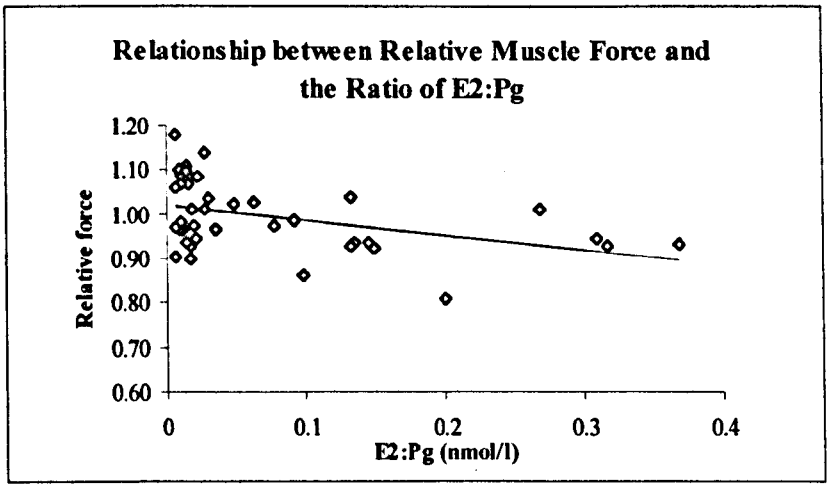


Fig. 4.2.9: The relation between relative muscle force and the oestrogen:progesterone ratio (E2:Pg) for individual subjects across the menstrual cycle when measured at the same time. The solid black line represents the regression line.

4.2.3.4. Maximal voluntary strength of the first dorsal interosseus (FDI)

4.2.3.4.1. Absolute force

The pattern of force production for the FDI differed from that of the quadriceps across the menstrual cycle in both groups (Fig. 4.2.10). Further, there were no significant changes in maximal strength of the non-OC group over 10 measurements ($F_{9,72}$=1.15: $p>0.05$) or between the 7 main phases ($F_{6,48}$=1.37: $p>0.05$). The strength of the FDI was greater in the non-OC group although there were no significant differences between groups for all measurements made ($F_{1,17}$=0.28: $p>0.05$). Maximal force of the FDI in the OC group did not change significantly across the cycle ($p>0.05$). Differences in mean maximal strength between combined and triphasic subjects are shown in Fig. 4.2.11.

4.2.3.4.2. Relative force of the FDI

The relative force of the FDI in non-OC and OC users is plotted in Fig. 4.2.12. There were no significant differences when proportional changes in strength were considered for the non-OC ($F_{9,72}$ = 1.25) and OC ($F_{9,81}$ = 0.31) groups ($p>0.05$) across 10 sessions. The relative force of the OC group, subdivided into combined and triphasic groups, is shown in Fig. 4.2.13.

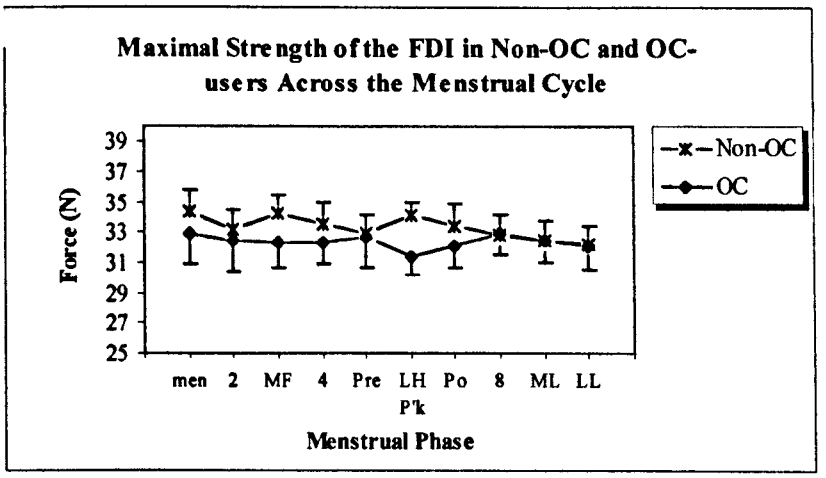


Fig. 4.2.10: Comparison of mean (±SE) maximal strength of the first dorsal interosseus muscle (FDI) across the menstrual cycle of non-OC and OC-users. men = menses; MF = mid-follicular; Pre = pre-LH peak; LH peak; Po = post-LH peak; ML = mid-luteal; LL = late-luteal.

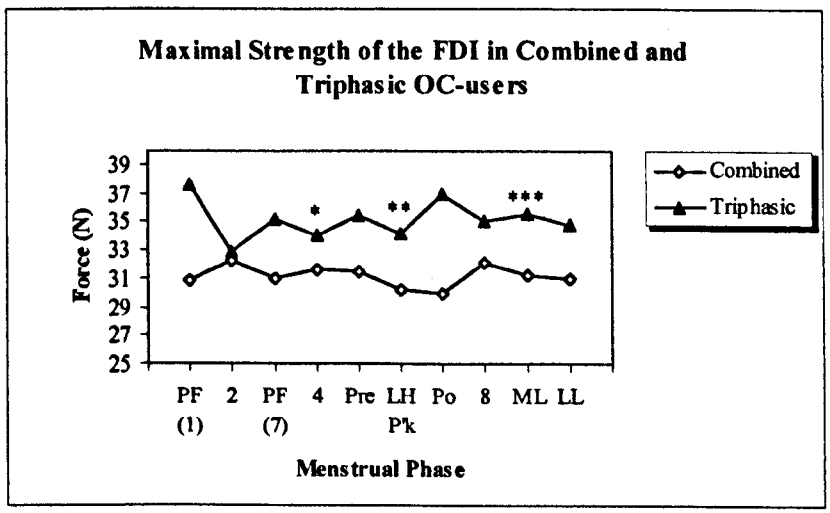


*Fig. 4.2.11: Comparison of maximal strength of the first dorsal interosseus muscle (FDI) between combined and triphasic OC-users. The two first data points represent pill free days (PF). men = menses; MF = mid-follicular; Pre = pre-LH peak; LH peak; Po = post-LH peak; ML = mid-luteal; LL = late luteal. * = low oestrogen/progestogen; ** = high oestrogen/medium progestogen; *** = low dose oestrogen/high progestogen components of triphasic preparations (Table 4.2.1).*

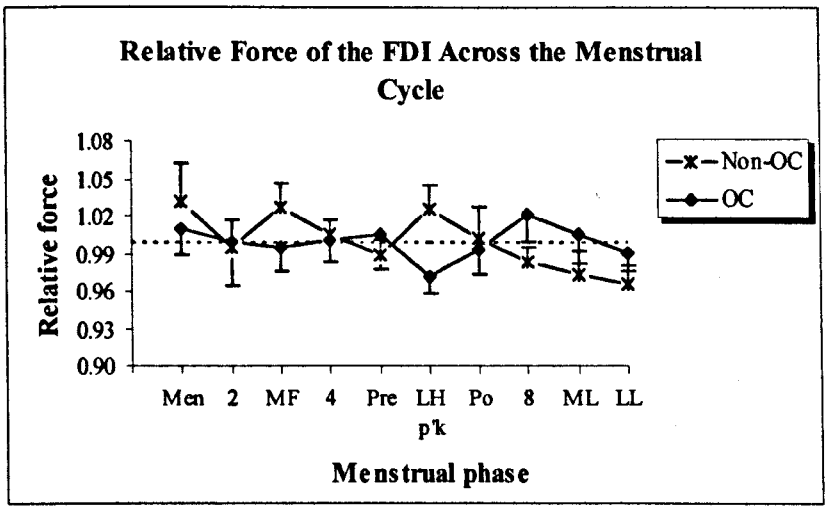


Fig. 4.2.12: Mean (±SE) relative muscle strength of the FDI in non-OC and OC users across the menstrual cycle. Force is expressed relative to the mean of all measurements (dashed line) for that subject.

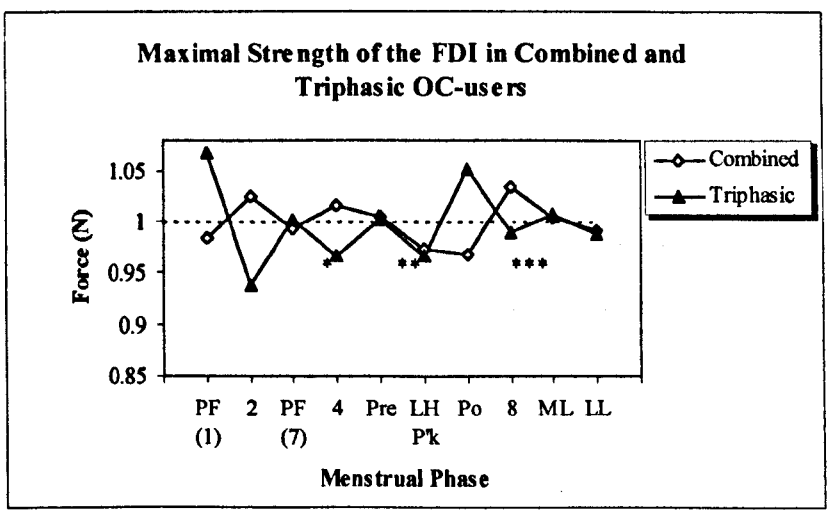


Fig. 4.2.13: Comparison of maximal strength of the first dorsal interosseus muscle (FDI) between combined and triphasic OC-users. The two first data points represent pill-free days (PF).

4.2.3.5. Contractile properties of the quadriceps

4.2.3.5.1. 20/50% - Fresh muscle

The ANOVA did not reveal any trend in the 20/50% ratio across the menstrual cycle in the non-OC group ($F_{6,48}$=2.15: p>0.05). There were no changes between phases for the OC group ($F_{6,54}$=1.32: p>0.05). The mean (±SE) ratio across the cycle between the two groups is shown in Fig 4.2.14.

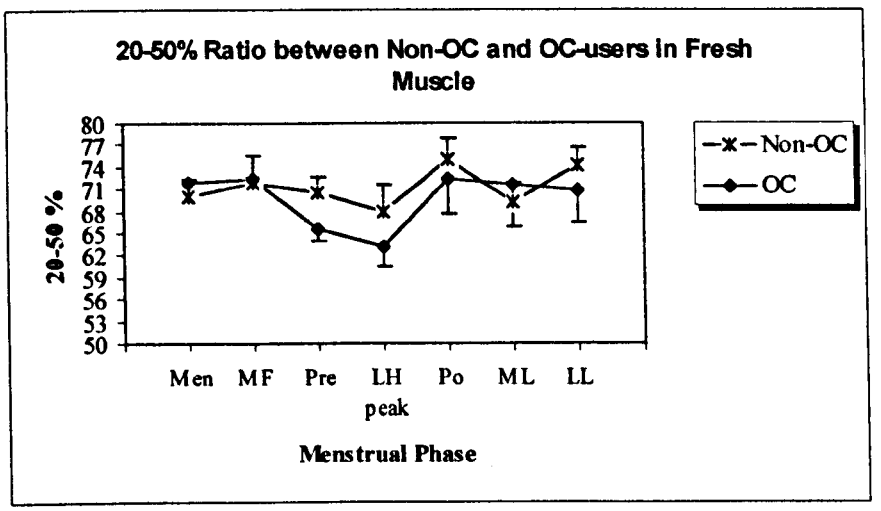


Fig. 4.2.14: A comparison of the mean (±SE) 20-50% ratio between non-OC and OC-users measured in fresh muscle across the menstrual cycle. The forces generated at 20 Hz was expressed as a percentage of force at 50 Hz, calculated from a train of increasing frequencies of electrical stimulation.

4.2.3.5.2. 20-50% - Fatigued muscle

The 20-50% ratio in fatigued muscle did not change significantly across all phases tested for the non-OC ($F_{6,48}$=1.15: p>0.05) and OC groups ($F_{6,54}$=1.33: p>0.05). The 20-50% ratio in non-OC and OC users is shown in Fig 4.2.15. A comparison of this ratio between fresh and fatigued muscle is illustrated in Fig 4.2.16 and 4.2.17 for the non-OC and OC groups respectively. In non-OC's, the ratio was higher in fresh muscle across all phases compared with fatigued muscle. There was greater variability in the OC-users, with a mid-cycle decrease in mean 20-50% ratio.

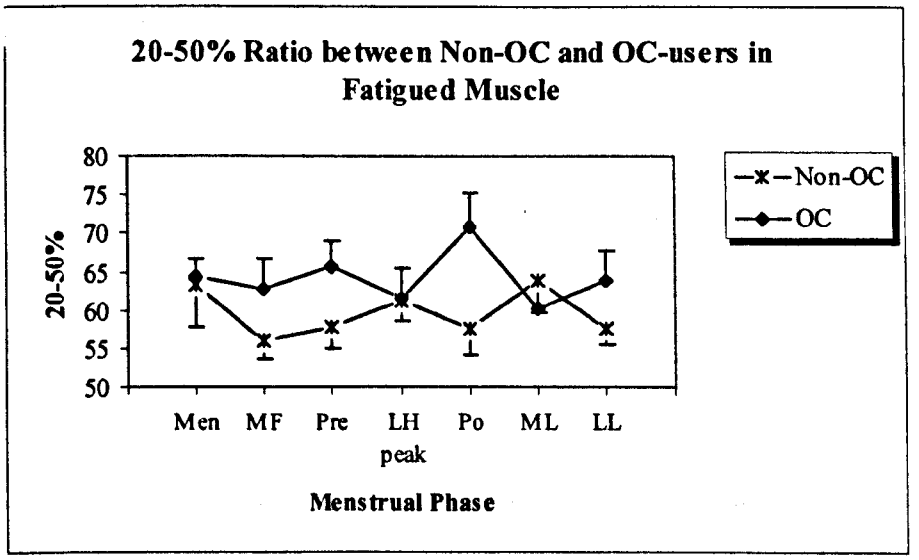


Fig. 4.2.15: A comparison of the mean (±SE) 20-50% ratio between non-OC and OC-users measured in fatigued muscle across the menstrual cycle. The forces generated at 20 Hz was expressed as a percentage of force at 50 Hz, calculated from a train of increasing frequencies of electrical stimulation.

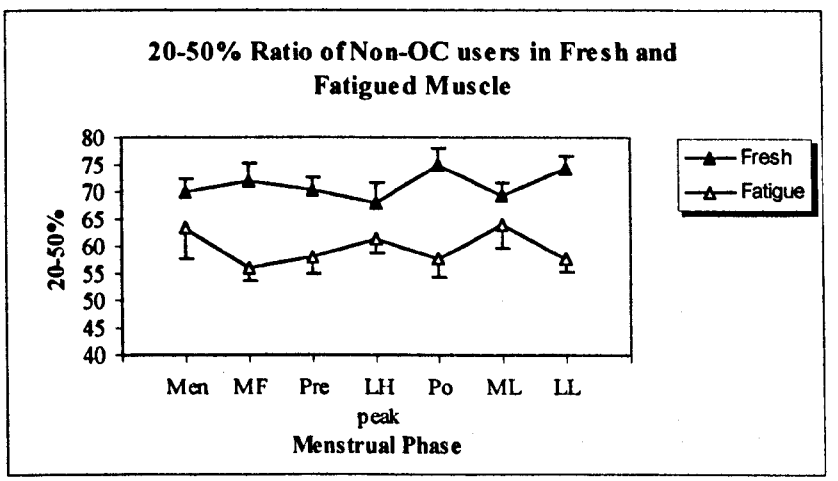


Fig. 4.2.16: A comparison of the mean (±SE) 20-50% ratio in fresh and fatigued muscle of the non-OC group. The forces generated at 20 Hz was expressed as a percentage of force at 50 Hz, calculated from a train of increasing frequencies of electrical stimulation.

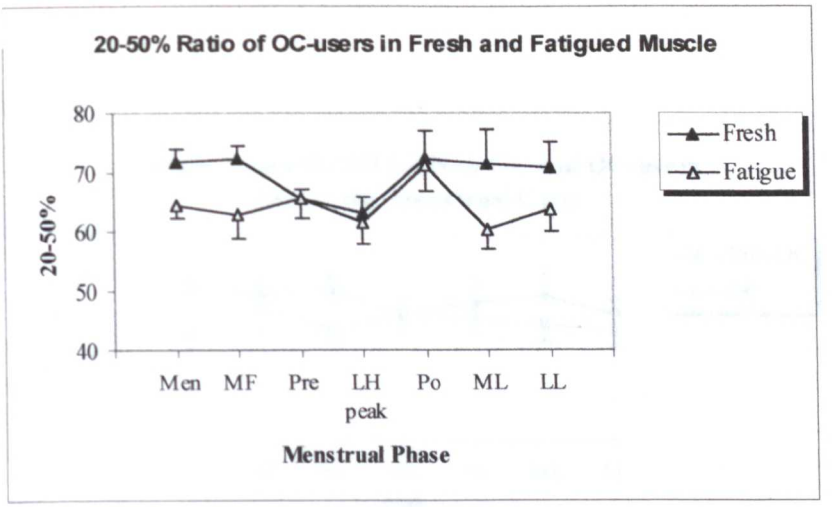


Fig. 4.2.17: A comparison of the mean (±SE) 20-50% ratio in fresh and fatigued muscle of the OC group. The forces generated at 20 Hz was expressed as a percentage of force at 50 Hz, calculated from a train of increasing frequencies of electrical stimulation.

4.2.3.6. Fatigue Index (%)

There were no significant differences in peak tension of the non-OC ($F_{6,48}$=1.13: p>0.05) or OC ($F_{6,54}$=0.57: p>0.05) groups across seven phases of the menstrual cycle. Mean values of peak tension in relation to oestrogen is shown in Fig. 4.2.13. Changes in mean tension of the non-OC and OC groups are shown in Fig. 4.2.19.

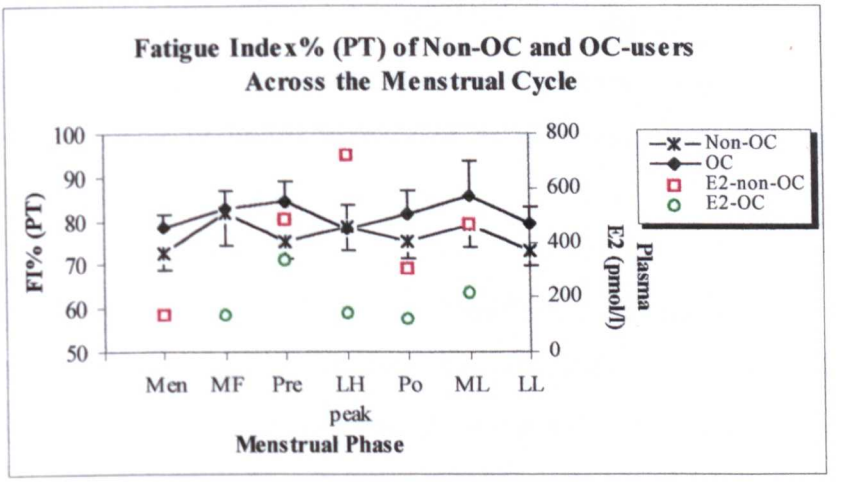


Fig. 4.2.18: Fatigue index (%) of peak tension (PT) in non-OC and OC across the menstrual cycle. Plasma oestradiol (E2) level values of each group are superimposed to compared the pattern of fatigue between phases with circulating oestrogen.

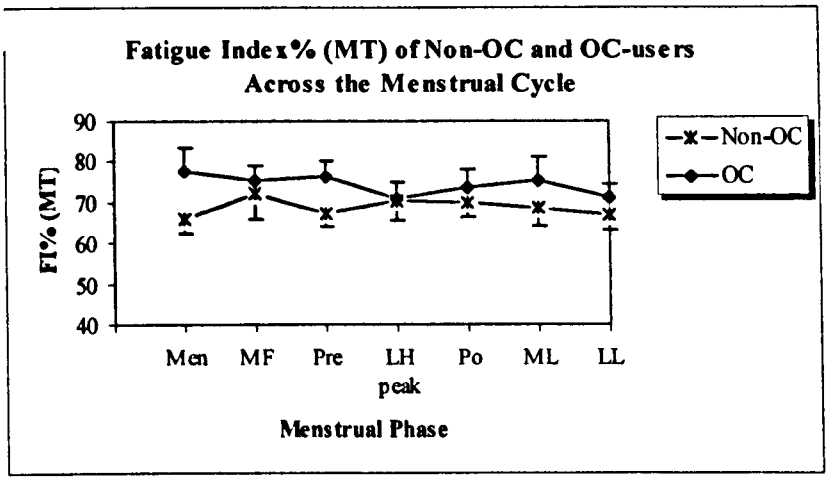


Fig. 4.2.19: Fatigue index (%) of mean tension (MT) in non-OC and OC across the menstrual cycle. Mean tension represents the mean force of the tetanic contraction.

4.2.4. Discussion

Maximal force of the quadriceps in females not taking oral contraceptive preparations clearly showed cyclical changes when measured across the menstrual cycle. Strength was analysed a] across 6 time points of the cycle with all absolute scores and b] at 7 time points with values interpolated at one phase due to missing data. These time points were ascertained according to predicted changes in hormones which were retrospectively confirmed with plasma hormonal levels taken at the time of strength measurements. The mid-cycle LH peak was used as a reference point for a physiological marker to synchronise cycles. Maximal force was also correlated with hormones for individual data when both strength and hormone samples were obtained.

When each time point was considered, significant changes in force of the quadriceps were detected across the cycle. Force was stable during early-mid follicular, but droped dramatically 1 to 3 days prior to the LH peak. The speed of this force loss was not realised here since 5 to 6 days elapsed between mid-follicular and pre-LH peak measurements. A steady increase in strength occurred thereafter, reaching its peak mid-luteal. Maximal force mid-luteal was greater than the force at the mid-follicular phase. Significant differences were confirmed between the force generated pre-LH peak and menses, mid-follicular, mid-luteal and late-luteal phases. There were also significant changes with strength measured at the LH peak the mid-follicular, mid-luteal and late-luteal phases.

In the OC group, there were no significant changes in the strength of the quadriceps. There was a notable decrease from menses to mid-follicular, during the pill-free week although this did not influence the overall performance in strength. The results from the non-OC group, however, do not agree with the strength changes reported in past studies when measurements were made 2 to 3 times during the cycle for handgrip (Wirth and Lohman, 1982; Davies et al., 1991) or leg strength (Dibrezzo et al., 1991; Richardson and George, 1993) or when more detailed measurements were undertaken (Sarwar et al., 1995; Phillips et al., 1996).

The concept that hormones influence physical performance has received much attention over the past decades. However, the variability in protocols of testing and definitions of cycle phases have precluded the comparison of results between studies with consequential inconclusive findings. For example, greatest strength has been reported for handgrip during the follicular compared with the luteal phase (Wirth and Lohman, 1982), and at menses compared with the follicular and luteal phase (Davies et al., 1991). No differences in grip strength were reported when measured at the premenstrual, menses, postmenses and mid-cycle phases (Allen and Bailey, 1982) or between pre-ovulatory and luteal phases (Petrofsky et al., 1976). Sarwar et al. (1995) tested isometric leg strength and handgrip five times across the cycle in regularly menstruating females. Measurements were taken early follicular (days 1-7), mid-follicular, mid-cycle (days 7-12), mid-luteal and late-luteal. Maximal strength, confirmed in the quadriceps using superimposed electrical impulses, was highest mid-cycle compared with the other phases measured. The greatest difference of 11% occurred with the late luteal phase. Mid-cycle in their study corresponded with ovulation, estimated from temperature charts and counting back 14 days from onset of next menses. These findings support the preliminary results of Phillips et al. (1993a) who reported a 20% increase in force of the adductor pollicis (AP) muscle from day 1 to day 14 of the cycle. There were no significant differences between day 1 and 21. Oestrogen, a predominant hormone during the follicular phase, undergoes rapid short-term changes in production mid-cycle as a result of the feedback effects of the gonadotropins secreted at this time. Oestrogen peaks prior to the LH surge (Ojeda, 1992) and declines to its lowest 2 to 3 days later in concert with ovulation. These changes in oestrogen can therefore be easily miscalculated. Recently Phillips et al. (1996) reported a marked reduction in force to its lowest level, 1-2 days after the preovulatory peak in strength, coinciding with ovulation. These results support the strengthening role of oestrogen.

In the present study the time course of strength changes were also examined in greater detail, focusing on the mid-cycle hormonal fluctuations. However, in contrast to Phillips et al. (1996), weakest strength measures of the quadriceps were found 1 to 2

days prior to the LH peak, with peak MVC observed mid-luteal (days 20-22). The higher force values at this point were not significantly different to the follicular phase.

Unless hormonal measures are undertaken, any endocrine effect on the changes in strength are based on conjecture. To examine the role of the cyclical hormones further, relative force values (to mean measurements across cycle) of the quadriceps were correlated with plasma oestrogen (17-β oestradiol), progesterone, FSH and LH concentrations measured on the same occasion. There was no significant relationship between strength and circulating oestrogen levels, although a negative tendency was observed (Fig. 4.2.7). A significant negative correlation between oestrogen and isometric handgrip strength has been reported recently (Bassey et al., 1995) and has been implicated in previous studies (Davies et al., 1991; Wirth and Lohman, 1982). Strength of the biceps brachii, triceps surae and handgrip were measured in nine eumenorrheic females 8 times over 4 weeks. A 5% fall in handgrip strength for a 200 pmol/l increase in oestrogen was documented (Bassey et al., 1995). Rice et al. (1988) undertook a battery of strength tests and correlated performances during days 1 to 5 with oestradiol levels. No significant relationship was found, although measurements were made at only one phase.

Although Phillips et al. (1996) outlined the role of oestrogen in enhancing force production they were unable to detect a significant association between relative force of the adductor pollicis muscle and plasma oestradiol when measured around ovulation. They attributed this to a time lag between the rapid changes in force and the effects of oestrogen. The results from the present study do not support these indications. A significant relationship was detected between relative strength of the quadriceps and progesterone levels and it is believed that this hormone is linked with the increase in force production. There was an increase in strength from the pre-LH peak to the mid-luteal phase which corresponded to rising progesterone levels. Previous studies investigating strength across the menstrual cycle have not measured progesterone levels. A further noteworthy observation was a significant negative relationship between relative force and the ratio of oestrogen:progesterone. There was no relationship between strength and FSH or LH.

The possibility of progesterone influencing force production of skeletal muscle is not implausible. There is increasing evidence that progesterone is actively involved in bone metabolism (Prior, 1990) and has been associated with ovulatory disturbances in young active females (Prior et al., 1990a). Spinal bone loss of 2% per annum was reported in healthy young women with progesterone deprived, but normal endogenous oestradiol production (Prior et al., 1990a). Given the relationship between bone and muscle strength in postmenopausal women (Rutherford and Jones, 1992), it is possible that muscle weakness postmenopause, and during the menstrual cycle is caused through progesterone deficient/reduced states.

The possible mechanisms by which progesterone exerts its effects on skeletal muscle are indicated in studies examining its actions on other tissues. Progesterone has been associated with calcium mobilisation from intracellular stores of the pituitary in rats (Ortmann et al., 1995), which has significant ramaifications on the availability of calcium for contraction in skeletal muscle. Further, progesterone exposure to excised myometrial strips of pregnant women increases the frequency and tonus of this smooth muscle (Fu et al., 1994). It has been proposed that hormones (oestrogen) may influence the sensitivity of the cross-bridges to phosphorus metabolites i.e. inorganic phosphate (Pi) (Phillips et al., 1993), an increase of which has been reported to alter the equilibrium of low and high force states of the cross-bridge (Pate and Cooke, 1989). During the oestrus cycle of hamsters, Shivaji et al. (1995) did not find any significant difference in Pi or pH using ^{31}P NMR. An increase in phosphocreatinine (PCr) and ATP in the uteri of oestrus animals was found with increasing levels of oestradiol, although progesterone *per se* did not cause an increase in the concentration of PCr and ATP.

The mechanisms of actions of progesterone are still highly speculative and progesterone receptors have not been identified on skeletal muscle. Given the above discussion it is still important to state that oestrogen is still implicated in force production since a negative relationship was found between strength and the ratio of oestrogen to progesterone. When oestrogen was unopposed (pre-LH peak), strength

was at its lowest. With high progesterone and increasing oestrogen levels, strength was greatest. Whether progesterone and oestrogen combined or progesterone alone enhances force production is not known, since under no physiological conditions is progesterone secreted in isolation. It is apparent, however, that muscle weakness occurs under an "oestrogen-only milieu".

The force patterns produced by the quadriceps in this study are not consistent with those of Sarwar et al. (1995) who also tested the quadriceps. It does not seem likely therefore that the changes in strength are dependent on the muscle group tested. Phillips et al. (1996) measured force production of the adductor pollicis muscle, a small parallel fibred muscle of the hand. Whereas the pattern of force is similar to that obtained with the quadriceps in this study, the onset and magnitude of change are not. However, maximal strength of the first dorsal interosseus (FDI) was also assessed on the same occasion as the quadriceps, with extra measurements undertaken between the main phases. There were no significant changes in force of the FDI for the non-OC group. These results were surprising given the effects of hormonal changes on the AP. This contradict the suggestion that smaller muscles are more sensitive to changes in the hormonal milieu compared with a complex, multi-pennated muscle group such as the quadriceps.

Maximal strength is one method of assessing muscle function. The measurement of force production in response to electrically stimulated contractions at increasing frequencies determines the contractile properties of muscle. When expressed numerically, the forces generated at 20 Hz are calculated as a percentage of 50 Hz. The effects of hormonal status on the contractility of muscle was assessed.There were no significant changes in the 20/50 % across the cycle for the non-OC group (or OC users). The fluctuations that were observed were therefore not attributable to hormonal changes. Even though the electrical stimulation technique is a common method for identifying muscular dysfunction in patients with myopathic disorders (Edwards, 1978), it has only been employed in one study to assess the effects of reproductive hormones on muscle function. Sarwar et al. (1995) reported a left-shift in the force-frequency curve from mid-luteal to mid-cycle. This was attributed to the slowing of

relaxation mid-cycle. Whether mid-cycle in their study is representative of the peak or the trough of this hormone is not known, although they assumed that this measurement was undertaken at ovulation. If so, oestrogen is at its lowest at this time, which does not uphold their proposal of an effect of oestrogen. Relaxation time was not assessed in this study, although the changes observed by Sarwar et al. (1995) are more likely to be due to the secondary effects of hormones via changes in core temperature rather than directly.

Muscle fatigue of the quadriceps was induced through electrically stimulated contractions over 3 min. The end force as a percentage of the initial force was expressed as the fatigue index. There were no significant differences in the fatigue index measured for the quadriceps across the cycle. Both volitional respiratory fatigue (Chen and Tang, 1989) and electrically elicited fatigue of the quadriceps (Sarwar et al., 1995) have been reported to be lowest at mid-luteal phase, probably due to the higher circulating progesterone levels as a result of the increase in core temperature (Prior et al., 1990b). The modified fatigue protocol used in this study (stimulating for 1 s with a 1 s rest) would be expected to be induce ischaemia similarly to the protocol used by Sarwar et al. in which the muscle was stimulated for 0.25 s every second for 3 min. The greater proportion of stimulated muscle (20-30 % of MVC) elicited by Sarwar and co-workers, compared with ~20 % in this study, may account for greater variability in force production and as a consequence fatiguability.

These results support a hormonal influence on force production of the quadriceps. Further, they demonstate that force loss can be acute and unless frequent measurements are made across the cycle, these changes can be missed. There were no corresponding changes in strength of the first dorsal interosseus muscle or in the contractility or fatiguability of the quadriceps. Despite the relationship between strength and progesterone, the menstrual cycle does not render itself a good method for examining hormones. The changes in oestrogen are particularly acute and temporary, with accompanying changes in the gonadotropins and progesterone. A clearer model for isolating the effects of reproductive hormones is needed to investigate fully these phenomena.

4.4. THE EFFECT OF HORMONE REPLACEMENT THERAPY (HRT) ON MUSCLE FUNCTION OF THE FIRST DORSAL INTEROSSEUS MUSCLE (FDI)

4.4.1. Introduction

It has been established that maximal voluntary contraction (MVC) of the quadriceps is reduced in post-menopausal women. The strength loss, at a rate of 9-10% per annum, is manifest within 1 to 3 years of the menopause in association with the reduction in reproductive hormones. Females taking hormone replacement therapy do not experience this weakness. The mechanisms of action are not known, although under hypo- and hyperoestrogenic conditions no change in strength of the FDI is observed (study 4.3). This indicates that oestrogen is not the sole mediator for influencing force production. During the menstrual cycle, MVC was greatest during the mid-luteal phase corresponding with high progesterone levels (study 4.2).

The objective of this study was to investigate the effect of HRT on muscle function administered from an oestrogen and progesterone deficient state, with a focus on the differences in oestrogen alone, and the oestrogen/progestogen component. Oestrogen was administered during the first 14 days of HRT and a progestogen component is added during last 7 days in attempts to mimic the normal menstrual cycle, allowing for a more controlled method of examining cyclical changes. Whether these synthetic hormones act as a substitute to endogenous reproductive hormones is not known. The following hypotheses were devised to undertake this study:

Hypothesis 9: Force production of the FDI increases from baseline levels during HRT

Hypothesis 10: The oestrogen and progestogen phase of HRT results in greater force production of the FDI compared with the oestrogen-only phase.

Hypothesis 11: Contractile properties of the FDI differ with the administration of HRT.

Hypothesis 12: Adminstration of HRT induces greater fatigue resistance compared with baseline values.

The aims of this study were to:

1) Examine the changes in strength at baseline and during HRT.

2) Investigate fatiguability of muscle.

3) Monitor changes in contractile properties.

4) Compare the difference in muscle function between oestrogen alone, and oestrogen and progestogen components of HRT.

4.4.2. Methods

(i) Subjects

Nine healthy post-menopausal women (age: 55.78±6.74; mass: 64.8±10.2: height: 1.59±0.05 (mean±SD)) gave informed written consent to participate in the study. Subjects were recruited from the Menopause Clinic at Liverpool Women's Hospital, where blood samples were taken to confirm their hormonal status (mean bloods are shown in Table 4.4.1). Following consultation with the doctor, subjects were referred for a baseline measurement, prior to taking hormone replacement therapy (HRT). All subjects were prescribed oestrogen and oestrogen-progestogen treatment (preparations are shown in Table 4.4.2). The dominant hand was selected for assessment, except in one subject in whom the FDI of the non-dominant hand was tested due to rheumatism of the fingers of the dominant hand. All other subjects were free of pain or disease of the hand.

Table 4.4.1. Mean (±SD) oestradiol, luteinizing hormone (LH) and follicle stimulating hormone (FSH) in post-menopausal women (n=9).

VALUES	Oestrogen (pmol/l)	FSH (U/l)	LH (u/l)
MEAN	68.33	70.5	53.5
±SD	46.45	29.2	18.9
RANGE	<37-190	26.2-113.7	30.6-88
Normal values †	<150	>20	>20

† Normal post-menopausal values

Table 4.4.2. Hormone replacement therapy preparations administered to subjects

ROUTE OF ADMINISTRATION	PREPARATION	OESTROGEN	PROGESTOGEN
ORAL	FEMOSTON (X3)	Oestradiol	Dydrogesterone **P**
ORAL	TRIDESTRA (X2)	Oestradiol valerate	Medroxyprogesterone acetate **P**
ORAL	PREMIQUE (X1)	Conjugated oestrogens	Medroxyprogesterone acetate **P**
PATCH	ESTRACOMBI (X2)	Oestradiol	Norethisterone **T**
TRANSDERMAL CREAM	PRO-GEST (X1)	Oestradiol/oestrone	Progesterone **P**

P = progesterone derivative

T= testosterone derivative

(ii) Procedure

Maximal voluntary contraction (MVC), contractile properties and fatiguability of the FDI were assessed on three occasions. The first measurement was taken on the same day HRT was prescribed to establish baseline strength. Subjects were requested back to the laboratory four months following the start of HRT. Two measurements were undertaken during treatment a) during the oestrogen phase and b) during the oestrogen/progestogen phase of treatment. The order of testing for measurements during HRT was counterbalanced.

Following the warm-up of the muscle in heated water, the electrodes were positioned on the hand and stimulated at 1 Hz, with increments of voltage to the highest, but tolerable, level of stimulation. An isolated lateral movement of the index finger was achieved prior to recording. A programmed stimulation of electrical impulses, from unfused to fused tetanic contractions was delivered to the muscle to ensure adjacent muscles were not recruited at higher frequencies of stimulation. Clear visible recordings of the myogram was also required. The data of 2/9 subjects for electrically stimulated contractions were excluded from analysis due to their intolerance of the sensation of the electrical stimulation. Details of the procedure for

assessment of MVC, contractility and fatigue resistance of the FDI are described in Chapter 3.5.

(iii) Data analysis

A repeated measures analysis of variance (ANOVA), with visit as the repeated factor, was employed to calculate trends over the three test sessions for all variables. Post-hoc analysis (Tukey's test) was used to localise differences between trials.

4.4.3. Results

4.4.3.1. Maximal voluntary contraction

The results of the ANOVA shows that there was no significant difference between baseline measurements of strength and phases of treatment ($F_{2,16}$=3.03: $p<0.1$). A t test undertaken between baseline measurements and the oestrogen/progestogen phase of treatment induced a significant difference (t_8 = -2.51;$p<0.05$). Mean (±SE) of maximal strength across test sessions are shown in Fig. 4.4.1.

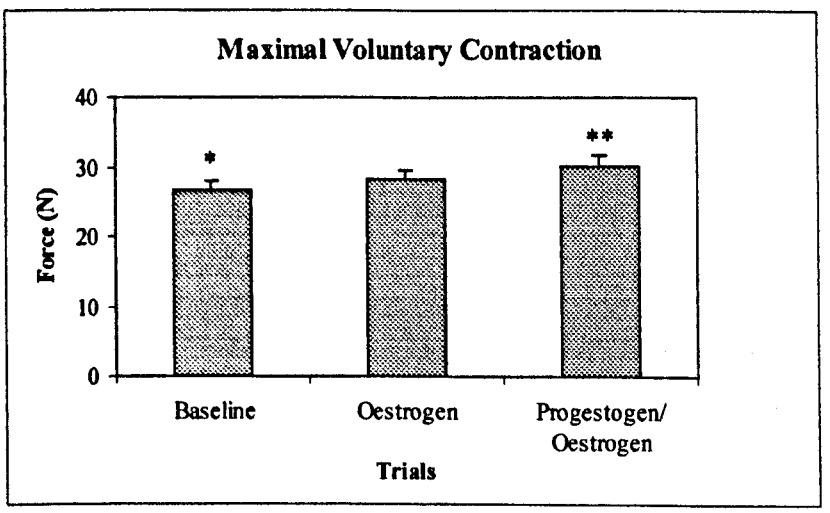


*Fig. 4.4.1: Mean (±SE) maximal voluntary contraction (N) of the first dorsal interosseus muscle (FDI) in post-menopausal women at baseline, and during the oestrogen and progestogen phases of hormone replacement treatment. The * indicate significant difference (difference between * and **at $p<0.05$)*

4.3.3.2. Contractile properties

The 20/50% ratio was calculated as the force generated from 20 Hz as percentage of 50 Hz. Whilst there were no significant difference in this ratio between treatments ($F_{2,12}$=0.1: p>0.05), the mean (±SD) was higher for the oestrogen phase (70.5 9±19.5% compared with 67.0±12.8 and 67.4±18.8% for baseline and progestogen, respectively). The comparison between conditions in the force-frequency curve as an indicator of the contractile properties of muscle, is shown in Fig. 4.4.2. At lower frequencies (up to 20 Hz) greater force as a percentage of 100Hz was generated during HRT phases. This was maintained in the progestogen phase at high frequencies, although force for oestrogen-only declined. There were no significant differences in forces between phases at each frequency (p>0.05).

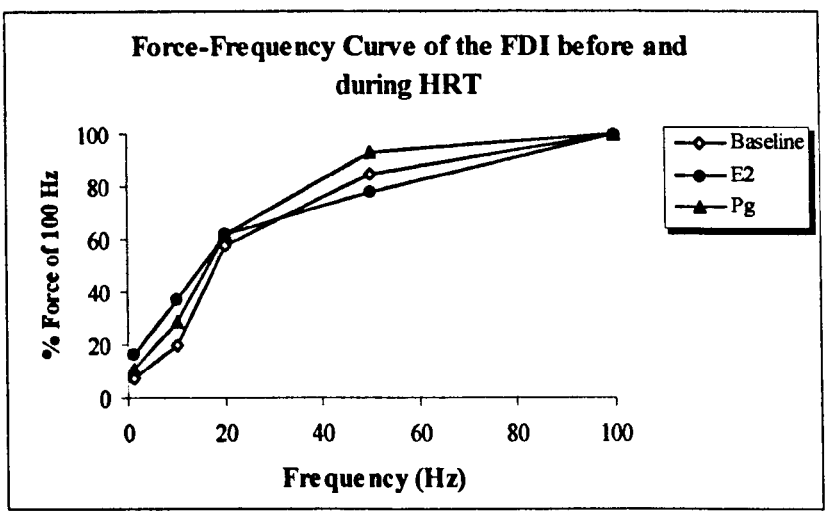


Fig. 4.4.2: The force-frequency curve across increasing frequencies of electrical stimulation between baseline and the oestrogen and progestogen phases of hormone replacement therapy (HRT). Forces at lower frequencies is expressed as a percentage of force at 100 Hz.

4.4.3.3. Fatigue Index (%)

There were no significant differences in fatigue index for peak tension ($F_{2,12}$=2.43; p>0.05) or mean tension ($F_{2,12}$=1.10; p>0.05) across the three treatment trials. Fig. 4.4.3 illustrates mean (±SE) FI% for baseline, oestrogen and progestogen phases of HRT.

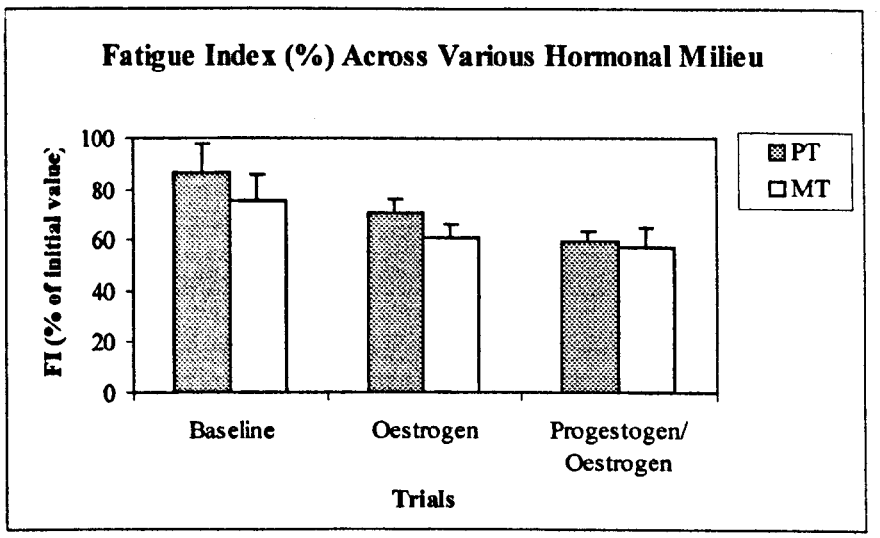


Fig. 4.4.3: *A comparison of mean (±SE) fatigue index for peak tension (PT) and mean tension (MT) between baseline measurements and subsequently at the oestrogen and progestogen phase of hormone replacement therapy (HRT).*

4.4.4. Discussion

The influence of reproductive hormones on force generation in skeletal muscle is becoming more apparent. Under hypo-oestrogenic/hypo-progestogenic conditions, muscle strength is compromised. In this study, muscle strength was measured prior to hormonal treatment and monitored during two phases of HRT following 4 months of administration. There was no significant increase in strength between baseline and the two phases of HRT. When the oestrogen-only phase was excluded, a significant increase in strength between baseline and the oestrogen/progestogen phase was observed.

The prevention of muscle weakness of the adductor pollicis (AP) has been reported in post-menopausal women taking hormone replacement therapy compared to a hypoestrogenic post-menopausal group (Phillips et al., 1993b). It is not clear if HRT maintains strength, or enhances the force-generating capacity as no studies have monitored performance before and after the administration of HRT. In this study, the results revealed that during the progestogen phase, muscle strength was higher than during the oestrogen-only phase of treatment. These findings have important implications in the administration of HRT, since hysterectomised women do not require progestogen, and are routinely administered unopposed oestrogen. Progestogens is only added to preparations for women with an intact uterus to prevent endometrial hyperplasia and reduce the occurrence of carcinoma (Whitehead and Godfree, 1992).

Since the mechanisms of hormonal action on muscle function are still speculative, it is not clear if endogenous reproductive hormones act in the same way on skeletal muscle as exogenous synthetic hormones. Due to the rapid hepatic metabolism of oral (micronised) progesterone (Whitehead et al., 1990), progestogens are administered in HRT preparations. They can either be of C-19 nortestosterone derivatives (e.g. norgestrel, norethindrone) which are androgenic or of the C-21 derivatives of progesterone (eg. medroxyprogesterone). The undesirable side effects of progestogens — breast tenderness, bloatedness, oedema, abdominal cramps, irritability and anxiety — implicate their effects on electrolyte imbalance and on the central nervous system. It is also believed that progestogens antagonize the beneficial effects of

oestrogen on the arterial system by decreasing HDL and increasing LDL. Even though this was initially attributed to androgenic progestogens, medroxyprogesterone, a progesterone derivative, has also been shown to reduce HDL-cholesterol (Ottosson et al., 1985). The antagonistic effects are not class-specific and dydrogesterone does not have the same effect as medroxyprogesterone, despite being a progesterone derivative.

It is therefore difficult to suggest what mechanisms are operating for progestogens to enhance strength. The difference in source of derivatives, and inter-class variability of progestogens makes it difficult to isolate its effects. Phillips et al. (1993b), whose work supports the role of oestrogen for influencing specific force, suggested that oestrogen exerts its effects through altering the sensitivity of the cross-bridges to inorganic phosphate (P_i) and pH. High P_i (and low pH) switches the equilibrium of cross-bridge attachment between low and high force states but when a rapid stretch is applied to the muscle, this weakness is absent. Stretching forces the cross-bridges into the high-force state regardless of the force state at the onset of the stretch (Lombardi and Piazzesi, 1990). The influence of progesterone on these metabolites warrants examination.

It may be construed that a systematic increase in strength occurred as a result of familiarisation, particularly since no practice session was undertaken. Given that the IVF patients were subject to the same protocol and no change in force production was found, and that the oestrogen and progestogen phases were counterbalanced, it is reasonable to speculate that reproductive hormones influenced muscle function.

The 20/50% is an indicator of the force-frequency curve, and any changes in force at these frequencies will be reflected in this ratio. The mean ratio across phases was higher for the oestrogen phase due to the reduction in force at 50 Hz (Fig 4.4.2). These differences were not significantly different, probably due to too few subjects and great inter-individual variation. The cause for a drop in force at this higher frequency for the oestrogen phase is not known. However, there is a clear left-shift in the force-frequency curve in the progestogen phase from baseline. This shows that greater force is generated across all frequencies as a percentage of 100 Hz compared to the hypo-hormonal state. A left-shift in the force-frequency curve has also been demonstrated during the menstrual cycle for the quadriceps at mid-cycle (corresponding

to high oestrogen, low progesterone) from mid-luteal (corresponding to high oestrogen, high progesterone) (Sarwar et al., 1995). These results do not concur with those obtained with exogenous hormones in post-menopausal women. Furthermore, it may be erroneous to compare responses from the quadriceps, a large multi-pennated muscle group with the FDI, a single-fibred muscle, despite the similar fibre composition (Johnson et al., 1973).

If the lack of hormonal influences at the level of the cross-bridge was responsible for muscle weakness, forces generated at low-frequencies would be expected to be affected, via excitation-contraction coupling. The observation that forces are also lower at higher frequencies of stimulation at baseline may indicate also that progestogen affects neuromuscular transmission, maybe mediated through acetlycholine (ACh) release, or propagation of the neurotransmitter signal. The role of oestrogen and progesterone on muscle function need to be established before their mechanisms of action can be investigated.

Fatigue of the FDI was induced through electrical impulses delivered over 3 min. There were no significant differences in the fatigue index (%) across phases of treatment, for peak tension (PT) or mean tension (MT). The lack of changes in fatigue resistance with hormonal manipulation has been found with each study using this measure (study 4.2 and 4.3). However, Sarwar et al. (1996) reported a pattern of change in fatiguability across the menstual cycle. The quadriceps were least fatiguable at the mid-luteal phase, when progesterone levels are at their highest. The authors attributed the fatigue resistance at this phase to progesterone, which in addition to its thermogenic properties, preserves glycogen stores during the luteal phase. The muscle would be expected to be reliant on glycogen due to the ischaemic conditions induced by the fatigue protocol. Whether progestogens have the same effect on glycogen storage as 'natural' progesterone is not known. However, the lack of trend in the fatigue index could be more easily explained by the some factor such as using percutaneous stimulation of the muscle rather than supramaximal stimulation via the motor nerve, where greater force output would be attained. This did not occur during the menstrual cycle examined in study 4.2.

In conclusion, there was a significant difference in strength before and during HRT, in which progestogen and oestrogen combined resulted in an increase in the force-generating capacity of the muscle. There were no other changes in either contractility or fatigue resistance of the

muscle as a result of HRT, and thus the question of the mechanism of action of hormones on muscle function is still unresolved. The positive role of progestogens on muscle strength is paradoxical to the many side effects of this synthetic hormone which can reduce the quality of life of affected post-menopausal women. However, without this component added to HRT preparations to prevent endometrial hyperplasia, carcinoma is highly likely.

4.5. Summary

1) Maximal force of the quadriceps declined over 9 months in hypoestrogenic post-menopausal females at 9 to 10%, measured isometrically and at 1.05 rad/s. There was no evidence of a reduction in strength at higher angular velocities, or for handgrip.

2) Muscle weakness was not observed in menopausal/post-menopausal females taking hormone replacement therapy (HRT). This implicates reproductive hormones in strength losses.

3) Isometric strength in young females declines during the menstrual cycle, corresponding with pre-LH peak. Strength peaked at the mid-luteal phase when progesterone was highest. The relationship between these parameters suggests that progesterone is involved in force generation.

4) The fluctuations in strength of the quadriceps are not manifest in the FDI. There were also no changes in contractility or fatigue resistance in the quadriceps related to changes in endogenous hormones.

5) Strength measurements across the cycle in oral contraceptive users were not unaltered, indicating that continuous administration of exogenous steroid sex hormones do not influence force production.

6) Acute changes in oestrogen were not accompanied by changes in strength or fatiguability of the FDI. Unopposed oestrogen does not therefore appear to be involved in force changes.

7) Following four months of oestrogen/progestogen replacement therapy, strength did not increase from baseline to different phases of hormone replacement therapy (HRT) in post-menopausal females. Strength of the FDI increased from baseline to the oestrogen/progestogen phase by 15.2±20.6% which may further implicate progesterone-related component in force production.

CHAPTER FIVE

SYNTHESIS OF FINDINGS

5.0. SYNTHESIS OF FINDINGS

The purpose of this chapter is to collate the findings from the studies undertaken in this thesis and determine the extent to which the hypotheses and aims have been realised. The outcome of the findings will be discussed in reference to the consequences and clinical application of the results.

5.1. REALISATION OF AIMS

The rate of strength loss in hypoestrogenic post-menopausal women was ascertained in the longitudinal study, fulfilling aim 1. Maximal strength of the quadriceps declined markedly at a rate of 10 and 9 % for 0 and 1.05 rad/s respectively, but was not evident at higher angular velocities or in grip strength.

In the menstrual cycle model peak strength of the quadriceps occurred at the mid-luteal phase in concert with greatest progesterone concentration (aim 2a), demonstrating changes in strength of the quadriceps but not for the first dorsal intersseus (FDI). In the IVF model, despite large acute changes in oestrogen, no changes in strength wree reported. These data suggest that oestrogen does not promote an independent effect on muscle strength (aim 3). The efficacy of hormone replacement therapy (HRT) in preventing post-menopausal loss in muscle strength was established in study 4.0. This indicated that combined oestrogen and progesterone had an effect (aim 4.0).

Other indices of muscle function i.e. contractile properties and fatigue resistance, were investigated during acute changes in endogenous hormones (aim 2b and 3b) and with the administration of hormone replacement therapy (HRT) (aim 4b). There were no changes in fatiguability or contractile properties under these conditions.

The secondary aims of the methodology section (Chapter 3) were also realised, establishing the reliability of the equipment, techniques and protocols employed in the experimental studies.

5.2. REVIEW OF HYPOTHESES

The hypotheses proposed throughout the experimental studies will be reviewed, and accepted or disproved according to the findings.

Hypothesis 1. *Maximal strength decline over 12 months in women within 1 to 3 years post-menopause.*

This hypothesis is accepted, a reduction in strength in post-menopausal women was reported for isometric and dynamic leg strength at 1.05 rad/s. This was only significant however, when the post-menopausal group was compared with the HRT group only. The peri-menopausal group had no effect.

Hypothesis 2. *There is no change in maximal strength in females taking hormone replacement therapy (HRT).*

Maximal strength remained stable for each strength parameter measured. This hypothesis was accepted.

Hypothesis 3. *Strength loss in hypoestrogenic post-menopausal women is of the same proportion with increasing angular velocities.*

This hypothesis was rejected. Strength loss in post-menopausal women was only significant at zero or slow angular velocities (1.05 rad/s).

Hypothesis 4. *Maximal strength of the quadriceps and FDI is greater when oestrogen concentrations are at their highest.*

Maximal strength of the quadriceps did not peak with high oestrogen levels. There was no significant relationship between muscle and circulating oestrogen. This hypothesis was rejected. Maximal force of the FDI did not change.

Hypothesis 5. *Changes in hormone levels result in a shift of the force-frequency curve.*

This hypothesis was rejected since there was no change in the 20/50 Hz ratio during the menstrual cycle.

Hypothesis 6. *The quadriceps are least fatiguable when progesterone levels are high.*

There were no changes in the fatigue resistance of the quadriceps during the menstrual cycle, and therefore progesterone did not exert the effect as previously postulated. This hypothesis was rejected.

Hypothesis 7. *Maximal force production increases from hypo- to hyperoestogenic conditions during in vitro fertilisation treatment.*

This hypothesis was rejected. The hypo- and hyperoestrogenic conditions experienced during *in vitro* fertilisation treatment did not have an effect on maximal force generated in the FDI.

Hypothesis 8. *The fatigue resistance of the FDI changes during hypo- and hyperoestrogenic conditions.*

The fatigue resistance of the FDI also remained stable across treatment and this hypothesis was therefore rejected.

Hypothesis 9. *Force production of the FDI increases from baseline levels during HRT.*

This hypothesis was rejected since force did not increase when both phases of treatment (oestrogen and oestrogen/progestogen) were considered. Exclusion of the oestrogen-only phase resulted in an increase in force between baseline and the oestrogen/progestogen phase.

Hypothesis 10. *The oestrogen — progestogen phase of HRT results in greater force production of the FDI compared with oestrogen-only.*

This hypothesis was rejected. There was no difference in maximal strength of the FDI between the two phases of HRT.

Hypothesis 11. *Contractile properties of the FDI differ with the adminstration of HRT.*

This hypothesis was rejected. The 20/50 Hz ratio was no different before or after the administration of HRT.

Hypothesis 12. *Administration of HRT induces greater fatigue resistance compared with baseline values.*

The fatiguability of the FDI during electrically stimulated contractions did not alter with hormonal replacement. This hypothesis was subsequently rejected.

5.2. GENERAL DISCUSSION

The longitudinal study has revealed that a significant loss of strength occurred in post-menopausal women of 9 and 10% per annum for dynamic (1.05 rad/s) and isometric contractions respectively. This rapid weakening of the quadriceps corresponds with the accelerated loss of bone mass reported at the menopause in response to the reduction in reproductive sex hormones. Whether this rate of force loss continued was not examined in this study, although it is predicted that it would reach a plateau similarly to that shown for menopausal bone loss. The group of peri/post-menopausal women who were undergoing hormone replacement therapy (HRT) did not experience any strength deficits, further implicating the involvement of reproductive hormones in regulating force production. These data suggest that HRT may confer protection against muscle weakness of the quadriceps, an important muscle group for ambulation and balance, and thereby reducing the risk of falling and sustaining an osteoporotic fracture.

The findings from this study corroborate cross-sectional data of Phillips et al. (1993b), who reported a significant reduction in specific force i.e. force per cross-sectional area (force/CSA) of the adductor pollicis (AP) in women at ~50 years. Furthermore, specific force was greater in post-menopausal women taking HRT compared with hypoestrogenic age-matched controls. Whilst both studies demonstrated an hormonally-related strength loss, the hormone responsible cannot be elucidated in the menopause/post-menopausal model. Phillips et al. (1993b) advocated oestrogen as the hormone affecting muscle strength. They verified this supposition from observations of strength changes during the menstrual cycle (Phillips et al., 1996). A pre-ovulatory peak in strength of the AP, followed by a rapid decline 1 to 2 days later was reported to parallel the acute changes in oestrogen characteristic of the ovulatory phase. However, they failed to find significant correlations in relative strength with circulating oestrogen levels (Phillips et al., 1996).

The changes in force production of the quadriceps during the menstrual cycle in this thesis did not support those reported by Phillips et al. (1996). A gradual increase in strength was observed during the follicular phase of the cycle, which was followed by a

rapid drop prior to the LH surge. A secondary, greater rise in force production reached its peak mid-luteal. These force changes were not coincident with fluctuations in oestrogen, and there was no relationship between relative strength and circulating oestradiol.

The menstrual cycle does not lend itself as a good model to isolate any independent effect of oestrogen. The *in vitro* fertilisation (IVF) model was therefore employed to assess muscle function of the first dorsal interosseus (FDI) during 1] down-regulation of the hypothalamic-pituitary-ovarian axis with suppression of pituitary and reproductive hormones and 2] during up-regulation with consequently supraphysiological oestrogen levels. No changes in strength were found during this treatment. The IVF model provides a unique method of assessing the independent effects of oestrogen. The massive, acute changes in this hormone, which exceed those of the menopause, are not observed during normal physiological events. These findings demonstrate unequivocally that oestrogen alone does not affect the force generating capacity of muscle.

Finally, there were also indications from the study assessing the effects and components of HRT on strength of the FDI, that oestrogen is not the sole regulator of force production. From baseline measurements in post-menopausal women, strength did not increase significantly after four months of treatment when assessed during the oestrogen-only phase. These findings are preliminary since few subjects were tested (n=9), and they only provide tenuous suggestions against oestrogen as the effects of exogenous hormones on muscle strength, particularly the role in restoring strength, are not fully understood.

There are reports of oestrogen receptors on skeletal muscle in animal (Dahlberg, 1982) and humans (Smith et al., 1990). If oestrogen is not the sole regulator of changes in strength, then some other hormone must be involved. Gonadotropins are important in the regulation of the reproductive cycle and the synthesis of oestrogen and progesterone, exerting their effects on reproductive tissues, principally the ovaries. Both luteinizing hormone (LH) and follicle stimulating hormone (FSH) are elevated in

post-menopausal women, increasing 18 fold pre-menopausal values. This is considerably higher for LH. The loss of strength in post-menopausal women could possibly be associated with the rapid, and very high increases in the gonadotropins. The trends in strength changes during the menstrual cycle do not contradict this, as strength peaked mid-luteal when gonadotropin levels were low. However, correlations between relative force and circulating LH and FSH were not significant, suggesting that gonadotropins were not responsible for changes in strength. This is further substantiated in the IVF study. Down-regulation suppressed the pituitary release of LH and FSH, as well as the reproductive hormones, and subsequent administration of exogenous pituitary hormones did not result in any reduction in strength.

Progesterone is the only other major hormone involved in reproductive functioning which could be involved in the strength changes observed so far. Progesterone is actually the hormone that first becomes deficient at the climacteric. Even though oestradiol declines at the menopause, a small amount is still produced from the peripheral conversion of androstenedione to oestrone whereas progesterone is very low. The findings from this thesis strongly implicate the involvement of progesterone in the changes of force production. This was initially observed in the menstrual cycle study, in which maximal strength of the quadriceps peaked mid-luteal when progesterone was at its highest. A significant correlation between strength and circulating progesterone confirmed this pattern of strength change with progesterone levels.

The IVF model also provided useful evidence supporting the role of progesterone. During IVF treatment, progesterone does not increase markedly from the baseline measurements until human chorionic gonadotropic (hCG) hormone is administered. In this study, the strength measurements were taken prior to hCG administration, and therefore at a time when progesterone concentration remains constant, as did the strength of the FDI.

The mechanism by which progesterone may affect muscle strength is conjectural at this time. There is no known evidence of the presence of progesterone receptors on

skeletal muscle, although given the recent proposals of a hormally-mediated effect on muscle function, this may not yet have been investigated. Progesterone and its metabolites e.g. 3α, 5α - tetrahydroprogesterone, exert diverse effects on various tissues such as the brain, uterus, smooth muscle and the oocyte. Their effects, which may be mediated via the classical intracellular receptor, membrane receptors or via the $GABA_A$ receptor system, depend upon the tissue involved, the dose of progesterone and time of administration. If progesterone is found to act rapidly, then the latter two mechanisms are probably involved. Prolonged effects would involve the intracelluar receptor through which progesterone inhibits uterine contractility.

The studies within this thesis do not favour the responsibility of oestrogen for regulating changes in force production. However, the involvement of oestrogen should not be ignored. Oestrogen may act together with progesterone to exert its effect on muscle. A significant relationship between the ratio of oestrogen to progesterone with leg strength during the present menstrual cycle study implicates the involvement of both hormones. It was observed that strength peaked mid-luteal when progesterone and oestrogen were both present. However, force production was also high during the follicular phase when oestrogen concentrations were greater than progesterone. Therefore, it may be that progesterone exerts its effects on an oestrogen-primed muscle. Indeed, this has been demonstrated in reproductive tissue such as the uterus; the presence of oestrogen is necessary to up-regulate progesterone receptors in the uterus in preparation for the proliferative luteal phase. Furthermore, the facilitative effect of progesterone is required for gonadotropin secretion. Oestradiol alone does not initiate the preovulatory gonadrotropin surge in the ovariectomized rat. Oestradiol priming is essential for progesterone to exert its action on gonadotropin secretion since oestrogens are required for the induction of hypothalamic and anterior pituitary progesterone receptors. Given the pattern of strength changes during the menstrual cycle it is plausible to suggest an effect upon skeletal muscle. This can be examined further using the IVF model, and measuring strength at hypo- and hyper oestrogenic conditions, and following hCG administration (when progesterone levels have increased). It would also be of use to examine the effects of progesterone derivatives in the form of the progestogen only pill on strength.

The change in strength across the menstrual cycle was not prolonged and appeared to fluctuate in concert with reproductive hormones. The temporal reduction in force observed mid-cycle was followed by an increase in strength which shortly attained its optimal point. This indicates that this is a temporary, but reversible effect. If the same mechanism operates in hormally-deficient muscle of post-menopausal women, this has important clinical implications for preventing or treating muscle weakness within this age-group. The most efficacious treatment for preventing bone loss is HRT, which has been reported to maintain muscle strength in post-menopausal women. This was confirmed in the longitudinal study since post-menopausal women taking HRT did not exhibit a reduction in strength.

The recovery of the loss of the force generating capacity of the quadriceps with fluctuations in endogenous hormones appears to occur with administation of exogenous hormones. An increase in strength of the FDI was observed in hypoestrogenic post-menopausal women following hormone replacement therapy from 26.6N at baseline to 30.0N of the oestrogen/progestogen phase, a difference of +15.2±20.6%. This further supports the role of oestrogen/progesterone as previously proposed, corroborated by the lower force values obtained during the oestrogen only phase. It therefore appears that HRT may restore strength rather than just preventing further weakening. An adjustment period greater than 4 months for taking HRT may be necessary for the complete restoration of strength. There is current evidence which indicates that an increase in strength of the adductor pollicis does occur over 12 months following HRT administration (Woledge, 1997. Unpublished findings, University College London).

The mean strength of the FDI before (26.6N) and after (30.0N) HRT are comparable with the differences in strength in young hypoestrogenic IVF patients (~27.0N) and young females during the menstrual cycle (32-34.0N). The low strength values observed in the IVF patients may therefore be the result of a decrease in strength in response to pituitary down regulation, and consequently low reproductive hormones.

An initial measurement prior to treatment, i.e. during the preceding cycle, would clarify this and is therefore a recommendation for future research.

If the preservation of muscle strength is conferred by HRT, then administration of exogenous hormones would reduce the muscle weakness which impairs performance and induces vulnerability to falling. The main benefits of this treatment would be two-fold including the reduction of the accelerated bone loss associated with the menopause. Unfortunately, this solution would only assist a small proportion of the female population. Hormone replacement therapy opposed with a progestin is accompanied by side-effects related to the progestogen component. Hence, this is not always a favourable treatment in progestogen sensitive females. Under these circumstances, the promotion of exercise regimens is important, and would not only increase muscle strength, but would provide a protective mechanism in bone and on the cardiovascular system.

CHAPTER SIX

RECOMMENDATIONS FOR FUTURE WORK

6.0. RECOMMENDATIONS FOR FUTURE WORK

The role of reproductive hormones in regulating muscle strength is a very new concept, and has important implications in both a clinical environment and in a sporting context. The findings from the experimental work has therefore lead to many unanswered questions, and has also generated many more. The recommendations for future work will focus on *in vivo* studies, although it is recognised that data from *in vitro* research is needed to fully understand the role of oestrogen and progesterone on skeletal muscle.

(1) A longitudinal study undertaken over a longer duration, from 5 to 10 years, is needed, to reveal in greater detail the time course of the strength changes associated with the menopause. This would also determine whether the rapid loss in strength observed over 1 year plateau as the post-menopausal era is prolonged. The beneficial effects of exercise and/or HRT assessed over this time period would determine the most effective prophylaxis in preventing hormonally-related muscle weakness.

(2) There is still speculation of whether reproductive hormones are involved in regulating muscle function. Assessment of strength before IVF treatment ie. during the preceding cycle, and during IVF treatment would demonstrate if a loss of strength occurred as a result of pituitary down-regulation. While this may further implicate the role of reproductive hormones, it would also justify the low strength values of the FDI attained in IVF patients.

(3) The outcome of this thesis is that reproductive hormones exert an effect on muscle strength, and that progestrone is likely to be involved. This needs to be investigated further. It is impossible to isolate the effects of progesterone *in vivo,* although the proposed synergistic effects of oestrogen and progesterone can be tested using the following models:

a) Utilising the IVF model, assessing strength during treatment when oestrogen levels are manipulated, and following the administration of hCG would allow the

comparison of strength when oestrogen and progesterone (and LH/FSH) are very low, when oestrogen is high but progesterone is low, and when oestrogen and progesterone are high. Strength can be measured confidently under these controlled conditions, unlike the endogenous fluctuations during the menstrual cycle.

b) Assessment of strength before and during the administration of the progestogen-only pill in young women would allow the effects of this progesterone-derivative to be examined. The progestogen-only pill suppresses ouvlation is some women and thus careful monitoring of hormone levels would be necessary.

c) Oestrogen and progesterone are elevated during pregnancy. Monitoring strength before and during pregnancy would elucidate the effects of a rise in hormones levels on muscle function. The rapid decline in these hormones on strength could be tested postpartum.

(4) Preliminary findings suggest that HRT may restore strength. This needs to be examined further using larger subject numbers, and controlling for the HRT preparation. In a large randomised study, baseline levels of strength in post-menopausal women should be compared with measurements taken after the stabilisation on HRT, using combined preparations (i.e. in women with an intact uterus).

(5) The reversible effects of hormonally-related strength deficits applicable to post-menopausal women can be measured with endogenous hormonal changes. Medically-induced amenorrhoea is used to relieve disorders such as endometriosis with the same methods as the IVF treatment, but for a longer duration (up to 6 months). Using these patients, strength before, during hypoestrogenia/progesteronia, and in a follow-up trial will allow the changes in strength to be characterised. This will also establish if any strength deficits are reversed with resumption of menses, which will have important applications for menopausal and amenorrhoeic sedentary and athletic women.

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APPENDICES

APPENDIX ONE

LEISURE TIME ACTIVITY QUESTIONNAIRE

5. Physical activity

5.1 Have the last two weeks been typical of your normal levels of leisure time physical activity? yes/no

5.2 If no, in what ways were they not typical?

5.3 On a scale of 1-5, rate the level of your present fitness compared to other people your age?

very poor	poor	average	good	excellent
1	2	3	4	5

Date of activity recall.....

Activity List

ACTIVITIES (1)	FREQUENCY	DURATION	SWEAT
Aerobic dancing (intense)	————	————	**yes/no**
Boxing:			
In ring	————	————	**yes/no**
Sparring	————	————	**yes/no**
Basketball	————	————	**yes/no**
Circuit training	————	————	**yes/no**
Hill climbing:			
With 5 kg load	————	————	**yes/no**
With 10 kg load	————	————	**yes/no**
With 20 kg load	————	————	**yes/no**
Cycling (race)	————	————	**yes/no**
Gardening (digging)	————	————	**yes/ no**
Hockey	————	————	**yes/no**
Jogging	————	————	**yes/no**
Judo	————	————	**yes/no**
Running			
11.5 min/mile	————	————	**yes/no**
9 min/mile	————	————	**yes/no**
7.5 min/mile	————	————	**yes/no**
6 min/mile	————	————	**yes/no**
5.5 min/mile	————	————	**yes/no**
cross country	————	————	**yes/no**
Soccer	————	————	**yes/no**
Squash	————	————	**yes/no**
Swimming:			
Back stroke	————	————	**yes/no**
Breast stroke	————	————	**yes/no**
Crawl (slow)	————	————	**yes/no**

Please indicate how often (frequency) the activity was performed, for how long (duration) and whether you sweated. For example: If you performed the activity once a day for 20 minutes but did not sweat then:

1 **20 mins** **yes/no**

ACTIVITIES (2)	FREQUENCY	DURATION	SWEAT
Aerobic dancing (moderate)	----------	----------	**yes/no**
Badminton	----------	----------	**yes/no**
Ballet	----------	----------	**yes/no**
Cricket:-			
Batting	----------	----------	**yes/no**
Bowling	----------	----------	**yes/no**
Cycling (leisurely: 9.4mph.)	----------	----------	**yes/no**
Dancing	----------	----------	**yes/no**
Hill climbing (no load)	----------	----------	**yes/no**
Ice skating (less than 9 mph.)	----------	----------	**yes/no**
Karate	----------	----------	**yes/no**
Roller skating	----------	----------	**yes/no**
Tennis:			
Singles	----------	----------	**yes/no**
Doubles	----------	----------	**yes/no**
Walking (4.5 mph. level)	----------	----------	**yes/no**
Weight lifting/training	----------	----------	**yes/no**

ACTIVITIES (3)	FREQUENCY	DURATION	SWEAT
Bowls	----------	----------	**yes/no**
Cricket (fielding)	----------	----------	**yes/no**
Cycling (leisurely: 5.5 mph.)	----------	----------	**yes/no**
Drumming	----------	----------	**yes/no**
Gardening			
Hedging	----------	----------	**yes/no**
Raking	----------	----------	**yes/no**
Golf	----------	----------	**yes/no**
Horse riding	----------	----------	**yes/no**
Lawn mowing (push-type)	----------	----------	**yes/no**
Table Tennis	----------	----------	**yes/no**
Sailing	----------	----------	**yes/no**
Volleyball	----------	----------	**yes/no**
Walking			
3.0 mph	----------	----------	**yes/no**
4.0 mph	----------	----------	**yes/no**
Window cleaning	----------	----------	**yes/no**

ACTIVITIES (4)	FREQUENCY	DURATION	SWEAT
Althetics (track and field)	----------	----------	yes/no
Bowling (ten pin)	----------	----------	yes/no
Canoeing	----------	----------	yes/no
Caving/potholing	----------	----------	yes/no
Fencing	----------	----------	yes/no
Five-a-side soccer	----------	----------	yes/no
Gymnastics	----------	----------	yes/no
Lacrosse	----------	----------	yes/no
Mountain climbing	----------	----------	yes/no
Netball	----------	----------	yes/no
Recreational swimming	----------	----------	yes/no
Trampolining	----------	----------	yes/no

APPENDIX TWO

MEAN ±SD OF FORCE FOR DIFFERENT MUSCLE GROUPS ACROSS ALL ANGULAR VELOCITIES.

A1=HRT

A2=PERI-MENOPAUSAL WOMEN

A3=POST-MENOPAUSAL WOMEN

Table A.1. *Mean values (± SD) of force across a range of angular velocities for the Hormone Replacement Therapy (HRT) group. All subjects completed the five testing sessions for leg (Nm) and grip (ft lbs) strength.*

HRT Group N=11	EXTENSORS (NM)					FLEXORS (NM)				
Angular velocity (rad/s)	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
0 (90° of flexion)	**114.7** (28.2)	**114.0** (24.0)	**113.8** (22.4)	**112.4** (23.4)	**111.8** (24.4)	*	*	*	*	*
1.04	**127.1** (25.2)	**125.9** (25.6)	**125.8** (31.8)	**128.8** (26.0)	**122.0** (29.9)	**67.4** (16.5)	**69.9** (20.6)	**66.7** (19.5)	**68.8** (19.8)	**64.8** (19.3)
2.09	**93.6** (14.0)	**94.8** (19.3)	**94.4** (19.2)	**93.7** (16.2)	**93.4** (20.9)	**54.1** (13.9)	**55.7** (15.4)	**54.8** (15.7)	**54.0** (15.4)	**52.4** (15.3)
3.13	**80.5** (14.9)	**80.4** (16.6)	**79.4** (14.4)	**79.9** (14.9)	**80.6** (16.8)	**46.6** (11.4)	**47.2** (14.4)	**45.8** (11.6)	**46.6** (12.7)	**45.1** (13.0)
5.22	*	*	*	*	**74.1** (16.3)	*	*	*	*	**48.1** (12.6)
0 (60° flexion)	*	*	*	*	**141.4** (28.5)	*	*	*	*	*
Grip Strength (Ft lbs)	**27.2** (4.6)	**26.2** (4.6)	**27.4** (3.7)	**26.2** (5.2)	**25.6** (4.2)					

Table A.2. *Mean values (± SD) of force across a range of angular velocities for the Perimenopausal group. All subjects completed the five testing sessions for leg (Nm) and grip (ft lbs) strength.*

Peri.M. Group N=9	EXTENSORS (NM)					FLEXORS (NM)				
Angular velocity (rad/s)	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
0 (90° of flexion)	**117.7** (28.4)	**115.9** (29.0)	**112.3** (30.9)	**111.3** (31.5)	**112.1** (29.0)	*	*	*	*	*
1.04	**136.8** (29.5)	**134.3** (27.7)	**131.3** (19.3)	**133.0** (30.0)	**133.9** (20.3)	**68.3** (22.1)	**70.1** (20.5)	**69.1** (15.6)	**72.2** (21.4)	**64.3** (15.0)
2.09	**100.9** (24.1)	**100.9** (18.9)	**101.4** (21.2)	**98.6** (16.7)	**103.1** (17.2)	**59.2** (15.8)	**58.7** (16.5)	**56.3** (14.8)	**58.7** (11.8)	**59.2** (10.4)
3.13	**87.4** (19.6)	**87.6** (16.7)	**88.6** (18.6)	**85.3** (16.4)	**89.7** (17.4)	**50.4** (11.9)	**51.1** (12.8)	**48.4** (14.2)	**50.6** (13.2)	**50.3** (10.8)
5.22	*	*	*	*	**79.3** (22.1)	*	*	*	*	**55.4** (17.0)
0 (60° flexion)	*	*	*	*	**145.8** (34.2)	*	*	*	*	*
Grip Strength (Ft lbs)	**29.3** (3.0)	**27.7** (3.2)	**29.0** (3.0)	**28.4** (2.9)	**27.1** (3.4)					

Table A.3: *Mean values (± SD) of force across a range of angular velocities for the Post-menopausal group. All subjects completed the first four testing sessions for leg (Nm) and grip (ft lbs) strength. Only 7 subjects completed every session (>12 months).*

Post.M. Group N=10	EXTENSORS (NM)					FLEXORS (NM)				
Angular velocity (rad/s)	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
0 (90° of flexion)	**122.3** (19.9)	**123.8** (20.8)	**113.5** (22.7)	**109.7** (20.4)	***109.4*** *(27.7)*	*	*	*	*	*
1.04	**127.5** (17.8)	**131.0** (27.4)	**122.8** (27.6)	**116.5** (26.5)	***124.1*** *(20.1)*	**68.2** (16.5)	**70.4** (20.6)	**67.2** (19.5)	**66.7** (19.8)	***69.0*** *(19.3)*
2.09	**90.9** (16.6)	**90.5** (22.1)	**89.7** (20.4)	**86.7** (20.3)	***89.4*** *(13.6)*	**54.1** (13.4)	**55.7** (15.4)	**54.8** (15.7)	**54.0** (15.4)	***52.4*** *(15.3)*
3.13	**76.8** (15.9)	**77.4** (13.8)	**78.0** (17.7)	**75.3** (20.0)	***80.1*** *(12.4)*	**44.8** (12.1)	**44.5** (7.32)	**44.9** (11.5)	**44.7** (11.3)	***47.3*** *(9.7)*
5.22	*	*	*	*	**72.0** (11.5)	*	*	*	*	**50.5** (10.7)
0 (60° flexion)	*	*	*	*	**146.7** (28.4)	*	*	*	*	*
Grip Strength (Ft lbs)	**28.5** (7.1)	**27.2** (6.8)	**27.6** (7.0)	**27.0** (6.6)	***27.4*** *(5.5)*					

Values in *italics* for the last test represents N=7.

APPENDIX THREE

COMMUNICATIONS

Communications rising from this thesis:

Greeves J., Atkinson, G., Reilly, T. and Cable, N.T. (1994) *Are leg measurements of leg strength reproducible at different angular velocities using the LIDO isokinetic dynamometer.* Communication at The 11th International Scientific Students Conference of Hungarian University of Physical Education, 13-14 May 1994, Budapest.

Atkinson G., Greeves, J., Reilly, T., and Cable, N.T. (1994) *Day-to-day and circadian variability of leg strength measured with the LIDO isokinetic dynamometer.* Communication at the Annual Conference of the British Association of Sport and Exercise Sciences, 12-21 July 1994, Aberdeen.

Greeves J. and Cable, N.T. (1994) *Are measurements of knee extensors and flexors strength reproducible at different angular velocities using the LIDO Active dynamometer?* Communication at The Physiotherapy Research Autumn Workshop, 10 November 1994, Manchester.

Greeves J., Biljan, M., Cable, N.T. and Luckas, M.J.M. (1996) *Effects of Acute changes in Oestrogen on Muscle Function of the First Dorsal Interosseus (FDI) Muscle in In Vitro Fertilisation Patients.* Communication at The British Fertility Conference, April 1996, Cardiff.

Greeves J., Cable, N.T. and Reilly, T. (1996) *Hormonal Influences on Muscle Function in Healthy Women Aged 45-55 Years of Different Oestrogen Status.* Communication at The European Congress of Sports Science, May 1996, Nice.

Greeves J., Cable, N.T., Nevill, A.M. and Kingsland, C. (1997) *A longitudinal Analysis of Muscle Strength in Middle-Aged Women of Different Hormonal Status.* Communication at The Physiological Society, March 1997, Dublin.

Greeves J., Cable, N.T., Luckas, M.J.M., Reilly, T. and Biljan, M.M. (1997) Effects of Acute Changes in Oestrogen on Muscle Function of the First Dorsal Interosseus (FDI) Muscle in Humans. *Journal of Physiology*, **500**, 265-270.

Greeves J., Cable, N.T., Nevill, A.M. and Kingsland, C. (1997) A longitudinal Analysis of Muscle Strength in Middle-Aged Women of Different Hormonal Status. *Journal of Physiology*, **501P**, 170P.

APPENDIX FOUR

JOURNAL OF PHYSIOLOGY PAPER

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

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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

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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 ULA000, 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

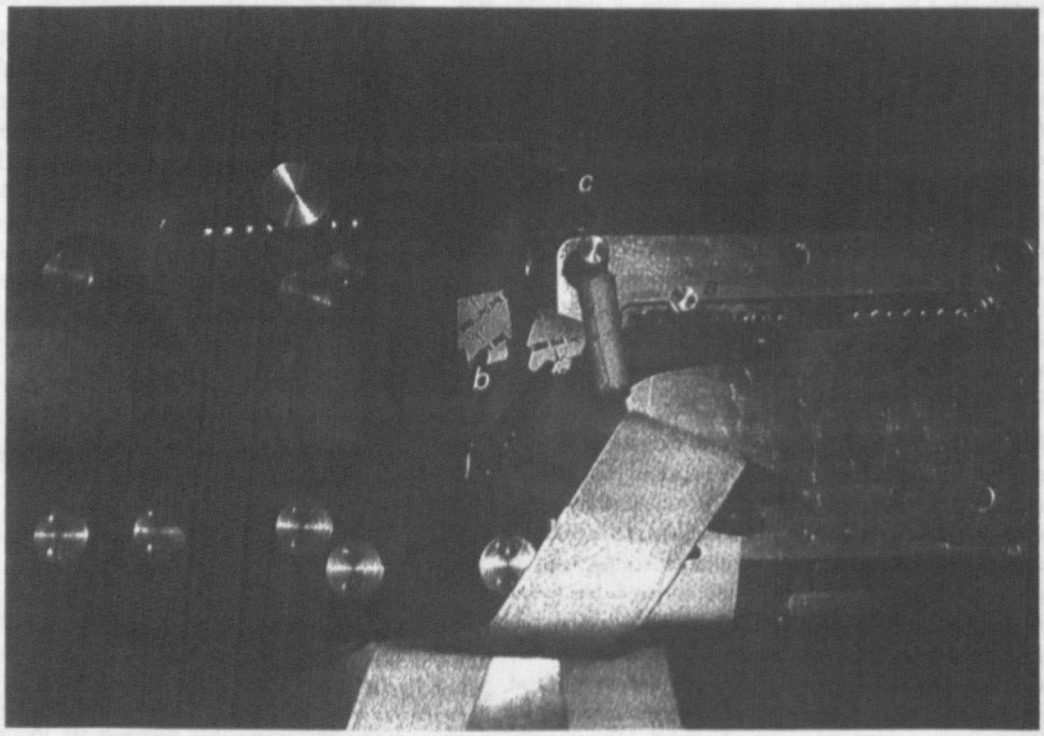

Figure 1

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

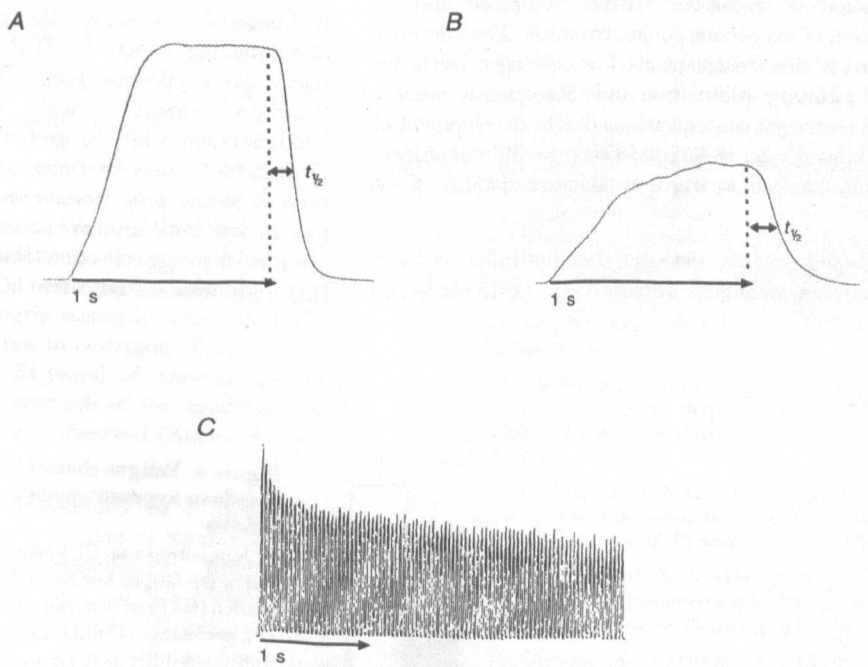

Figure 2. Typical myograms from the fatigue test

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).

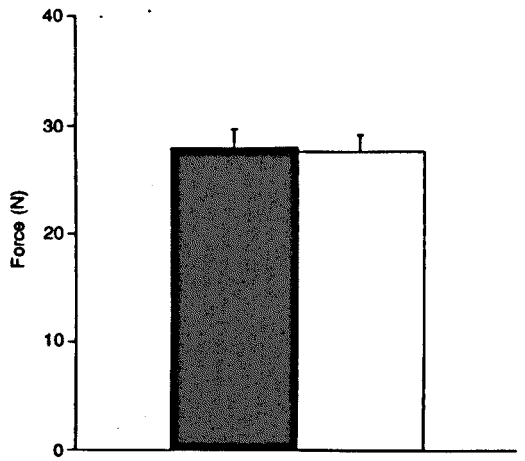

Figure 3

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 (Phillips *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,

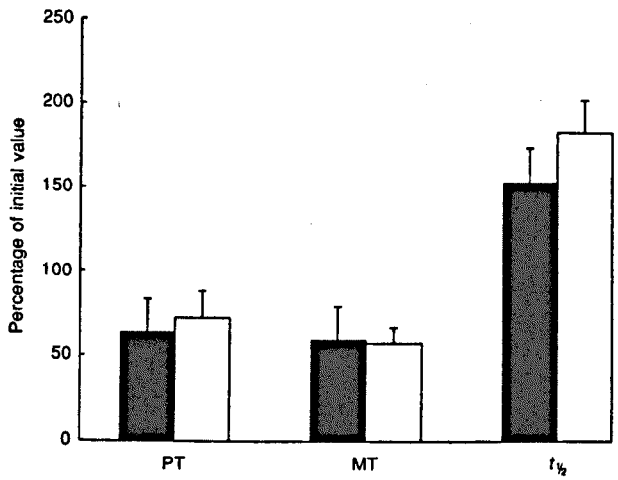

Figure 4. Fatigue characteristics of the FDI muscle in hypoestrogenic and hyperoestrogenic females

■, 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.

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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.

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Received 20 August 1996; accepted 6 December 1996.

APPENDIX FIVE

INFORMED CONSENT FORMS

INFORMED CONSENT FORM

Title: The reliability of day-to-day testing of isokinetic leg strength

Researcher: Julie Greeves

The study aims to evaluate test-retest variation of leg strength using the isokinetic dynamometer at 3 different velocities. You will be required to attend the laboratory on 4 different occasions separated by at least 2-3 days. The first session will familiarise you with the equipment and procedures involved.

You will initially complete a 5 minute warm-up on the cycle ergometer (65 rpm) and 3 minutes of static stretching of the relevant muscle groups (quadriceps and hamstrings). Leg strength will be measured on the computerised isokinetic dynamometer and peak torque of the left and right knee extensors and flexors will be recorded. You will perform two submaximal and four reciprocal maximal voluntary contractions (MVC) at angular velocities of 60, 180 and 300°/s. Each trial will be conducted at the same time of day (10:00±1 hour) for each session.

All information will remain confidential. Feedback on performance will be given after the completion of the experiment.

I.....understand the experimental protocol and have no objection to participating in this study. I also reserve the right to withdraw from testing at any time.

SIGNED..... **DATE.....**

SUBJECT INFORMED CONSENT

Title: The reliability of day-to-day testing of leg and grip strength in post-menopausal women with and without hormone replacement therapy

Researcher: Julie Greeves

Experimental protocol

The aim of the study is to assess the test-retest variation of leg and grip strength in post-menopausal women. One group will be receiving hormone replacement therapy (HRT) and the other group will be oestrogen deficient ie. not receiving HRT.

Initially a questionnaire will be distributed to determine your present health and gynaecological details. You will then be required to visit the laboratory on 3 different occasions each lasting 25-30 minutes, separated by 3-4 days. The first session will familiarise you with the equipment and procedures involved.

You will undertake a 5 minute warm-up on a cycle ergometer at a low resistance, followed by 3 minutes of static stretching of the upper leg muscles. Leg strength will be measured at 3 different speeds of the preferred leg of the knee extensors and flexors for 4 maximal contractions. This will be preceded by 2 submaximal contractions as a practice run. Each trial will be separated by one minute of passive recovery.

Following a 5 minute rest period, you will be required to contract your muscles maximally against a non-movable resistance. To ensure you are working maximally (as hard as possible), electrodes will be positioned on the involved muscle group. This will cause no discomfort or pain. The final test will measure your maximal hand grip strength and the best of 3 trials will be recorded.

You will be free of injury to the lower body and dominant arm, or of pain/discomfort which may debilitate performance. Also, you must refrain from strenuous activity 24 hours prior to testing. It is important that constant exercise levels are maintained throughout the experimental period. Testing will be conducted at the same time of day for each session.

All information will remain confidential. If you would like a fitness assessment ie. Blood pressure, skinfolds (fat measurements) and lung function, this can be arranged (this is not obligatory).

I.....am willing to participate in this study having read and understood the experimental protocol. I reserve the right to withdraw my consent at any time.

SIGNED..... **DATE.....**

INFORMED CONSENT FORM

Title: *Day-to-day variation in* muscle function of the *quadriceps* determined by programmed stimulation electromyography (PSEM) and fatiguability

Researcher: Julie Greeves

This study aims to evaluate the variability in muscle function of the quadriceps assessed using programmed stimulation electromyogram (PSEM) and fatiguability tests. The PSEM test involves delivering electrical impulses of two second duration at frequencies of 1, 10, 20, 50 and 100 Hz. Fatiguability will be measured with 40 Hz impulsed per second for 3 minutes. You will be required to visit the laboratory on three occasions. The first sesstion will be a familiarisation trial with the electrical stimulation techniques. This will be followed one week later by two visits, separated by 3-4 days. It is important that exercise is not undertaken on the day of testing as this will affect performance. Exercise should also be controlled the day preceding the trial.

The test protocol will involve:-

- 5 minutes warm-up on the cycle ergometer at 60 rpm
- PSEM
- 5 minute rest
- Fatigue test of 40 Hz/s with one second rest intervals
- PSEM

The PSEM test will cause some discomfort. If this is not tolerated, the experiment will be terminated. There may be some muscle soreness the following day and thus tests will be scheduled carefully.

SUBJECT DECLARATION

I.....understand the experimental protocol and have no objection to participating in the study. I also reserve the right to withdraw from testing at any time.

SIGNED..... **DATE.....**

EXPERIMENTERS SIGNATURE.....

SUBJECT INFORMED CONSENT FORM

Title: The Reliability of the hand dynamometer for measuring Strength and Contractile Properties of the First Dorsal Interosseus Muscle (Index Finger)

Researcher: Julie Greeves

The aim of this study is to determine the reliability of repeated measurements of maximal strength and contractile properties of the first dorsal interosseus (FDI) muscle. The FDI functions to move the index finger away from the middle finger. You will be required to visit the laboratory on three occasions at 2 to 3 day intervals.

The following tests will be undertaken on each visit:-

- The hand will be immersed in hot water for 10 minutes to stabilise the temperature of the finger
- Three maximal voluntary contractions with the index finger - (pushing as hard as you can away from the middle finger)
- Five minute rest
- Electrical stimulation test, whereby the finger is stimulated at increasing frequencies. Each frequency will last 2 seconds, with a 5 second rest interval.
- Five minute rest
- Three minute fatigue test. The muscle will be fatigued for 3 minutes with one second interval impulses.

Each test session will last approximately 30-35 minutes. If the electrical stimulation is not tolerated, the experiment will be terminated. All information will be confidential.

SUBJECT DECLARATION

I.....AM WILLING TO PARTICIPATE IN THIS STUDY, HAVING READ AND UNDERSTOOD THE EXPERIMENTAL PROTOCOL. HOWEVER, I RESERVE THE RIGHT TO WITHDRAW FROM TESTING AT ANY TIME WITHOUT FURTHER EXPLANATION.

SIGNATURES:-

Subject:..... Date:.....

Experimenter:.....

LIVERPOOL JOHN MOORES UNIVERSITY

INFORMED CONSENT FORM

Researcher: Julie Greeves

Title: The Effect of Hormone Replacement Therapy on Muscle Strength in Postmenopausal Women

Experimental Protocol

The aim of this project is to determine whether a decline in the reproductive hormone **oestrogen** associated with the menopause, causes a reduction in the strength of the quadriceps (thigh) muscle. Differences in strength will be compared in two groups of postmenopausal women: One group will be receiving hormone replacement therapy (HRT) and the other group will be oestrogen deficient ie. not receiving, or have not received any form of HRT.

Initially, a questionnaire will be distributed to determine your present health, family medical history and gynaecological details. Following this, you will be asked to attend the laboratories at John Moores University, The Royal Liverpool University Hospital and The Liverpool University, prior to HRT and once every three months over a 12 month period.

Body composition will be assessed at John Moores University. This will involve height and weight, and skinfold measurements taken at four sites of the body ie. front and back of the arm, above the hipbone and under the shoulder blade. Blood pressure, grip and leg strength and venous blood samples will also be taken. Grip strength will be measured using a hand-held device, and leg strength will be determined whilst performing a kicking action and contracting against a non-movable resistance. To ensure you are working maximally (as hard as possible), electrodes will be positioned on the involved muscle group. This will cause no discomfort or pain. Blood will be drawn from the antecubital vein (of the forearm) to determine hormonal levels. For subjects receiving HRT, blood samples may possibly be taken at the gynaecology clinic.

All information will be kept strictly confidential and your identity will not be revealed in any way. If you would like a fitness test then this will be arranged.

SUBJECT DECLARATION

I ..... am willing to participate in the study having read and understood the experimental protocol. I am aware of the length of time the experiment will last and reserve the right to withdraw my consent at any time,

SIGNATURES: SUBJECT..... *DATE .....*

UNBIASED WITNESS.....

EXPERIMENTER.....

INFORMED CONSENT FORM

Researcher: Julie Greeves

Title: The effects of the menstrual cycle on strength and contractile properties of a distal and proximal muscle group

Experimental protocol

1. Each stage of the menstrual cycle will be determined by monitoring oral temperature. This will be taken on awakening each morning (± 2 hours) for two complete cycles using a digital thermometer provided. Instructions will be given separately. The data will be recorded on the sheet provided, and temperature readings will be plotted on the attached temperature chart starting from day 1 of menses. A questionnaire will also be completed prior to testing.

2. You will be required to attend the research laboratory at John Moores University for 8 visits. These include:
i] A familiarisation session with the electrical stimulation procedure and finger strength technique.
ii] Once during menses (between day 1-5)
iii] Mid-follicular (day 12)
iv] Pre-ovulation (day 14)
v] Ovulation
vi] Post-ovulation
vii] Mid-luteal
vi] Late-luteal
On each visit you will be monitored as follows:-

Strength measurements

Index finger: Maximal strength of the index finger will be measured during a pushing movement away from the middle finger. To ensure maximal effort is employed, the muscle will be stimulated percutaneously using electrodes attached to the hand. This will cause minimum discomfort.

Quadriceps: You will be secured in a chair with the leg suspended at 90°. The quadriceps will be electrically stimulated during a maximal effort of pushing against a non-movable resistance. The muscle group will then be stimulated whilst relaxed at increasing frequencies (1, 10, 20, 50 and 100 Hz) for 2 seconds at each frequency. You will be rested for 5 minutes and the procedure will be repeated 6 times in 30 minutes. This test will cause some discomfort and may affect results if a familiarisation session is not performed.

All information will be confidential.

I .....agree to participate in this study having read and understood the experimental protocol as described above. I do, however, reserve the right to withdraw from testing at any time without further explanation.

SIGNED..... DATE.....

INFORMED CONSENT FORM

Title: The Effect of Oestrogen Changes on Strength and Contractile Properties of the First Dorsal Interosseus Muscle (Index Finger)

Researcher: Julie Greeves (BSc Hons)

The aim of this study is to determine whether a decline in the reproductive hormone *oestrogen* causes a change in strength and contractile properties of the first dorsal interosseus (FDI) muscle. This muscle functions to move the index finger away from the middle finger. Differences in performance will be monitored before, during and after treatment. Maximal voluntary effort and electrical impulses of increasing frequencies to the muscle (called programmed stimulation electromyogram (PSEM)), will be performed.

The test protocol will involve:-

i] Immersion of the hand and forearm in water for 10 minutes at 45° to stabilize muscle temperature

ii] One Hz twitches (electrical impulses) delivered to the finger muscle via electrodes to ensure correct stimulation of the muscle

iii] Maximal voluntary contraction superimposed with 1 Hz twitches

iv] A PSEM (increasing frequencies of electrical impulses of 1, 10, 20, 50, 100 Hz and 1 Hz for 2 seconds each)

v] Fatigue test of frequent stimulation for 3 minutes

vi] A PSEM

A rest period of 3 minutes will be given between each phase of testing. The PSEM test will cause some discomfort. If this is not tolerated, the experiment will be terminated. All information will remain confidential. The test will last approximately 20-30 minutes.

SUBJECT DECLARATION

I.....understand the experimental protocol and have no objection to participating in this study. I also reserve the right to withdraw from testing at any time.

SIGNATURES

SUBJECT.....
DATE.....

EXPERIMENTER.....

UNBIASED
WITNESS.....

SUBJECT INFORMED CONSENT FORM

TITLE: The effects of hormone replacement therapy on muscle function of the first dorsal interosseus muscle (FDI) in post-menopausal women before and during treatment.

The aim of this study is to assess the effect of hormone replacement therapy on muscle function of the index finger (first dorsal interosseus). You will be required to visit the laboratory on 3 occasions over 4 months. The first visit will follow your appointment at the menopause clinic. You will then be asked to attend the laboratory for two visits four months later, following a routine visit to the menopause clinic. The following tests will be undertaken on each visit:-

- The hand will be immersed in hot water for 10 minutes to stabilise the temperature of the finger
- Three maximal voluntary contractions with the index finger - (pushing as hard as you can away from the middle finger)
- Five minute rest
- Electrical stimulation test, whereby the finger is stimulated at increasing frequencies. Each frequency will last 2 seconds, with a 5 second rest interval.
- Five minute rest
- Three minute fatigue test. The muscle will be fatigued for 3 minutes with one second interval impulses.
- Skinfolds, estimating your percentage body fat, will also be assessed at four sites:- the front and back of the arm, shoulder blade and hip bone.

Each test session will last approximately 30-35 minutes. If the electrical stimulation is not tolerated, the experiment will be terminated. All information will be confidential.

SUBJECT DECLARATION

I.....AM WILLING TO PARTICIPATE IN THIS STUDY, HAVING READ AND UNDERSTOOD THE EXPERIMENTAL PROTOCOL. HOWEVER, I RESERVE THE RIGHT TO WITHDRAW FROM TESTING AT ANY TIME WITHOUT FURTHER EXPLANATION.

SIGNATURES:-

Subject:..... Date:.....

Experimenter:.....

Unbiased witness:.....