MUSCULOSKELETAL SYSTEM IN TURNER'S SYNDROME

MUSCLE MORPHOLOGY, FUNCTION AND BONE MINERALIZATION IN GIRLS WITH TURNER'S SYNDROME

BY

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ABSTRACT

The purposes of this research were i) to compare skeletal muscle development, function and bone mineralization in girls with Turner's syndrome (TS) (n=7) and healthy control girls (n=13), and ii) to examine the effects of growth hormone (GH) and estrogen (E2) therapy on musculoskeletal variables using a case study approach in two TS girls and one healthy control.

Anthropometric measurements included: height, body mass, percent fat, and muscle and bone cross-sectional areas and muscle density from computed axial tomography. Evoked peak twitch torque (TT), maximal voluntary strength (MVC), contractile properties and motor unit activation (MUA) were determined for the elbow flexors (EF), plantar flexors (PF) and the knee extensors (KE). Total body and segmental bone mineral content (BMC) and density (BMD) were measured with dual photon absorptiometry. Dietary intake and participation in physical activity were assessed from questionnaires.

Absolute strength (TT and MVC) for the TS patients was lower than that of the control girls' for EF, PF and KE and could not be accounted for by differences in muscle density, contractile properties, MUA, diet or level of physical activity. There were no significant differences in evoked and

voluntary strength corrected for muscle area and lever length between the TS and control girls. Total body, leg and trunk BMC were lower in the TS girls compared to the controls; however, when normalized for body mass and bone width, total body BMC (g/kg) and BMD respectively were comparable between the TS and control girls.

Growth hormone therapy increased height and lean mass, and reduced adiposity. All measures of arm strength increased but leg strength (PF & KE) was reduced. These may reflect the lack of GH effects on the leg muscle or possibly a detraining effect from the subject's withdrawl from a skating program. Growth hormone therapy resulted in increased leg BMC which may reflect a lag time between bone growth and subsequent mineralization.

Estrogen therapy resulted in increased muscle area, fat mass and strength at all 3 muscle groups. The latter may be due to the laying down of muscle proteins as a result of estrogen therapy. The lack of major changes in BMC or BMD probably reflects the short duration of the follow-up period.

Further studies are required with larger numbers for longer treatment periods in order to make conclusive statements about the effects of hormonal therapy on muscle function and bone mineralization in Turner's patients.

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Introduction

A. Description of Turner's Syndrome

Turner's syndrome (TS) occurs as a result of a genetic abnormality caused by the loss of all or part of one X chromosome. The main features include short stature, sexual infantilism, ovarian dysgenesis and associated congenital malformations (shield chest, webbing of the neck, low posterior hairline, skeletal abnormalities and peripheral lymphoedema; Haddad & Wilkins, 1959; Lemli & Smith, 1963). The phenotype depends on the extent and localization of the deletion of the X chromosome. Complete Turner's syndrome (monosomy 45,XO) occurs when the entire X chromosome is deficient. Turner's mosaicism (structural abnormalities or a partial deletion of the X) exists in numerous forms such as 45,XO/46,XX; 45,XO/46Xi(Xq) or 45,XO/46XY (Lippe, 1987). Turner's syndrome is one of the most common chromosomal abnormalities with a frequency at conception estimated at 1.5% (Connor & Loughlin, 1989). The vast majority of affected fetuses, however, are spontaneously miscarried and the residual birth frequency is between 1/2500 and 1/3500 females (de la Chappelle, 1983).

Short stature (less than 2 standard deviations below the mean for height) occurs in nearly 100% of children with 45, XO karyotype and in 75-100% of the children with mosaicism (Ferguson-Smith, 1965; Park et al 1983). Rappaport and Sauvion (1989) reviewed numerous reports on stature in TS and found the range in adult height achieved without therapeutic intervention to be between 143cm and 146cm, depending on mid-parental height (the average of the mother's and father's heights). Besides their diminished long bone growth there are numerous reports of skeletal abnormalities such as shortening of the fourth metacarpal, epiphyseal dysplasia, reduced bone mineralization and osteoporosis (Finby & Archibald, 1963; Shore et al, 1982; Zseli et al, 1986; Stepan et al, 1989).

Obesity as defined by clinical impression has been mentioned frequently as a characteristic of TS girls (Lippe, 1987). Yet Delgado and colleagues (1986) measured per cent body fat (% BF) in 14 TS females and found that 7 had slender phenotypes and 7 were obese. Elliott and Cheek (1968) using open muscle biopsies found that TS girls had a significantly greater percent of fat per unit muscle when compared to normal females ($5.2 \pm 2.16\%$ vs $2.6 \pm 1.4\%$).

Physical activity patterns have not been critically evaluated in this population. Some investigators, such as Warkany (1971) suggest that TS females are quite often robust despite their endocrine and growth problems. Yet others such

as Drash and colleagues (1968) claimed that TS girls manifest low levels of activity.

Muscle size and function have only been briefly alluded to in girls with Turner's syndrome. Cheek (1968) noted that TS girls had a reduction in muscle cell population when compared to age matched controls but when expressed relative to height there were no significant differences between the groups. Tanner and colleagues (1971) noted a reduction in limb muscle width of -1.12 standard deviations (SD) below the mean, yet they only measured 2 girls. Maximal voluntary strength of the elbow flexors (EF) and knee extensors (KE) in 5 TS girls (ages 11.5 to 15.5 years) was recently measured by Parker (1990). She found that EF strength of these girls was normal for their age, and the KE's were weaker in relation to age but of normal strength when related to height.

B. Hormone Profile in Turner's Syndrome

An increased rate of death of ovarian germ cells occurs postnatally in Turner's patients (Fedeerman, 1987) and by age 4 their ovaries are merely streaks of tissue (Massarano et al, 1989). Thereafter, urinary and serum estrogen levels are undetectable in both monosomy and mosaic TS patients (Almqvist et al, 1963; Ross et al, 1983 & 1988). This is manifested phenotypically by sexual infantilism. Numerous investigators believe that this chronic estrogen deficiency in

TS females is the etiologic factor in their reduced bone mineralization, osteoporotic-like bones and lack of a pubertal skeletal growth spurt (Brown et al, 1974; Park, 1977; Ranke et al, 1986; Stepan et al, 1989).

Growth hormone (GH) values in TS girls, in response to various physiological and pharmacological stimuli, have been reported to be either normal or elevated (Donaldson et al, 1968; Root et al, 1967; Kaplan et al, 1968; Tzagournis, 1969) or reduced (Brook, 1978; Butenandt, 1980; Laczi et al, 1979). Levine-Ross and colleagues (1985) attempted to clarify this controversy by examining the serum 24-hour GH response in females ages 2 to 20 years old. They found that by 9 years of age the TS girls had significantly lower mean 24 hour GH levels, a reduction in peak frequencies and peak amplitudes of GH secretion. This perturbed GH profile is hypothesized to contribute to their reduced skeletal growth (Almqvist et al, 1963; Laczi et al, 1979: Lin et al, 1988; Kirkland et al, 1990).

Somatomedins (SM) are the critical link between GH release and the anabolic actions of GH at the tissue level. Somatomedin-C (SM-C), the most powerful growth stimulator, has been extensively measured in TS girls but the findings are not consistent. Low levels of SM-C have been reported (Ross et al, 1985; Martin et al, 1977). However, others (Cuttler et al, 1985; Ranke et al, 1987) claim that the low levels of SM-C do

not occur until after the age of 10 years and prior to this GH levels appear to be normal. Despite the growth retardation in TS females, numerous investigators have demonstrated normal basal serum SM-C concentrations (Daughaday & Parker, 1963; Almqvist et al, 1963; Rudman et al, 1981; Takano et al, 1986). This discrepancy may be due to the fact that circulating SM levels may only approximately reflect the local concentrations or activity of SM's in various tissues (Bala et al, 1981). As well, since SM-C is regulated through a feedback system an acute high or low value may only be a transient response to a previous stimulus.

It has been demonstrated that TS females have a normal rise in SM-C concentrations after administration of a standard dose of exogenous human growth hormone (hGH), which suggests that they have a normal capacity for SM synthesis and release (Cuttler et al, 1985). Additionally, Rosenfeld and coworkers (1983) reported normal SM-C binding, normal thymidine incorporation and cell replication after SM-C stimulation in TS girls suggesting that there is no evidence of peripheral resistance to SM's in these patients.

Other hormones important for growth such as insulin, parathyroid and thyroid hormones are characteristically normal in TS patients (Almqvist et al, 1963; Donaldson et al, 1968).

C. Skeletal Growth in Turner's Syndrome

The impaired skeletal growth in these patients has been divided into three phases (Ranke et al, 1983 & 1988). Intrauterine growth is slightly retarded with a reduction of about 1 SD in birth weight and height. After birth and up to the age of 3 years, there is a decline in the ratio of bone age to chronological age but a normal growth velocity occurs. Then after the age of 3 years, height velocity deviates from normal values reaching the lower limit by the age of 10. Finally at puberty, the lack of an adolescent growth spurt puts the TS females even further behind their peers in terms of height. Additionally, Beals (1973) noted a decline in their bone age progression reaching a maximum of a 2-year delay by age 16, with late epiphyseal closure in the early twenties.

Specific skeletal abnormalities include a less dense appearance of the bones with an abnormal trabecular pattern (Park, 1977), epiphyseal dysplasia (Finby & Archibald, 1963), low bone mineralization (Stepan et al, 1989) and osteoporosis (Zseli et al, 1986). TS patients also have an abnormal development of the facial skeleton (micrognathia) which bears some similarity to the facial development of children with growth hormone deficiency (GHD; Lippe, 1987).

In reviewing these studies, most were done on TS adults and many did not control for their delayed skeletal age, bone width or estrogen status. Many of the reports on

children used older, less precise tools for quantification of bone mineralization. In Beals' (1973) report on children he admits that osteoporosis is difficult to quantitate and is a subjective diagnosis. This probably reflects his use of radiography which is not precise enough to accurately assess osteoporosis (Webber, personal communication). Brown and colleagues reported a significantly decreased bone density of the midshaft of the radius in 6 out of 8 patients (1974), but they did not control for the delayed skeletal maturation when comparing TS girls with controls. As well they assumed that the estrogen profiles were comparable between the 9 and 19 year old patients, thus rendering invalid their assumptions as to the causative role of estrogen deficiency in bone density. Stepan and coworkers (1989) recently documented a reduced spinal bone mineral density (BMD) in adult TS women as compared to age- and sex-predicted means. They also examined the effect of estrogen therapy on BMD and found that the treated group had the highest BMD, followed by the insufficiently treated (irregular and low dose therapy) and the untreated patients. Shore and colleagues (1982) found a decrease in bone mineral content (BMC) of 25.4% having controlled for age, gender, height and bone width. Their groups of TS patients included those on estrogen therapy who had an increase in height but no subsequent increase in radial BMC. All but two of their patients were older than 14 years of

age which does not provide any details about bone development during puberty.

A perturbed GH secretion and absence of ovarian estrogens have been postulated as causative factors for the abnormal skeletal growth and development in TS patients. However growth is influenced not only by hormones but also by nutritional intake and levels of physical activity, neither of which have been carefully measured in this population.

D. Nutrition and Growth

The musculoskeletal growth of children is influenced by nutrition and the nutritionally derived serum growth factors (Brook, 1988).

D.1 Effects of Nutrition on the Skeleton

Optimal skeletal integrity requires adequate dietary calcium to maintain calcium homeostasis and prevent excessive resorption from bones (Sandler, 1988). A decreased dietary intake of calcium has been implicated as one of the factors in the pathogenesis of osteoporosis (Brown et al, 1974). Vitamin C supplementation has resulted in higher dry bone weights and improved breaking strength of long bones in rats (Weiser et al, 1989). Animal studies have also shown that vitamin D_3 is necessary to mineralize bone and maintain normal serum and tissue concentrations of calcium (Baylink et al, 1970; Canas

et al, 1969).

Children with protein-calories malnutrition (Kwashiorkor) have low SM activity and poor growth (Rallsion, 1986) which is due to the fact that protein deficiency prevents SM's from stimulating cartilage production (Phillips & Unterman, 1984).

The adequacy of nutrient intake in TS patients has only been briefly described. Cheek (1968) observed a reduced caloric intake in TS girls and claimed that this was probably a function of their decreased growth rate.

D.2 Effects of Nutrition on Muscle

Energy needs during cell growth are higher than normal and thus when caloric intake is restricted, cell multiplication ceases (Cheek, 1968). Protein deprivation without caloric deprivation leads to a reduction in cell size and muscle mass in rats (Mendes & Waterlow, 1953). A lack of nutrients results in a reduced body weight, skinfold thickness and arm muscle circumference, in humans (Jeejeebhoy, 1986). An adequate protein intake is essential for growth hormone to exert its anabolic effects (Pruden et al, 1956; Stearns et al, 1958). Hypocaloric feeding in obese adults, (21 days of dieting) induced changes in muscle ultrastructure, as evidenced by fibre atrophy and degeneration of the Z bands (Russell et al, 1983 & 1984). The effects of these structural

changes were a slower rate of muscle relaxation, reduction in force output and a greater fatiguability (Jeejeebhoy et al, 1986; Russell et al, 1983).

E. Physical Activity and Growth

E.1 Effects of Physical Activity on the Skeleton

Physical activity is important for normal musculoskeletal growth. During growth, skeletal mass increases 20 times from the newborn to the adult and muscle mass increases 40 times (Urist, 1980). Activity level and exercise are, for healthy young persons, major factors in the regulation of bone density in weight bearing and heavily loaded bones (Whalen et al, 1988). This is based partially on the findings that skeletal loading is mediated by muscle contractions (Sandler, 1988). More specifically transduction, the physical process of converting mechanical stimuli (stress/strain) into a cellular response, is a fundamental event in the physiological control of bone modelling or remodelling (Drachman, 1974).

The concept of muscle contractions mediating skeletal remodelling has been demonstrated in adult athletes from various sports such as tennis, weight lifting and running. Typically these athletes have increased BMC with site specific bone hypertrophy. This occurs in the first lumbar vertebrae in runners (Lane, 1986), in the dominant humeral bone of tennis

players (Jones et al, 1977) and in the femur of weight lifters (Nilson & Westlin, 1971).

The effects of physical activity on bone mineralization in children has only recently been examined since most research in this area has dealt primarily with post menopausal women. Onoe et al (1988) examined the influence of anticonvulsant therapy for epileptic children and their level of physical activity on bone mineralization. They found that physical activity and the duration of treatment were related to the prevalence of delayed bone development, which is indicative of a reduced bone mineral content. Actually, the more limited the physical activity, the more likely that bone development was delayed. However there were only 3 general categories for physical activity; fully ambulatory, ambulatory (walking but with cerebral palsy or mental retardation) and non ambulatory (inability to sit).

Gilsanz and colleagues (1989) discovered a lower vertebral bone density in children with Tarsal Coalition. These children are forced to limp and place more weight on one leg because of painful ambulation or an unequal leg length. Thus the authors suggested that the reduced bone density is probably a consequence of the reduction in weight bearing physical activity in the abnormal limb.

Bone loss in children is accelerated with a decrease in physical activity because of the greater trabecular bone

turnover (Montoye, 1987). Yet the optimum level of mechanical loading or physical activity to induce bone mineral deposition in children is unknown.

E.2 Effects of Physical Activity on Muscle

Early biochemical studies on growing animals undergoing regular physical training indicated a significant rise in skeletal muscle DNA concentration above that expected for normal growth (Buchanan & Pritchard, 1970; Bailey et al, 1973; Hubbard et al, 1974). Muscle hypertrophy following periods of exercise training is accompanied by an increase in contractile substances (Helander, 1961), myofibrils (Goldspink, 1964), enzyme activity (Holloszy, 1967) and absolute strength.

In children, Parizkova (1977) found that the most active boys had significantly more lean body mass and less fat than did the least and moderately active boys.

Numerous researchers have demonstrated increases in strength in response to resistance exercise in the prepubescent age group. Weltman and coworkers (1986) trained 16 prepubertal males 3 times per week for 14-weeks using hydraulic resistance equipment. There was an increase in the average isokinetic work of 18.5% to 36.6% in the trained group. Yet there were no significant changes in anthropometric parameters with training.

Vrijens (1978) used a strength training program of concentric isotonic exercises at ± 75% of maximum performing 8 to 12 repetitions, 3 times per week for 8 weeks. He found no improvements for the 16 prepubertal boys in their upper and lower extremities, whereas isometric strength of the trunk muscles increased significantly. The lack of strength development in the extremities was confirmed by the unchanged lean cross-sectional areas of the arm and thigh. However the 12 adolescents in the post-pubertal group had an increased strength in all muscle groups tested, as well as a significant increase in muscle cross-sectional area.

Ramsay and coworkers (1989) found that progressive resistance training performed 3 times per week for 20 weeks in 13 prepubertal boys resulted in a significant increase of isometric elbow flexion (37%) and knee extension (25% at 90° and 13% at 120°) strength. However there were no significant effects of training on muscle cross-sectional area.

Sewall & Micheli (1986) followed 18 boys and girls, between 10 and 11 years of age, in a progressive resistive strength training study, 3 times per week for 9 weeks. They performed 3 sets of exercises at 50, 80 and 100% of a 10-repetition maximum, with training sessions lasting 25-30 minutes. The study group showed significant gains in shoulder flexion when compared to the control group. There were no significant differences in knee extension or shoulder

extension, between the groups. It was not possible to examine the effect of gender since the groups were not separated for statistical analyses.

Siegel et al (1989) studied girls (n=24) and boys (n=26) with a mean age of 8.4 years (± 0.5 S.D.) involved in 12 weeks of strength training concentrating on upper body resistance exercises. Following the training period, significantly greater gains were made by the experimental group for right handgrip, flexed arm hang and pull-ups. The training response was similar for boys and girls.

Funato et al (1987) examined the effect of strength training in prepubescent boys and girls on muscle strength and cross-sectional area of upper arm muscle. For the girls they found an increase in muscle area but the increase in strength occurred only during isometric elbow flexion and extension for the first grade girls. Muscle strength per cross-sectional area did not increase with training in the girls.

Thus it appears that prior to puberty strength gains are possible in girls and boys with resistance training. Whether there is a concomitant gain in cross-sectional area, however, requires further study.

The relationship between hypoactivity and muscle strength in children has not been widely studied. However, in adults it is known that inactivity will reduce muscle protein synthesis and cause muscle wasting (Shonheyder et al, 1954).

F. Hormones and Normal Growth

F.1 Growth Hormone

Growth hormone is a protein consisting of 191 amino acids. Despite being very similar in structure to human GH (hGH), animal GH from sheep, cattle or pigs is ineffective in man (Marshall, 1977), thus it is species specific. Growth hormone is secreted from the anterior pituitary cells, somatotropes, by a complex interaction of stimulatory and inhibitory neural influences (Martin et al, 1973). This control occurs by at least two hypothalamic hormones; growth hormone releasing hormone (GRH) and growth hormone inhibiting hormone (somatostatin) via a short-loop feedback system (Sakuma & Knobil, 1970).

There are numerous physiologic, pharmacologic and pathologic factors which stimulate or inhibit GH secretion (Martin et al, 1977). Growth hormone has both direct and indirect actions; the former effect is the anti-insulin like action which enhances lipolysis, decreases glucose oxidation and promotes cellular uptake of amino acids (Daughaday et al, 1975). The indirect actions of GH are mediated by insulin-like growth factors called somatomedins (SM or IGF). Somatomedins are small peptides formed in organs such as the liver, lungs and heart and in target tissues under the stimulation of GH (D'Ercole et al, 1984). Somatomedin-C (or IGF-I) is of major interest because it is a critical link between GH release and

its anabolic actions, and it is the most powerful growth stimulator. As well it is considered analogous to insulin because it has close structural and functional homologies (Kaplowitz, 1987).

Relatively few studies have clearly defined the normal profile of GH release during childhood and adult life (Thorner & Vance, 1988). This is partly due to problems in separating subjects by age and gender. Results have been expressed as absolute values, total 24 hour secretion rates or peak GH responses, which prevents comparisons from being made between studies.

Growth hormone secretion is markedly elevated in the immediate neonatal period (30-180 ng/ml) then it appears to be low during infancy and increases during early childhood to a low prepubertal level of 2.9 $\pm$ 0.2 ng/ml or a peak of 18 ng/ml (Glick et al, 1965; Ross et al, 1985; Shaywitz et al, 1971). At puberty GH secretion is greatly enhanced with a peak of 64 ng/ml. This marked increase occurs throughout the 24-hour period. As well, the daily secretion rate and the duration of secretion are markedly increased (Finklestein, 1972). Growth hormone decreases in late adolescence and remains stable until about 30 years (7.3 ng/ml or a mean peak GH of 30 ng/ml). Then a progressive decline ensues and by late adulthood (>50 yrs) the average peak serum GH is reduced dramatically to less than 4 ng/ml (Rudman et al, 1981).

Women have a greater GH secretion than men in both younger (18-33 yrs) and older age groups (>55 yrs). Stepwise regression analysis using the independent variables; age, gender, body mass index (BMI) and estradiol concentration, demonstrated that when the effect of estrogen was removed there was no significant correlation between GH secretion and age, gender or BMI (Ho et al, 1987). This provides evidence for the stimulatory effect of estrogen on GH secretion which is thought to occur via a stimulatory or permissive role of estrogen on somatotrope secretion (Thorner & Vance, 1988).

Somatomedins are low at birth and increase progressively to a maximum at puberty which coincides with the adolescent growth spurt (0 to 2 years old, 0.4 $\pm$ 0.04 U/ml; 11-14 years, 2.81 $\pm$ 0.4 U/ml; Rosenfeld et al, 1983; Ross et al, 1985). Luna and co workers (1983) found that the increase in SM-C/IGF-I levels corresponded better with children's Tanner stage than with their chronological age. After puberty there is a significant decrease to low levels which are maintained throughout adulthood (Luna et al, 1983).

It has been demonstrated in both rats and rabbits that cartilage is much more sensitive to the anabolic effects of GH and SM in young organisms. Thus it is conceivable that the low levels of GH and SM in small children are sufficient for supporting their high growth rate (Hall & Frykland, 1979). Radioreceptor assays for SM-C show that the mean levels of SM

are significantly higher in patients with acromegaly (a condition of supraphysiological GH levels) than in healthy controls; and significantly lower in patients with growth hormone deficiency (Marshall et al, 1974; Hall & Luft, 1974).

F.2 Effects of Growth Hormone on Muscle

In isolated muscles, two groups reported that the rat soleus has receptors for the insulin-like growth factor I (IGF-I) and these receptors respond to GH by exhibiting an increase in glucose uptake with a reduced glucose oxidation, enhanced lipolysis and increased cellular uptake of amino acids (Poggi et al, 1979; Yu & Czech, 1984).

Bigland and Jehring (1952) found that growth hormone treatment in healthy rats resulted in a greater growth rate of the proximal muscles (gastrocnemius) than the distal muscles (soleus and tibialis anterior). The treated muscles were 15-40% heavier and had fibres 6-20% greater in cross-sectional area than the controls. However GH therapy did not confer any functional advantage as evidenced by a larger increase in tetanic tension in the controls compared to the treated muscles. They claimed that the new substance laid down was not contractile material and actually impaired the efficiency of the contractile mechanism.

Apostolakis and colleagues (1980) immobilized adult rat hind limbs for 15 days by insertion of a metal pin, then

administered GH for 2 weeks. They found an increase in electrical myographic (EMG) activity of atrophied muscle by 73%, an increase in isometric twitch tension by 58% and restored work capacity by 44% compared to the atrophied untreated group.

In humans the absence of circulating GH in growth hormone deficient (GHD) patients results in a decreased muscle mass as noted by reduced circumference measures (Tanner et al, 1971). Preece and coworkers (1987) examined the effect of the withdrawal of GH in GHD males (18 & 19 years old) who had received therapy for 11 and 8 years respectively. The 18 year old patient, was severely GH deficient and had a reduction in strength and muscle area following withdrawal of treatment. The 19 year old subject, who was only partially GH deficient, however, had no reduction in muscle strength or area with the cessation of GH treatment. The authors thought that this may also reflect the fact that this male was quite robust and may have maintained strength because he was involved in a weight training program.

In acromegalic patients the supraphysiological levels of GH lead to an initial increase in muscle power and bulk. However with time proximal muscle weakness and easy fatiguability occur (Shy, 1967). This is probably due to a disturbance in muscle integrity as a result of an accumulation of glycogen and other non contractile substances.

F.3 Effects of Growth Hormone on the Skeleton

Growth hormone increases the activity of osteoblasts and osteocytes which leads to bone formation (Cameron & Sorenson, 1963). This occurs both directly and indirectly via the somatomedins.

Studies of GH infusion in dogs have found an increased mineral incorporation in the skeleton (Mankin, 1978; Heaney et al, 1972). As well increases in both bone formation and resorption have been reported in acromegalic subjects (Riggs et al, 1972) which were directly proportional to serum GH levels. In these patients there are also reports of hypertrophy of the frontal bones, carpal tunnel syndrome and increases in the area of the flat bones and the shaft width of long bones (Rasmussen & et al, 1974; Melmed, 1987 & 1990).

Children with GHD manifest a reduction in bone mineral content (Shore et al, 1980) and with 3 months of GH treatment there is a marked increase in the width of the band of calcifying tissue at the radial metaphyseal-epiphysis junction (Edlin et al, 1976).

F.4 Growth Hormone and Somatomedin Receptors

A detailed characterization of GH receptors has been limited to hepatic receptors in rats and rabbits (Posner et al, 1974) and the human lymphocyte line, IM-9 (Lesniak et al, 1974). The cellular receptors for GH are widely distributed in

target tissues such as skeletal tissue, kidney, liver, adipose tissue and the adrenal cortex (Schulster & Levitzki, 1980). The distribution probably reflects a role of these organs in the metabolism and removal of GH from circulation.

F.4i Growth Hormone and Somatomedin Receptors in Muscle

In rat diaphragm muscle, Lev and Holland (1986) noted specific binding of GH to culture cells. However, the location of the binding site remains unclear. In vitro work by Dodson and coworkers (1987) demonstrated that SM-C from sheep interacts with membrane receptors on ovine skeletal muscle fibroblasts. Beguinot et al (1985) confirmed the finding of IGF-I receptors in cultured rat skeletal muscle cells. Knowing that IGF stimulates uptake of hexose and amino acids and promotes DNA synthesis, the presence of IGF-I receptors may represent an important event in skeletal muscle development (Rechler et al, 1981; Zapf et al, 1981; Beguinot et al, 1985). Yet the presence of SM receptors in humans has not been established.

F.4ii Growth Hormone and Somatomedin Receptors in Bone

It has been observed that cartilage cells isolated from rabbit epiphyseal plates have specific binding sites for GH (Eden, 1979). More specifically, infusion of radioactively labelled GH in rats has shown GH to bind to cells in the

germinal zone of the growth plate (Mayberry et al, 1971; Isaksson et al, 1985). Hintz and coworkers (1972) found cartilage receptors which are particularly sensitive to SM's.

G. Estrogen

Plasma estradiol levels are very low throughout childhood and increase during the initial stages of breast growth which occurs on the average at 10.8 years in North American girls (Tanner, 1969; Jenner et al, 1972). The timing of this depends on many factors such as genetics, nutrition, levels of physical activity and environmental factors.

The role of estrogen in promoting development of female secondary sexual characteristics and maintaining them is well established. They stimulate uterine, endometrial and vaginal growth and influence fat deposition (Root, 1973). Estrogen is also known to stimulate GH secretion, hence the lack of estrogen in Turner's patients may contribute to the perturbed pattern of GH secretion typical of girls with TS (Ross et al, 1985).

G.1 Effects of Estrogen on the Skeleton

Estrogen lowers serum calcium (Aitken et al, 1971) and inhibits bone resorption (Riggs et al, 1969) yet the specific mechanism by which this occurs remains unclear (Colston et al, 1989). In healthy girls the measurement of plasma estradiol

correlates significantly with chronological age and skeletal maturation. Estrogen levels are low throughout childhood and do not increase until the initial stages of breast growth (Root, 1973). Thus prior to puberty E2 levels are comparable between TS and control girls. Even though there are reports of osteoporosis and reduced bone mineralization in TS children (Brown et al, 1974; Beals, 1973) this becomes more prevalent after 14 years of age. Thus it seems that the lack of estrogen exacerbates an already serious condition.

The presence of estrogen receptors in osteoblast-like cells (Komm et al, 1988; Eriksen et al, 1988) suggests that estrogen may be an important regulator of long bone growth and development. As well, measurements of plasma estradiol correlate significantly with skeletal maturation and timing of peak height velocity (Jenner et al, 1972; Levine-Ross et al, 1983).

A study by Levine-Ross and colleagues (1983) demonstrated a biphasic response of ethinyl estradiol (EE) on the ulnar growth rate (UGR). They examined 19 TS females, 5 to 15 years of age and found that low doses of EE (100ng/kg body weight) caused a doubling of the UGR whereas higher doses of 400 to 800 ng/kg did not have a significant effect on the growth rate. Perhaps the suraphysiologic dosage had an inhibitory effect on GH receptor concentration or activity which prevented growth from occurring.

High doses of estrogen are known to accelerate closure of the epiphyseal plates and bone maturation which in turn leads to a shortening of the total growth phase and reduction in adult height (Ranke et al, 1986; Tanner, 1969). This is observed in clinical practice with estrogen being prescribed for tall girls to reduce their rapid growth rate (Wettenhall & Roche, 1965).

G.2 Effects of Estrogen on Muscle

Estrogen induces cellular hypertrophy and increases mitotic activity in rat uterine and epithelial cells (Tepperman, 1987). Agricultural research on the growth of cattle has demonstrated that both androgens and estrogens are necessary to achieve the maximum growth rate (Heitzman, 1979). This is probably because estrogen promotes growth by regulating the concentrations of plasma GH and insulin at the muscle cell which in turn increases protein accretion (Lorenz, 1954; Trenkel, 1976). Estrogen receptors have been found in rat skeletal muscle (Dahlberg, 1982). However the presence of estrogen receptors and the effects of estrogen on muscle tissue in humans has not been reported.

Studies on castrated female rats suggest that estrogens may have an inhibitory effect on muscle growth insofar as ovariectomy caused a subsequent increase in muscle cell population to a level comparable to that in the male rat

(8.5 x 10^9 cells in normal females, 12.1 x 10^9 cells in castrated females and 12.5 x 10^9 cells in normal males; Cheek et al, 1968). The effects of low levels of estrogen on the musculoskeletal system in TS females would probably not be apparent until after the age of 13.

H. Hormonal Therapies

H.1 History of Growth Hormone Therapy

In 1951 Escamilla and Bennett used GH prepared from pituitaries of cattle and swine to stimulate growth in human dwarfism. The results were disappointing with an increase in height of only 3/8 of an inch in 3 months. Five years later human growth hormone (hGH) was first isolated and partially characterized by Li and colleagues (1956; Li, 1957). Using hGH extracted at autopsy from a human subject, Raben (1958) treated a pituitary dwarf. His patient's initial height at age 17 was equal to that of an average 8-year-old child (less than 50 inches). One year of GH therapy resulted in an increase of only 2.5 inches.

Despite having isolated hGH and demonstrating its metabolic and growth promoting effects, the National Pituitary Agency (USA) was only able to supply limited amounts because it's synthesis was such a costly procedure. Therefore the clinical and laboratory criteria for diagnosing GHD were rigid (Aceto et al, 1972). Growth hormone deficiency has been

defined as a failure of growth hormone concentration to rise above 15 mU/L following a pharmacological and/or physiological stimulus in a child with an abnormal growth velocity in whom other causes of growth failure have been excluded (Milner & Burns, 1982).

Growth hormone has been used in numerous studies with GHD patients to increase their height velocity from low values of less than 3.3 cm/year to a maximum of 11.9 cm/yr (Ferrandez et al, 1970; Aceto et al, 1972; Frasier et al, 1981; Wilton & Gunnarrsson, 1988). Typically the initial rapid rate of growth is markedly decreased in the second year of therapy (Soyka et al, 1970; Frasier et al, 1977) and there does not seem to be an improvement in predicted adult height. The limited information available on the final height does indicate that it remains at least -1.5 standard deviations below the population mean (Burns et al, 1981; Job et al, 1987; Bourginon et al, 1986; Hibi et al, 1989).

Until recently the only source of hGH was that extracted from human pituitaries. However its use was curtailed because it was suspected to be contaminated with slow virus particles possibly accounting for the new cases of Jacob-Creutzfeldt disease in some children treated with the extract (Hintz et al, 1985; Underwood et al, 1985). Jacob-Creutzfeldt disease is a progressive degenerative brain disease, due to infection by a transmissible agent which

results in peripheral muscle wasting, degeneration of the pyramidal & extrapyramidal systems, giving spasticity and tremor or other involuntary movements (MacNalty, 1965).

Fortunately, two groups of research teams in California developed synthetic hGH (shGH) in bacterial cells using recombinant DNA technology (Gonzalez, 1979). Since then, the criteria for administering GH became more lenient, which has lead to the use of GH for therapeutic treatment of short, non GHD children, TS girls and elderly adults, as well as its abuse in athletics.

In short children without classical GHD, shGH therapy increased growth rate from 4.6 $\pm$ 1.1 cm/year to 7.5 $\pm$ 1.2 cm/year. There was no significant change in growth rate for the untreated children (4.2 $\pm$ 1.3 cm/yr versus 5.0 $\pm$ 1.4 cm/yr; Genetech study, 1989). The effects of exogenous hGH on bone mineralization, muscle size and strength were not examined.

Synthetic hGH was administered to elderly men and women with the hopes of preserving bone and muscle mass (Marcus et al, 1990). The results were encouraging in that there was a decrease in nitrogen excretion, thought to represent the anabolic effects that GH has on muscle. As well the increase in osteocalcin concentration is suggestive of an increase in bone formation but no precise measures of bone mineral content were carried out.

H.2 Growth Hormone Therapy in Turner's Syndrome

Growth hormone has been used in TS girls solely to stimulate linear growth. The first paper on GH treatment in TS was published in 1951 (Escamilla & Bennett). A 14-year old girl with a height below the 3rd percentile, but with a normal bone age, was given 5 mg of GH daily for 3.5 months. Growth during therapy amounted to 7.5 cm/yr compared to a height velocity of 3.8 cm/yr before treatment. Since then GH has been widely used alone and in combination with either estrogen or anabolic steroids (Joss & Zuppinger, 1984; Sybert, 1984; Lenko et al, 1988; Rosenfeld et al, 1986; Brook, 1986; Ross et al, 1986, 1988; Takano et al, 1990).

The studies examining the effect of hGH alone have numerous inconsistencies such as different durations of treatment, varied combinations of hormone treatments, different dosages and previous hormonal therapy. Buchanan and coworkers (1987) studied 9 TS girls with a mean age of 9.8 $\pm$ 2.78 years ($\pm$ SD) and a mean height of 116.3 $\pm$ 12.0 cm. The growth velocity at entry was -0.3 standard deviations below the mean and increased to a rate of 1.4 SD above the mean, after 0.49 years of treatment which was significantly different from placebo controls. Unfortunately this study was aborted prematurely since GH was withdrawn from use in the United Kingdom after reports of Jacob-Creutzfeldt disease in TS patients receiving hGH therapy.

Butenandt (1980) examined 5 TS girls with a mean age of approximately 11.4 years, with a growth rate prior to therapy of 3.26 cm/year and a growth rate after treatment of 3.66 cm/year. He concluded that GH therapy was unsuccessful in promoting linear growth in all his TS patients.

Numerous other investigators have found a significant increase in growth rate with up to 2 years of shGH therapy (Lin et al, 1988; Rongen-Westerlaken et al, 1988; Takano et al, 1986, & 1989). Rosenfeld and collegues (1988) examined 17 TS girls, ages 4 to 12 years old receiving hGH for 3 years. The TS girls receiving hGH alone had a significant increase in growth rate during the first 2 years as compared to controls. Yet the growth rate during the third year was not significantly increased from the pre treatment scores (pre treatment 4.5 $\pm$ 0.8 cm/yr to 6.6 $\pm$ 1.2 cm/yr year I, 5.4 $\pm$ 1.1 cm/yr year II and 4.6 $\pm$ 1.4 cm/yr year III).

Thus it is the general belief that GH therapy does increase height velocity in TS girls, at least in the first two years. However whether this will result in an increase in predicted final adult height has yet to be established. Despite the numerous trials using GH in GHD and TS patients there is a lack of information about the effects of GH treatment on the musculoskeletal system.

H.3 Estrogen Therapy in Turner's Syndrome

Various forms of estrogen therapy have been used to induce development of secondary sexual characteristics in TS females, in addition estrogen therapy in low doses increases height velocity during the first year (Ross et al, 1986b; Ranke et al, 1986; Martinez et al, 1987). However, there is a subsequent growth deceleration. This fall in growth velocity as well as the regression of breast tissue and the decrease in urocytogram index (maturation score) in most patients suggests a diminishing effect of continuous ethinyl estradiol therapy (Martinez et al, 1987). Though estrogen therapy does induce a growth spurt and near normal development of secondary sexual characteristics, there does not appear to be a significant increase in predicted adult height. Theoretically this would not be expected as the sex steroids primarily modulate the tempo of pubertal growth. They do not control the final height (Kastrup et al, 1988).

Despite the possibility of a perturbed hormonal profile in TS females there is a paucity of information regarding the effects of this impairment on musculoskeletal development and function in TS. Therefore it is the purpose of this cross-sectional study to compare bone mineralization, muscle morphology and function, in girls with TS and healthy controls. In the longitudinal study, the effects of hGH and estrogen therapy in 2 TS patients will be described.

CHAPTER II

METHODS

There are two distinct parts to this research. The first is a descriptive cross-sectional study comparing Turner's patients with healthy controls. The second is a prospective or longitudinal study which includes the retest of 3 girls from the first study: 1 TS patient (ST) who received 7 months of hGH therapy, another TS girl (JV) who received 4 months of estrogen therapy and 1 control girl (SB).

CROSS SECTIONAL STUDY

Fourteen healthy girls volunteered from a local school to serve as controls. Since it is very difficult to match the Turner's girls based solely on one factor, without biasing other variables, all control girls were selected based on pubertal stage. Then further corrections were made for height, weight, muscle and bone area where appropriate.

Five Turner's patients from Chedoke-McMaster Children's Hospital and 2 TS patients from St Joseph's Hospital in London, Ontario also volunteered to participate. A chromosomal karyotype from peripheral leukocytes was

performed by the genetics department of the respective Hospitals. This confirmed the diagnosis of TS based on the original observation of short stature (at least 2 standard deviations {SD} below normal for height). Three girls had the karyotype for complete TS (45,X0) and four girls had a mosaic karyotype (including ST and JV). Growth hormone levels were determined in 6 of the 7 Turner's patients using one or more of the accepted provocation tests; arginine infusion, exercise, sleep-related release or L-Dopa and propanolol (GH was not measured in the TS patient JV). As well, two other TS girls had lower than normal GH levels on their first GH provocation test. However results of a second, yet different provocation test, yielded normal GH levels for these two TS girls (ST and LAH).

The study was approved by the Chedoke-McMaster ethics committee as well as the committee at St Joseph's Hospital in Hamilton. Informed consent was obtained from the parents and children.

Chronological age in decimal years was calculated. Pubertal stage was assessed by parent and daughter using Tanner's pictogram for pubic hair development (Marshall & Tanner, 1969; Appendix A).

LONGITUDINAL STUDY

Subjects for the prospective case studies were

selected from the cross-sectional group according to their identified hormonal therapy program. Subject ST was participating in a multicentre shGH trial sponsored by Eli Lilly. She was randomly allocated to the treatment group and received 0.8 ml of biosynthetic hGH 6 days a week based on a protocol of 0.05 mg per kilogram body weight per day. She volunteered to be retested after 7 months of GH therapy. The other TS patient (JV), did not qualify for the trial as she was older than the cut off age of 13 years. However she was started on estrogen therapy, ethinyl estradiol 0.005 mg daily for 4 months and volunteered to be retested. The healthy control girl SB was also retested after 6 and a half months in order to control for any learning effects or time related maturational changes.

A. ANTHROPOMETRY & BODY COMPOSITION

Standing height and weight were measured with subjects in their bare feet, wearing only a t-shirt and shorts. Body mass index (BMI) was calculated from these two measurements ($kg \times m^{-2}$). Skinfolds (SF) were measured on the right side of the body using Harpenden callipers at 2 and 4 sites respectively (triceps and subscapular; triceps, subscapular, biceps and suprailliac). The percent body fat (%BF) was determined by the method of Boileau and coworkers (1985) using the sum of 2 SF and by the method of Durnin and Rahaman (1967)

with the sum of 4 SF. Girth measurements were taken with a spring loaded measuring tape at the (1) mid upper arm (halfway between the lateral epicondyle of the humerus and the acromion process), (2) 3/4 point up the lower leg (between the lateral maleolus and the tibial plateau) and (3) 3/4 point up the upper leg (between the tibial plateau and the symphysis pubis). Four skinfolds measurements were made at each of the girth measurement sites; posterior, anterior, lateral and medial. Lean cross-sectional area (bone and muscle) was calculated using the protocol of Moritani and DeVries (1980).

B. MUSCLE MORPHOLOGY

Muscle cross-sectional area and density were determined using computed axial tomography (CAT) scans (General Electric 9800 Quick 3rd generation) with a scanning time of 2 seconds and a scan thickness of 3mm. Total radiation dose was 55 mrem for subjects in the cross-sectional study and 110 mrem for the 3 subjects in the longitudinal study.

An initial scout scan was taken to determine the 3/4 point up the lower leg (from the lateral maleolus to the tibial plateau) and the 3/4 point up the thigh (from the tibial plateau to the symphysis pubis). The 1/2 way point for the upper arm was determined using anthropometry, mid-way between the lateral epicondyle of the humerus and the acromion process. A CAT scan image was taken at these 3 sites, a

positive was made from this film and this in turn was projected onto plain paper and a tracing of the limb cross-sectional compartments was made. Total limb area, bone, muscle and fat area were calculated from the tracings using manual planimetry. For the upper arm and thigh, additional tracings were made of the elbow flexors (biceps brachii and brachialis) and the knee extensors (rectus femoris, vastus lateralis, vastus intermedius, vastus medialis). It was not possible with the CAT scan technique to differentiate individual muscle groups in the lower leg.

Muscle density was calculated in Houndsfield units (Hu) from the mean of 3 measurements provided by the CAT scan machine at each muscle of interest: elbow flexors (EF), plantar flexors (PF) and knee extensors (KE). The Hu expresses the attenuation value of the x-rays relative to the known attenuation value for water. This provided an estimate of the density of the muscle for an area of approximately 0.08 cm^2 for the EF's, 0.40 cm^2 for the PF's and 0.75 cm^2 for the KE's. These 3 sites were standardized by the radiology technician using a manual cursor.

C. SKELETAL MUSCLE FUNCTION

Voluntary and stimulated isometric contractions of the EF, PF and KE's were studied. The EF's were studied with the subject seated in an adjustable chair. The right arm was

securely fastened into the custom made dynamometer (Appendix B) with the wrist in a supinated position and the upper arm parallel to the floor and aligned in the frontal plane with the shoulder. The left arm rested on the subject's lap to prevent bracing during the trial. In order to isolate the EF's an assistant placed their hands on the subject's shoulders and applied a downward pressure. The subject was securely fastened to the chair with a wide velcro strap around the waist to prevent body movement at the hips. Measurements were made at 80°, 110° and 150° of elbow flexion (180°= full extension) with the order of testing randomized for each subject.

In order to obtain the electrically evoked contractile properties rubber electrodes coated with conducting gel were taped securely on the forearm just distal to the elbow fossa (anode) and on the belly of the biceps over the motor end plate (cathode) after the skin had been scraped with an abrasive sponge and cleaned with peroxide. Then an electrical stimulus was delivered percutaneuosly to the resting muscle. The stimuli were rectangular pulses of 50 to 200 μs duration, produced by a Devices stimulator (Medical System Corp.). The voltage was increased in a step-wise fashion until the twitch torque viewed on the oscilloscope could not be increased further. This plateau was considered the peak twitch torque. The torque was transmitted via the arm straps and a steel plate to a strain gauge located at the rotational centre of

the dynamometer. This signal was amplified and analyzed on-line by computer (PDP 11-23 Digital Equipment Corporation Mass.). The average of two trials was used in the analysis of results. The stimulation intensity ranged from 50-200 volts for the 3 muscle groups.

The experimental procedure was similar for both the arm and leg measurements. The plantar flexors (PF) of the right foot were studied with the subject seated and the ankle securely fastened to the custom made dynamometer for the lower leg (Appendix C). The anode electrode was taped at the heel at the level of the maleolus and the cathode was placed on top of the widest part of the triceps surae. The joint angles were 70° and 90° of plantar flexion (90° = horizontal foot position) and the order of testing was randomized for each subject.

For the knee extensors (KE) the subject was seated and securely fastened to a custom-made chair (Appendix D). The anode was secured proximal to the patellar tendon over the quadriceps muscle. The cathode lay over the femoral nerve in the pelvic notch. The joint angles tested were 90° and 120° of KE (180° full extension) and the order for testing the joint angles was randomized for each subject.

The order of testing for the muscle groups was consistent for all subjects; EF, PF, KE, to allow the children to get accustomized to the smaller twitches first.

Twitch torque (TT), time to peak torque (TPT), half relaxation time (HRT) and total contraction time (TCT) were measured using a custom programmed software package. The twitch contractions were measured before the maximal voluntary efforts to avoid potentiation effects (Vandervort et al, 1983).

Maximal voluntary contractions (MVC) were performed for all muscle groups at the same joint angles as in the stimulated protocol. To determine whether a maximal effort was made the interpolated twitch technique (ITT) was used to determine the degree of motor unit activation (MUA), (Belanger & McComas, 1981). The maximal MUA was determined from 2 MVC trials at 110° of EF, 90° of PF and 90° of KE. A supramaximal stimulus (twitch) was delivered to the muscle at the peak of a maximal voluntary contraction. Motor unit activation was obtained by comparing the magnitude of the torque increment following the stimulation (interpolated twitch torque-ITT) to that of the evoked peak twitch torque response :

$$\% \text{ MUA} = \frac{\text{TT} - \text{ITT}}{\text{TT}} \times 100 \ \%$$

Twitch torque and MVC were expressed absolutely in Newton meters (Nm) and corrected for muscle area and height, the latter being a correction for moment arm effects

on strength. The specific muscle groups used in this normalization procedure were the biceps brachii and brachialis for the EF's, the soleus, gastrocnemius, tibialis anterior, peroneus longus, extensor digitorum longus, extensor hallucis longus, tibialis posterior and the flexor digitorum longus for the plantar flexors and the rectus femoris, vastus lateralis, vastus intermedius and the vastus medialis for the knee extensors.

Strength measures for the longitudinal study (evoked peak torque, maximal voluntary strength and contractile properties) were expressed as one mean score collapsed across the respective joint angles (EF 80°, 110° and 150°; PF 70° and 90°; KE 90° and 120°).

D. BONE MINERALIZATION

Whole body dual photon scans were performed with a Norland 2600 dichromatic densitometer to quantify bone mineral mass (Webber, 1989: Appendix E). With this system a pencil beam of gamma rays is emitted from a radioisotope source (Gd-153) which is scanned throughout the body in a rectilinear pattern at a speed of 30mm/sec with a line spacing of 15mm. The "Bonestar" software program 3.4.1. is incorporated. The technique of DPA is based upon the assumption that the object is a two component system, bone mineral and soft tissue. At sites where no mineral is present the soft tissue is divided

into lean tissue and fat. The reproducibility of this technique for total body bone mineral on our apparatus has been tested and the coefficient of variation is 2% (Omerod et al, 1990) which is well within the range reported by others (Mazess et al, 1984; Gallagher et al, 1987). For segmental bone mineral analysis, the operator sets square or rectangular boxes over the regions of interest (head, spine, trunk, pelvis and leg areas). The reproducibility for regional assessment of bone mineralization has been measured in adults in our laboratory to be within 4%. Bone mineral density is expressed in g/cm^2 and is converted by the system software to BMC (g) by multiplying the number of pixels and the area per pixel (bone width). All DPA measurements were analyzed by one observer.

E. PHYSICAL ACTIVITY QUESTIONNAIRES

Physical activity scores (PAS) were determined from (i) standard personal interviews conducted with each girl and (ii) questionnaires that the parents completed about their daughter's habitual physical activity patterns and participation in physical activity relative to her peers (Appendix F).

F. DIETARY ANALYSIS

Three day food records were kept by the girls and analyzed for total calories, protein, carbohydrate, fat,

vitamins and minerals with the assistance of the Nutrient Data Bank computer program at the University of Guelph (Appendix G).

G. STATISTICAL ANALYSES

Comparisons were made between the Turner's patients and the control girls using Student's independent t-tests for the following variables; age, height, weight %BF, BMI, cross-sectional area measurements, muscle morphology, physical activity scores and nutritional status.

Two factor analyses of variance with repeated measures on the second factor, were performed on the evoked peak twitch torques, maximal voluntary strength measures and time related contractile properties (group x joint angle), to compare the TS girls with the control girls (Appendix H). Bone mineral content and density values were also analyzed with a 2 factor ANOVA (group x body segment) with repeated measures on the second factor. Tukey's HSD post hoc analyses were performed on significant ANOVA effects.

Pearson's "r" correlations were carried out to examine the relationship between bone mineralization and independent factors such as age, body mass, height and strength. Multiple linear regression analysis was carried out to determine the best predictor of variability in bone mineralization scores. Differences were accepted as significant at $p<0.05$.

CHAPTER III

RESULTS

CROSS-SECTIONAL STUDY
A. Anthropometric and Descriptive Data

What are the effects of Turner's syndrome on growth and development ?

The Turner's girls were significantly shorter and lighter than the control girls despite being older (Table 1). There were no significant differences between the groups for body mass index, the sum of 2 and 4 skinfolds or percent body fat. Fat mass was not different in TS versus controls using the sum of 2 and 4 skinfolds respectively. The TS girls had a smaller lean body mass when compared to the control girls using both the sum of 2 and 4 SF's (Appendix I). As suspected Turner's syndrome is associated with a reduced growth, yet adiposity values were comparable between the groups.

B. Cross-Sectional Area and Muscle Morphology

What are the effects of a possible GH perturbation on muscle area and morphology in TS girls ?

Computed tomography scans revealed that the TS girls had a significantly reduced total limb area at the lower leg (Appendix J). The smaller upper arm and upper leg girths of

Table 1. Descriptive data of patients with Turner's syndrome and control girls.

	TURNERS n=7	CONTROLS n=13	
AGE yrs	11.2 ± 0.83	9.5 ± 0.26	p<0.05*
HEIGHT cm	124.3 ± 2.79	137.8 ± 2.25	p<0.01
WEIGHT kg	27.4 ± 2.12	36.3 ± 2.57	p<0.05
BMI	17.6 ± 0.89	19.0 ± 0.99	

Values are mean ± SE
* indicates a significant difference between TS and controls

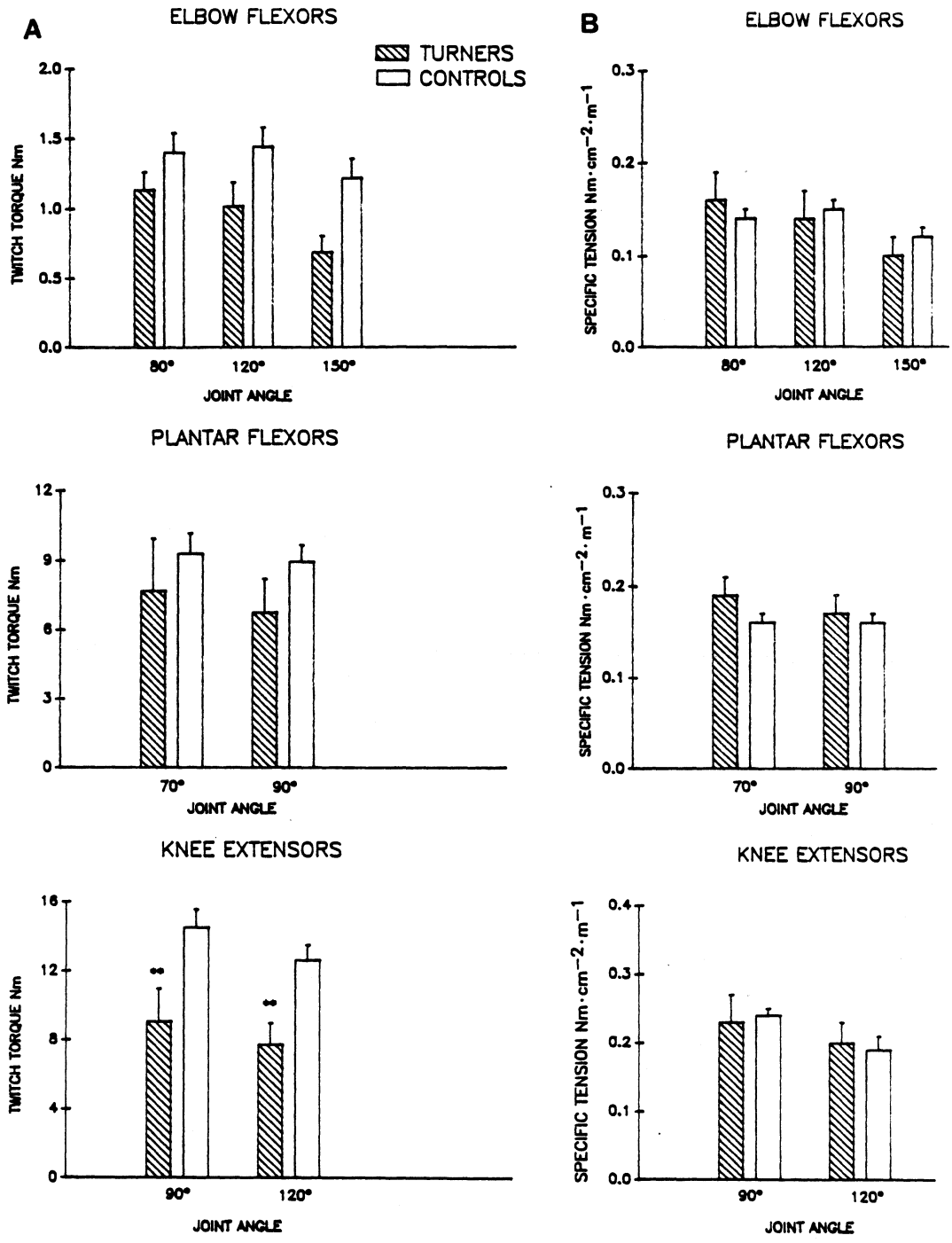
the TS girls were not however, statistically significant when compared to controls. Subcutaneous fat areas for the arms and legs were comparable between Turner's and control girls. Bone area was significantly smaller for the Turner's girls at the humerus, tibia, fibula and at the femur. The TS girls had a smaller muscle area for the EF's, KE's and lower leg muscles when compared to the control girls. However the smaller EF muscle area was not statistically different between the groups. Muscle density values, expressed in Houndsfield units, were similar between groups for all 3 muscle groups. Again the reduced growth in the TS girls is manifest by their smaller bone and muscle areas. However fat area and muscle density values were comparable between the TS and control girls.

C. Isometric Strength Measurements

What are the effects of a possible GH perturbation on muscle function in TS girls ?

The Turner's girls had consistently lower peak twitch torques than the control girls for both the EF's and PF's at all joint angles (Fig 1 & Appendix K), however these did not reach statistical significance. Knee extensor peak twitch torque was significantly lower in the TS girls compared to the controls. When corrected for the influence of muscle size (muscle cross-sectional area) and possible differences in

Figure 1. Electrically evoked peak isometric twitch torque (A) and specific tension (B) for the elbow flexors plantar flexors and the knee extensors in Turner's patients and controls (** $p<0.01$).


A
ELBOW FLEXORS
TURNERS
CONTROLS
TWITCH TORQUE Nm
2.0
1.5
1.0
0.5
0.0
80°
120°
150°
JOINT ANGLE
B
ELBOW FLEXORS
SPECIFIC TENSION Nm·cm⁻²·m⁻¹
0.3
0.2
0.1
0.0
80°
120°
150°
JOINT ANGLE
PLANTAR FLEXORS
TWITCH TORQUE Nm
12
9
6
3
0
70°
90°
JOINT ANGLE
PLANTAR FLEXORS
SPECIFIC TENSION Nm·cm⁻²·m⁻¹
0.3
0.2
0.1
0.0
70°
90°
JOINT ANGLE
KNEE EXTENSORS
TWITCH TORQUE Nm
16
12
8
4
0
**
**
90°
120°
JOINT ANGLE
KNEE EXTENSORS
SPECIFIC TENSION Nm·cm⁻²·m⁻¹
0.4
0.3
0.2
0.1
0.0
90°
120°
JOINT ANGLE

moment arm (height), there were no significant differences in normalized strength, expressed as twitch specific tension ($Nm \times cm^{-2} \times m^{-1}$) for the EF's, PF's or for the KE's at any joint angle.

Absolute maximal voluntary torque in the TS girls was significantly lower at all joint angles for the EF's, for the PF's and for the KE's (Fig 2 & Appendix L). When normalized for muscle area and moment arm, all maximal voluntary specific tension scores were comparable between the groups. There appears to be quantitative not qualitative differences in muscle strength since the TS patients had lower absolute strength values and comparable specific tension values to those of the control girls.

D. Motor Unit Activation

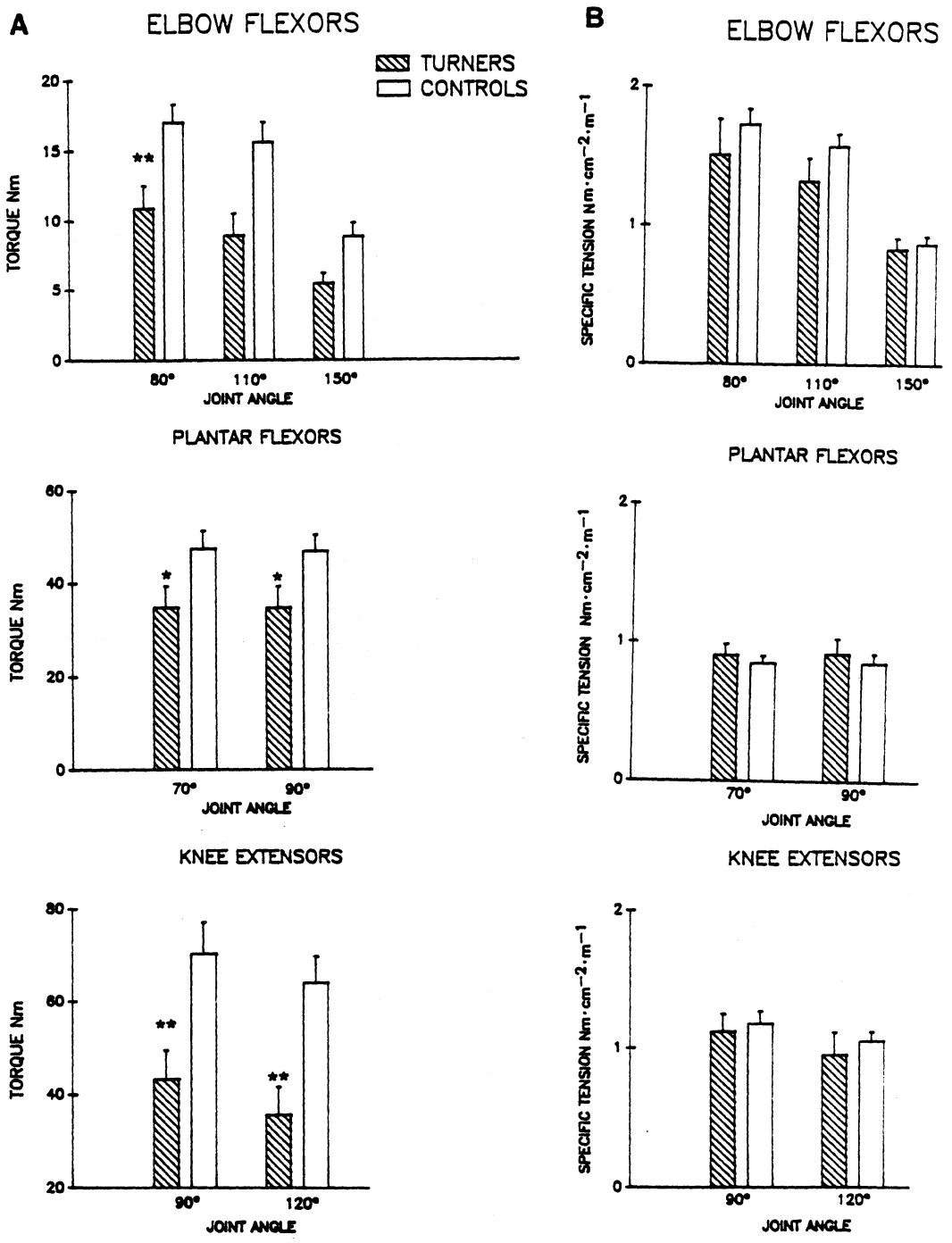
Are there differences in neuromuscular activation between the TS and control girls ?

Motor unit activation was not significantly different between groups for EF's at 110°, PF's at 90° or for the KE's at 90° (Appendix M). Mean values ranged between 90.8 % and 95.4 % for the 3 muscle groups.

E. Time-Related Contractile Properties

What effect does a possible GH perturbation have on muscle contractile properties in TS patients ?

Figure 2. Maximal voluntary isometric torque (A) and specific tension (B) of the elbow flexors, plantar flexors and knee extensors in Turner's patients and control girls (** $p<0.01$, * $p<0.05$).


A
ELBOW FLEXORS
TURNERS
CONTROLS
TORQUE Nm
20
15
10
5
0
**
80°
110°
150°
JOINT ANGLE
B
ELBOW FLEXORS
SPECIFIC TENSION Nm·cm⁻²·m⁻¹
2
1
0
80°
110°
150°
JOINT ANGLE
PLANTAR FLEXORS
TORQUE Nm
60
40
20
0
*
*
70°
90°
JOINT ANGLE
PLANTAR FLEXORS
SPECIFIC TENSION Nm·cm⁻²·m⁻¹
2
1
0
70°
90°
JOINT ANGLE
KNEE EXTENSORS
TORQUE Nm
80
60
40
20
**
**
90°
120°
JOINT ANGLE
KNEE EXTENSORS
SPECIFIC TENSION Nm·cm⁻²·m⁻¹
2
1
0
90°
120°
JOINT ANGLE

Time to peak torque was significantly longer in the elbow flexors of the TS girls when compared to controls (Appendix N). There were no significant differences between the groups, however, for TPT at the PF's or for the KE's. Half relaxation time was not significantly different between the groups for any of the 3 muscle groups. Total contraction time was longer in the TS girls at 80° and 110° of EF but it was not significantly different from the controls for the EF's at 150° or for the PF's or the KE's at any joint angle.

F. Bone Mineralization

Are there differences in bone mineralization in TS girls prior to puberty ?

Total body bone mineral content (BMC) was significantly lower for the TS girls compared to the control girls (Fig 3: Table 2). Likewise, BMC was significantly lower in TS patients versus the control girls at the legs and trunk. Total body BMC normalized for body mass (g/kg) was comparable between the TS and the control girls. However, on segmental analysis, leg and head BMC normalized for body mass g/kg) were slightly higher for the TS girls as compared to the control girls. Total body and segmental bone mineral density were comparable between the TS and the control girls.

Correlational analysis revealed that body mass was significantly correlated with BMC for both the TS and the

Figure 3. Total body bone mineral content (top insert) and segmental bone mineral content in Turner's patients and control girls (** $p<0.01$, * $p<0.05$).

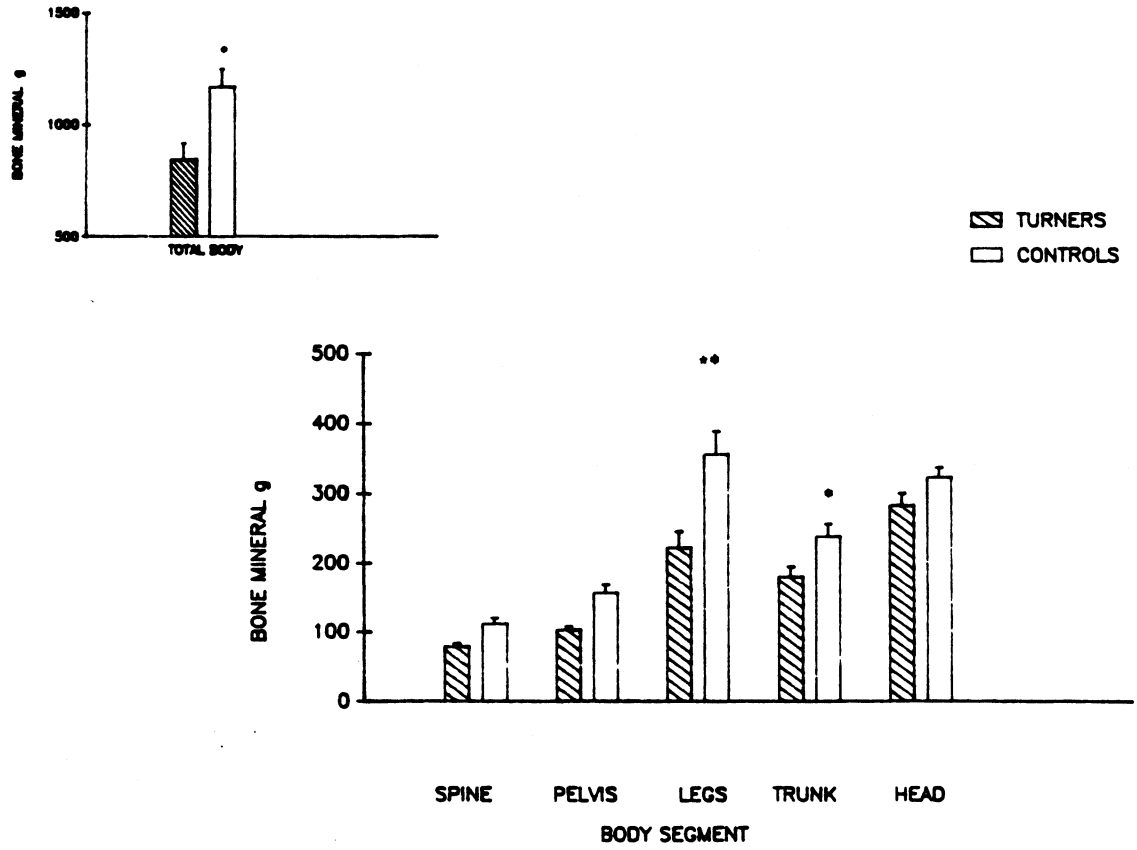

1500
1000
500
BONE MINERAL g
TOTAL BODY
TURNERS
CONTROLS
500
400
300
200
100
0
BONE MINERAL g
SPINE
PELVIS
LEGS
TRUNK
HEAD
BODY SEGMENT

Table 2. Bone mineral content and density in Turner's patients and controls.

	TURNERS n=6	CONTROLS n=13	
BONE MINERAL CONTENT PER KILOGRAM BODY MASS g/kg^{-1}			
TOTAL	32.19 ± 0.80	32.38 ± 0.81	
head	10.90 ± 0.55	9.23 ± 0.49	$p<0.05$*
trunk	6.88 ± 0.28	6.60 ± 0.16	
pelvis	3.87 ± 0.38	4.33 ± 0.14	
legs	8.41 ± 0.32	9.66 ± 0.34	$p<0.05$
spine	3.06 ± 0.15	3.08 ± 0.12	
BONE MINERAL DENSITY g x cm^2			
TOTAL	0.659 ± 0.03	0.717 ± 0.02	
head	1.38 ± 0.62	1.41 ± 0.04	
trunk	0.30 ± 0.16	0.33 ± 0.01	
pelvis	1.06 ± 0.18	1.13 ± 0.08	
legs	1.15 ± 0.13	1.10 ± 0.06	
spine	0.49 ± 0.02	0.51 ± 0.02	

values are mean ± SEM
* indicates a significant difference between TS and controls

control girls (Fig 4). Multiple regression analysis indicated that of the independent variables age, height, percent body fat and physical activity score, body mass accounted for the largest proportion of the variation in BMC among TS and control girls (96.8 %). It appears that the reduced BMC in TS girls merely reflects their smaller size as their total body BMC relative to body mass and BMD values were comparable to those of the control girls'.

Are there any differences in habitual physical activity levels and nutritional intake between Turner's patients and control girls?

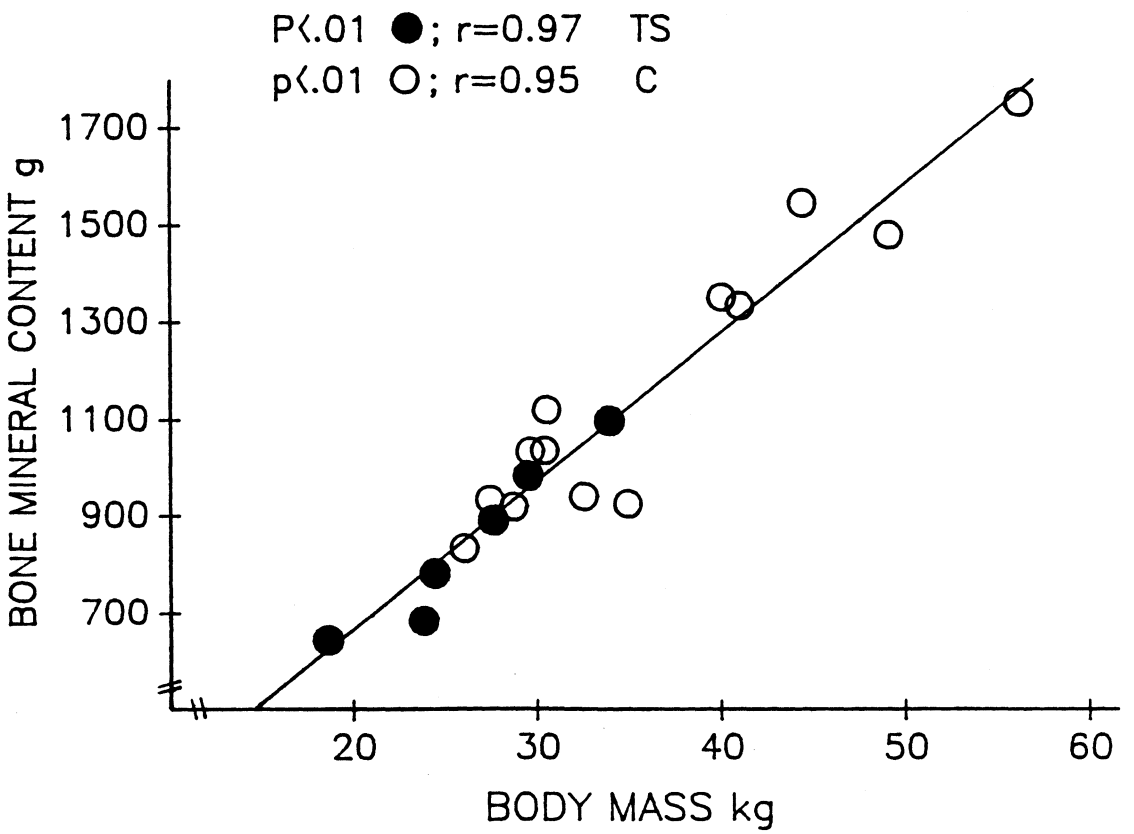
G. Physical Activity Profile

Physical activity scores, from the personal interview with each girl and the parental assessment, were not significantly different between the TS and the controls respectively (Appendix O).

H. Nutritional Status

There were no significant differences between the TS girls and the control group for any of the following nutritional indices; caloric intake, protein, carbohydrate, fat, calcium, vitamin D, phosphorus, zinc, iron (Appendix P). Although not statistically significant, caloric intake per kilogram of body weight, was higher for the TS girls than the control girls.

Figure 4. Correlation between bone mineral content and body mass in Turner's patients ($r = 0.97$, $p<0.01$) and control girls ($r = 0.96$, $p<0.01$).


P<.01 ●; r=0.97 TS
p<.01 O; r=0.95 C
BONE MINERAL CONTENT g
1700
1500
1300
1100
900
700
20
30
40
50
60
BODY MASS kg

LONGITUDINAL DATA

The results for the longitudinal component of this study are presented as case studies. Given the small sample sizes no statistical analyses were performed on these data. The results will be reported as trends and therefore will be based on relative differences amongst subjects.

A. Anthropometric and Descriptive Data

(i) Growth Hormone Effects

The patient who received hGH therapy (subject ST_{gh}) demonstrated a dramatic increase in height (6.7 cm) and in growth velocity (Fig 5; Appendix Q). Her percent body fat was dramatically reduced while the gain in body mass was minimal. This was the result of a balance between her decreased fat mass and a comparable gain in lean mass (based on the sum of 2 skinfolds measures).

(ii) Estrogen Effects

The patient who received estrogen therapy (subject JV_{e2}) demonstrated only a modest increase in height (2.8 cm) and growth velocity (Fig 6). She had a small increase in body mass consequent to the slight increase in fat mass and lean mass. $JV_{(e2)}$'s percent body fat increased slightly based on the sum of 2 skinfolds.

(iii) Control Effects

The control girl (subject SB_c) grew 5.1 cm and her

Figure 5. Changes in anthropometric data during growth hormone therapy.

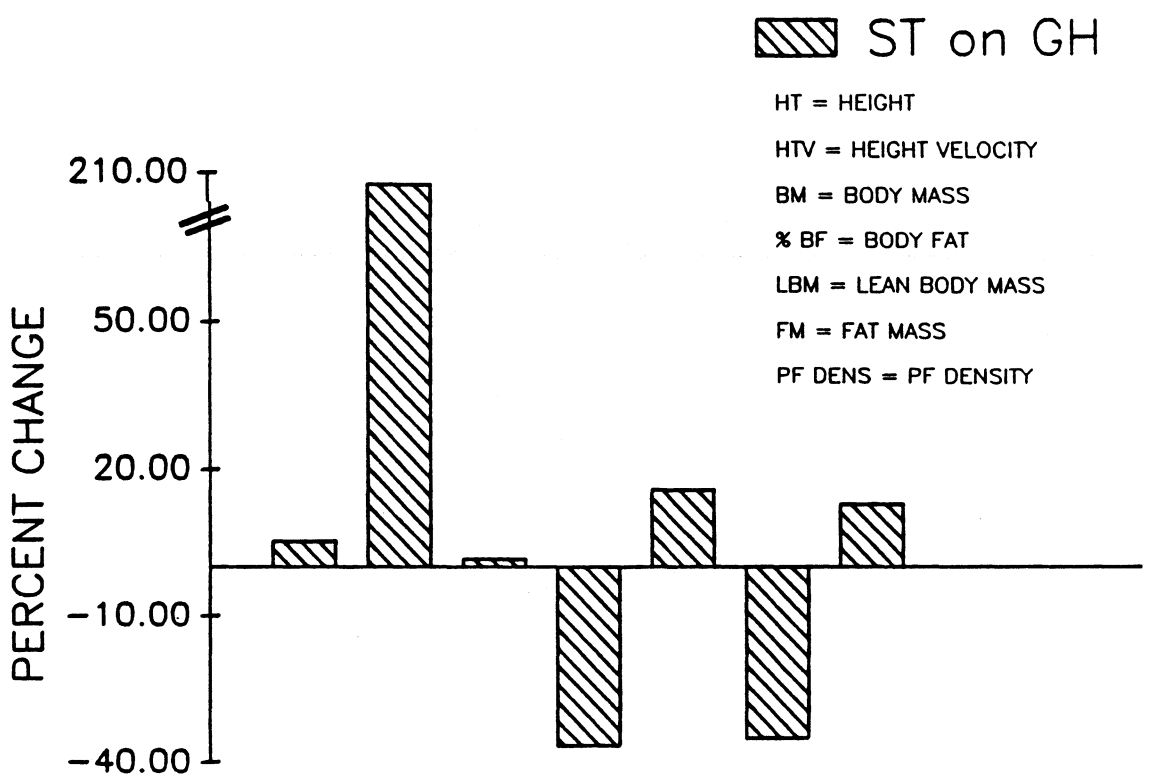
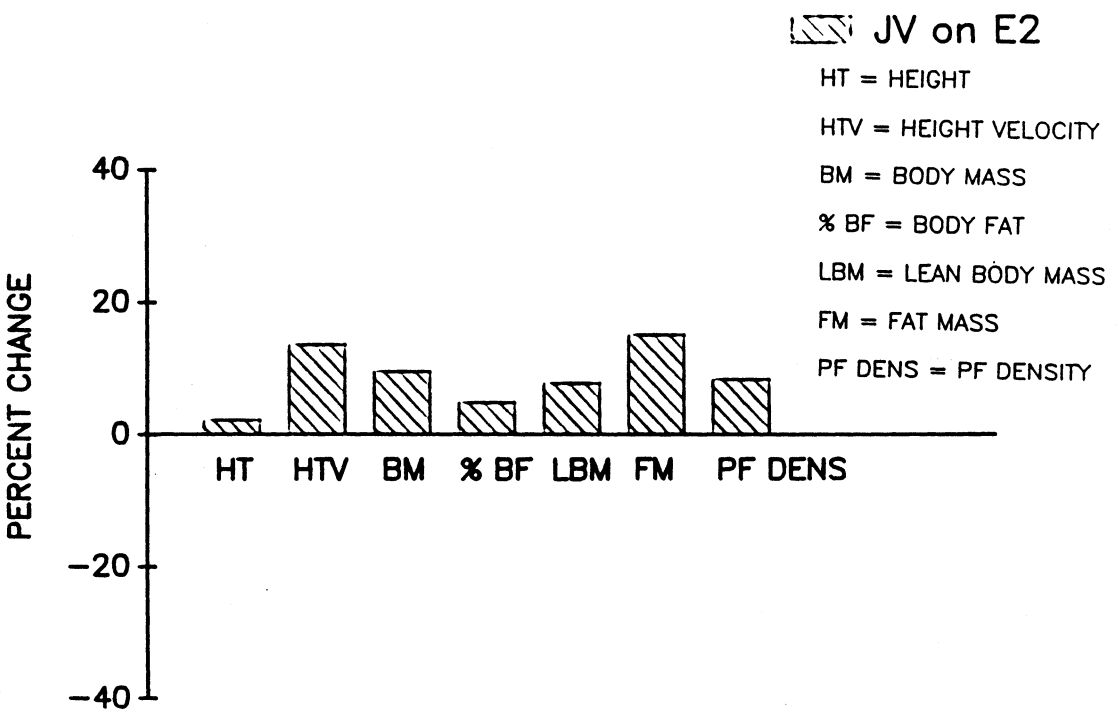

ST on GH
HT = HEIGHT
HTV = HEIGHT VELOCITY
BM = BODY MASS
% BF = BODY FAT
LBM = LEAN BODY MASS
FM = FAT MASS
PF DENS = PF DENSITY
PERCENT CHANGE
210.00
50.00
20.00
-10.00
-40.00

Figure 6. Changes in anthropometric data during estrogen therapy.


JV on E2
HT = HEIGHT
HTV = HEIGHT VELOCITY
BM = BODY MASS
% BF = BODY FAT
LBM = LEAN BODY MASS
FM = FAT MASS
PF DENS = PF DENSITY
PERCENT CHANGE
40
20
0
−20
−40
HT
HTV
BM
% BF
LBM
FM
PF DENS

height velocity from the pre-test to the post test was above the 97th percentile for her age (Tanner & Davies, 1985: Fig 7). After 7 months $SB_{(c)}$ was 7.9 kg heavier due to an increase in fat mass and lean mass. Yet the change in body fat (using the sum of 2 skinfolds) was minimal.

B. Cross-Sectional Area and Muscle Morphology

(i) Growth Hormone Effects

Total limb area and subcutaneous fat area decreased for all 3 limbs in $ST_{(gh)}$ (Appendix R). There were increases in bone area at the humerus, tibia, fibula and femur (Appendix S). Elbow flexor, knee extensor and total calve muscle area (MA) increased and muscle density increased slightly for the PF's in $ST_{(gh)}$ (Fig 5).

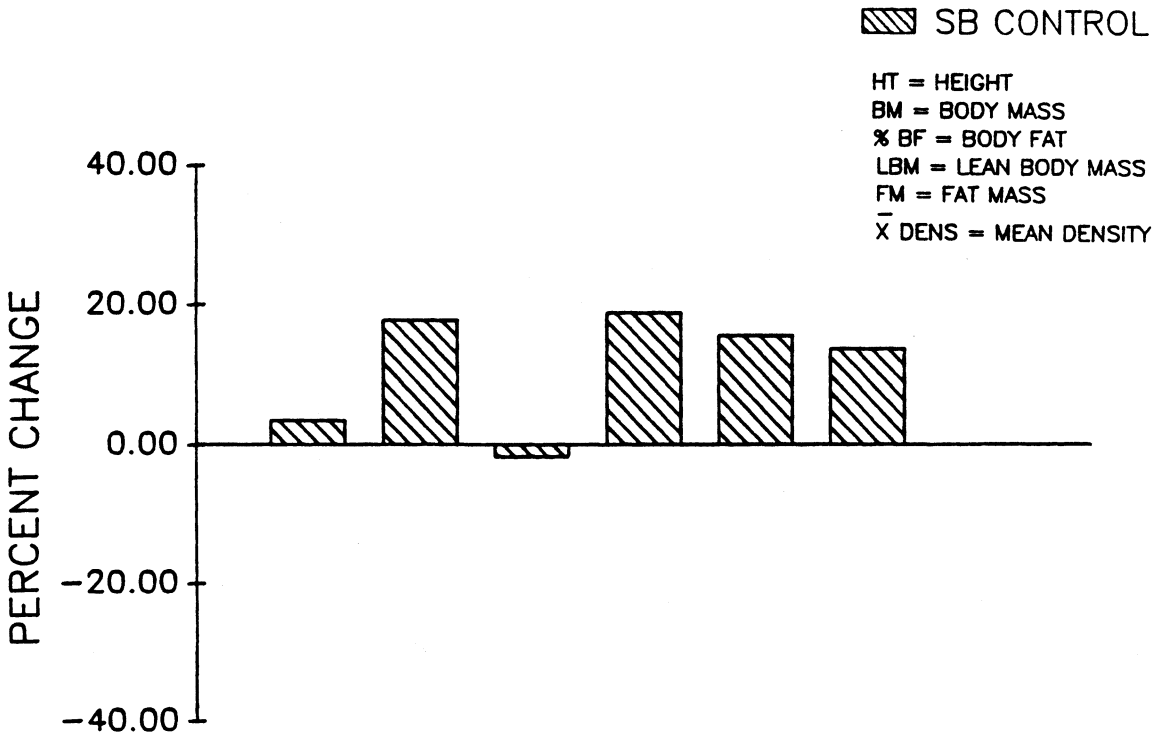
(ii) Estrogen Effects

Total limb and fat area increased for $JV_{(e2)}$ for all 3 limbs (Appendix R). Bone and muscle area (MA) increased at all three sites (Appendix S) and PF muscle density increased slightly (Fig 6).

(iii) Control Effects

Total limb, fat and bone area increased for $SB_{(c)}$ for all 3 limbs (Appendix R & S). She had a slight increase in calve MA with no major change in EF or KE muscle areas. Muscle density decreased substantially in all three muscle groups (Fig 7).

Figure 7. Changes in anthropometric data for the control subject.


SB CONTROL
HT = HEIGHT
BM = BODY MASS
% BF = BODY FAT
LBM = LEAN BODY MASS
FM = FAT MASS
X̄ DENS = MEAN DENSITY
PERCENT CHANGE
40.00
20.00
0.00
-20.00
-40.00

C. Isometric Strength Measurements

(i) Growth Hormone Effects

Growth hormone therapy resulted in a dramatic increase in EF evoked twitch torque and specific twitch tension (Fig 8; Appendix T). However $ST_{(gh)}$ had a decrease in PF and KE TT for absolute and specific tension. Absolute MVC strength for the EF's increased (Appendix U) and even when MVC strength was normalized for muscle area and lever length there was still a substantial increase in maximal voluntary specific tension. There was a decrease for relative and absolute PF and KE MVC for $ST_{(gh)}$ during GH therapy.

(ii) Estrogen Effects

Estrogen therapy resulted in an increase in EF TT and specific twitch tension (Fig 9; Appendix T). Absolute PF and KE twitch increased for $JV_{(e2)}$ yet twitch specific tension was essentially unchanged. Absolute MVC strength increased for the EF's, PF's and KE's but relative MVC strength was increased only for the PF's (Appendix U).

(iii) Control Effects

There was an increase in absolute and relative EF and KE twitch torque: PF TT was, however, essentially unchanged (Fig 10; Appendix T). Absolute MVC and specific tension increased for all 3 muscle groups (Appendix U).

D. Motor Unit Activation

Figure 8. Percent change in absolute and relative peak twitch (A) and maximal voluntary strength (B) for the elbow flexors (EF), plantar flexors (PF) and knee extensors (KE) during growth hormone therapy.

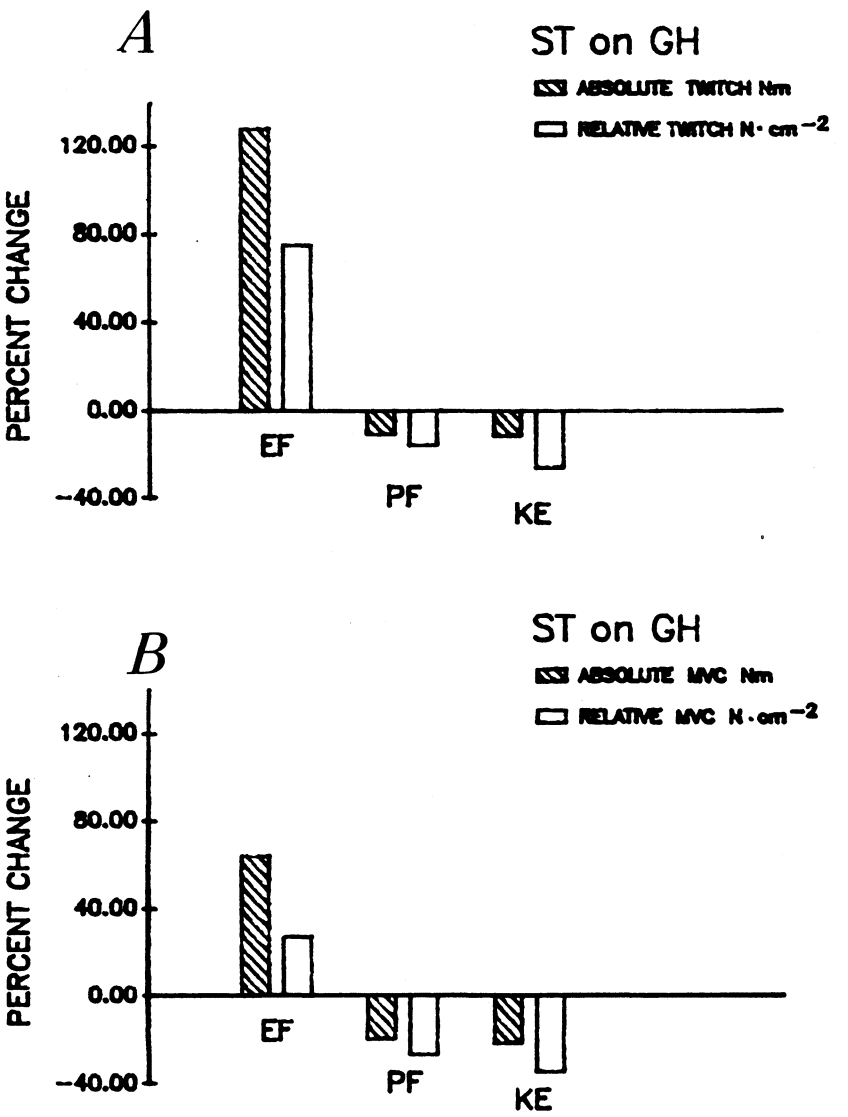

A
ST on GH
ABSOLUTE TWITCH Nm
RELATIVE TWITCH N·cm⁻²
PERCENT CHANGE
120.00
80.00
40.00
0.00
-40.00
EF
PF
KE
B
ST on GH
ABSOLUTE MVC Nm
RELATIVE MVC N·cm⁻²
PERCENT CHANGE
120.00
80.00
40.00
0.00
-40.00
EF
PF
KE

Figure 9. Percent change in absolute and relative peak twitch (A) and maximal voluntary strength (B) for the elbow flexors (EF), plantar flexors (PF) and knee extensors (KE) during estrogen therapy.

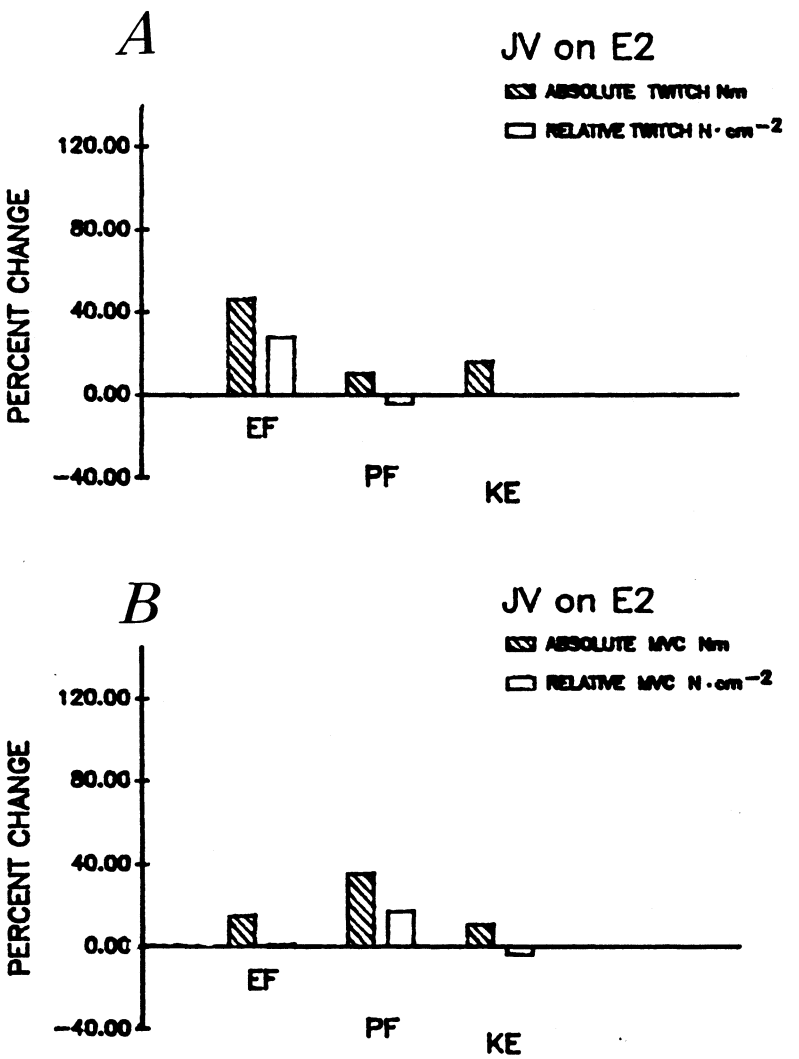
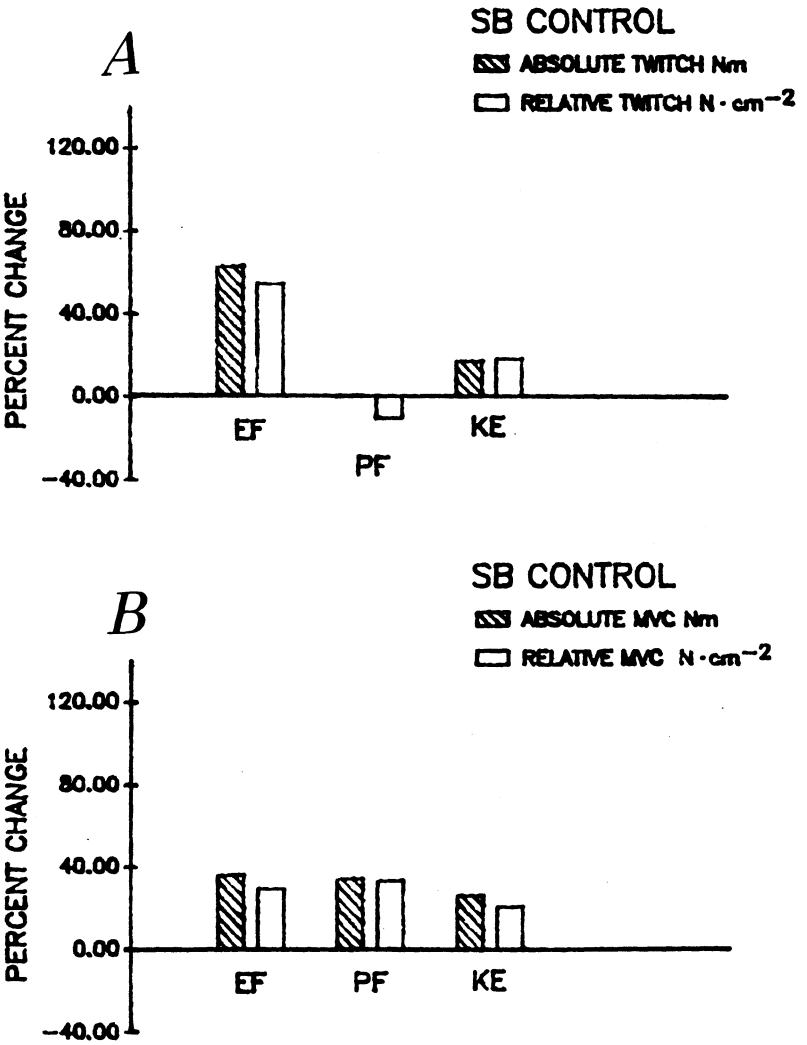

A
JV on E2
ABSOLUTE TWITCH Nm
RELATIVE TWITCH N·cm⁻²
PERCENT CHANGE
120.00
80.00
40.00
0.00
-40.00
EF
PF
KE
B
JV on E2
ABSOLUTE MVC Nm
RELATIVE MVC N·cm⁻²
PERCENT CHANGE
120.00
80.00
40.00
0.00
-40.00
EF
PF
KE

Figure 10. Percent change in absolute and relative peak twitch (A) and maximal voluntary strength (B) for the elbow flexors (EF), plantar flexors (PF) and knee extensors (KE) for the control girl.


A
SB CONTROL
ABSOLUTE TWITCH Nm
RELATIVE TWITCH N · cm⁻²
PERCENT CHANGE
120.00
80.00
40.00
0.00
-40.00
EF
PF
KE
B
SB CONTROL
ABSOLUTE MVC Nm
RELATIVE MVC N · cm⁻²
PERCENT CHANGE
120.00
80.00
40.00
0.00
-40.00
EF
PF
KE

Motor unit activation was consistently high for all subjects across all three muscle groups at pre-testing (>93.6 %), and did not change substantially as a result of hormonal therapy (Appendix V).

E. Time-Related Contractile Properties

Time to peak torque, collapsed across joint angles, was substantially reduced in the EF for all 3 girls. The only major change in half-relaxation time was a large increase at the EF's for all 3 girls and a slight increase for $ST_{(gh)}$ at the PF's. The percent changes for total contraction times (TCT) were consistently small (± 10.8 %) for all 3 girls across all 3 muscles (Appendix W).

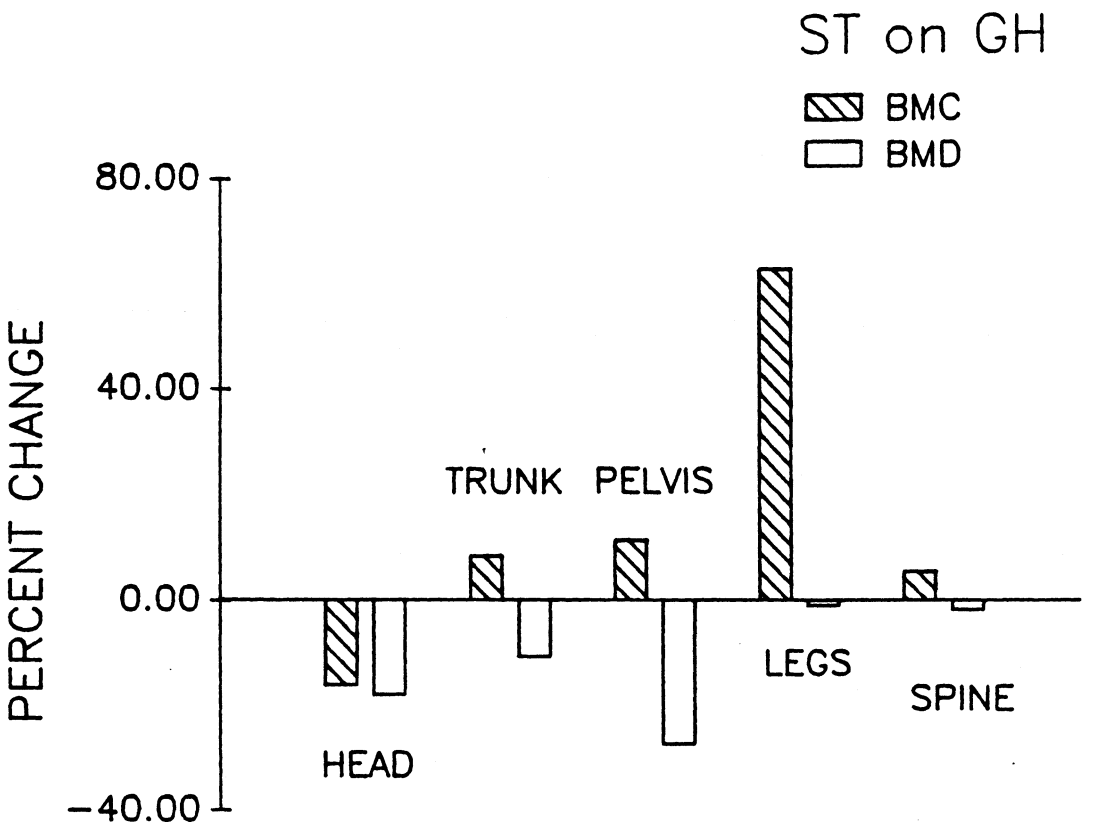
F. Bone Mineralization

The percent changes for total body BMC and BMD were all ± 10.8 % of the pre-test means (Appendix X & Y). The largest increase in total body bone mineralization occurred in $SB_{(c)}$, who also had the greatest gain in body mass.

(i) Growth Hormone Effects

The TS patient $ST_{(gh)}$, had a large increase in leg BMC, a minimal increase in pelvis BMC, whereas head BMC was slightly reduced (Fig 11). Bone density was reduced at the pelvis, moderately lower for the head and trunk but remained the same at the spine and legs.

Figure 11. Percent changes in segmental bone mineral content and bone mineral density during growth hormone therapy.


ST on GH
BMC
BMD
80.00
40.00
0.00
-40.00
PERCENT CHANGE
HEAD
TRUNK
PELVIS
LEGS
SPINE

(ii) Estrogen Effects

In the patient on estrogen therapy the only noticeable changes in bone mineralization were a slight increase in leg BMC and a substantial reduction in pelvis BMD (Fig 12).

(iii) Control Effects

The control girl, $SB_{(c)}$, demonstrated an increase in BMC at the pelvis, spine and trunk. Bone density was increased at the spine and trunk (Fig 13).

G. Physical Activity Profile

There were no major changes in self-assessed physical activity scores, for $ST(_{gh})$, $JV(_{e2})$ or $SB(_{c})$ (Appendix Z). Parental assessment of physical activity level decreased for $SB(_{c})$.

H. Nutritional Profile

With few exceptions, there was a reduction in almost every nutritional indice for all subjects from pre- to post-assessment (Appendix AA). Carbohydrate intake was slightly increased from the pre test for $ST(_{gh})$.

Figure 12. Percent changes in segmental bone mineral content and bone mineral density during estrogen therapy.

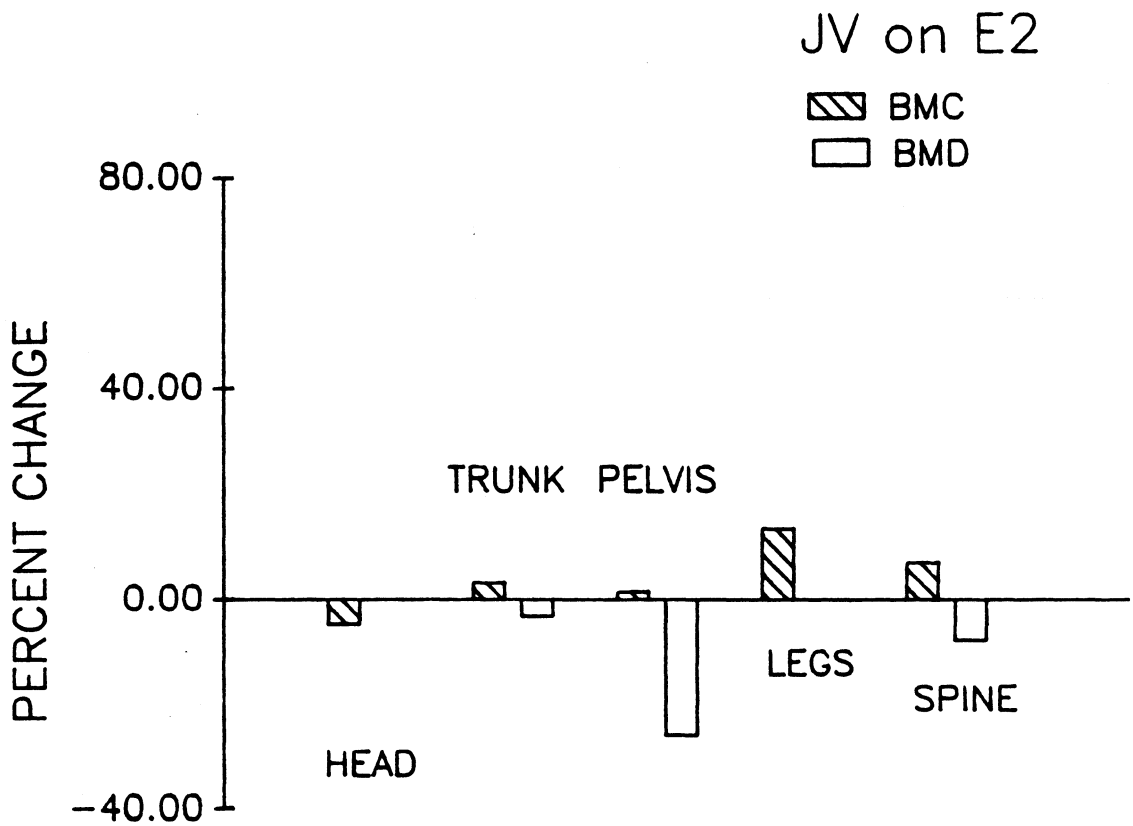
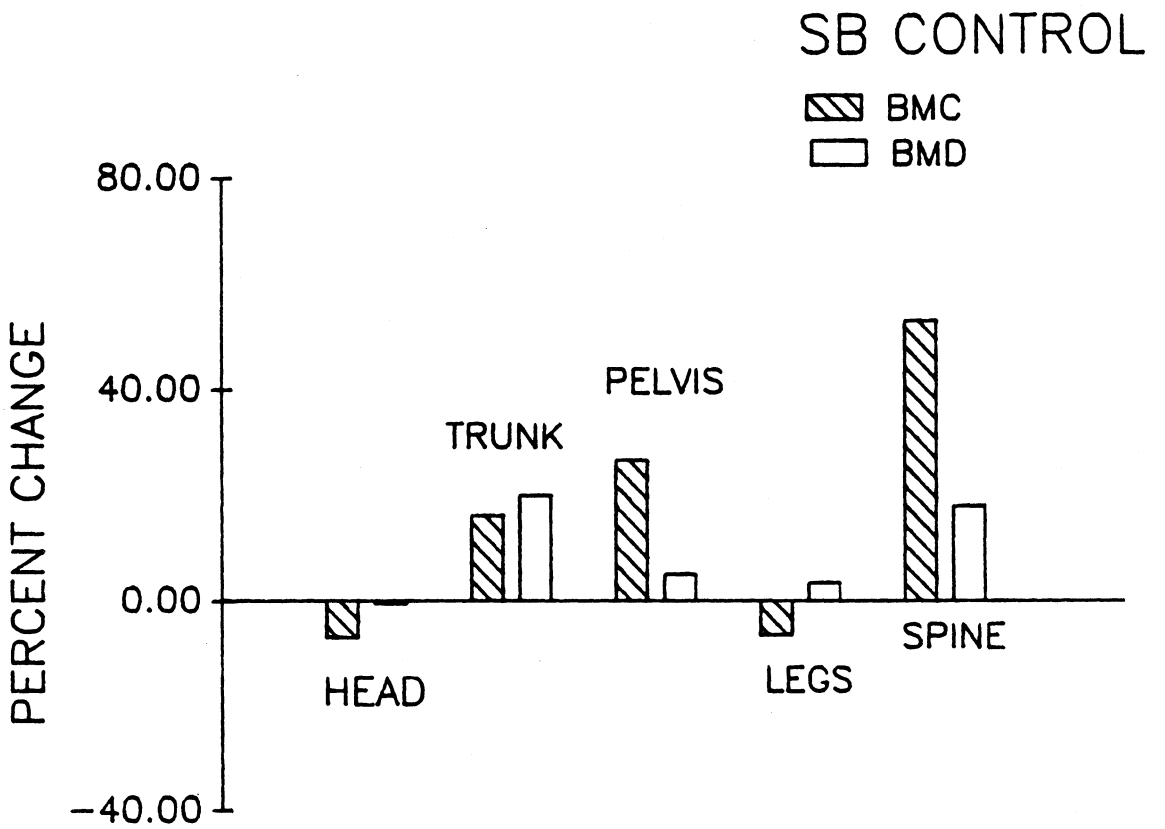

JV on E2
BMC
BMD
80.00
40.00
0.00
-40.00
PERCENT CHANGE
TRUNK
PELVIS
LEGS
SPINE
HEAD

Figure 13. Percent changes in segmental bone mineral content and bone mineral density for the control girl.


SB CONTROL
BMC
BMD
80.00
40.00
0.00
-40.00
PERCENT CHANGE
HEAD
TRUNK
PELVIS
LEGS
SPINE

CHAPTER IV

DISCUSSION

The most common feature of TS is short stature, with a mean height which is at least 2 standard deviations below the mean for age (Wilton, 1987). However the etiology of this characteristic is still controversial since numerous factors may influence growth and final height.

Some investigators believe that the skeletal abnormalities (reduced height, delayed bone age and low bone mineralization) in TS patients may be due to a perturbed GH profile since GH is essential for long bone growth and mineralization (Almqvist et al, 1963; Laczi et al, 1979; Lin et al, 1988; Kirkland et al, 1990). This belief is substantiated by the lower mean 24-hour GH levels in TS females after 9 years of age, due to reductions in GH peak amplitudes and peak frequencies (Ross et al, 1985;Ranke et al, 1987). As well, the fact that TS patients respond to hGH therapy with an increase in height velocity has added some strength to this hypothesis (Raiti et al, 1986; Buchanan et al, 1987; Takano et al, 1989; Rosenfeld et al, 1990). There are reports of classical GH deficiency in TS girls in response to GH provocation tests (Brook, 1978; Duke et al, 1981) but

these cases seem to be the exception, not the rule.

Van Vliet (1987) stated that the reduction in GH levels is caused by obesity in TS girls and not by a true deficiency in GH secretion. This hypothesis stems from the quantitatively abnormal GH response of obese subjects to a number of provocative stimuli (Glick et al, 1965).
Other investigators believe that the absence of ovarian steroids is the cause of the impaired GH profile in TS females (Frisch et al, 1988). This is based on the finding that estrogen stimulates GH and SM-C secretion (Copeland et al, 1984; Ho et al, 1987) and the fact that in normal girls the pubertal growth spurt correlates with the rise of estrogen (Ranke et al, 1986). On the other hand, the intrauterine growth retardation and delay in early childhood growth (Lubin et al, 1990), cannot be explained by GH deficiency since GH levels are normal in TS girls up to 9 years of age (Ross et al, 1985).

The final hypothesis is that the skeletal abnormalities are genetic in origin (Donaldson et al, 1968; Shore et al, 1982). It may be that the loss of X chromosome material results in the absence of a certain stimulus to achieve maximum height. This belief is substantiated by the finding that final height in TS females is strongly correlated with that of their parents, as is the case in healthy children (Brook et al, 1974). This observation suggests a fairly

constant proportion of height is lost through the absence of all or part of one X chromosome (Lemli & Smith, 1963).

There are also arguments against a genetic hypothesis from the finding that patients with defects of the autosomal chromosomes (ie; trisomy 21 and certain deletions) are also short in stature despite having a complete set of sex chromosomes. Therefore the growth promoting genes appear not to be limited to the X chromosome (Lubin et al, 1990). From these findings it has been stated that the hormonal and genetic hypotheses are not mutually exclusive and the skeletal abnormalities may be due to a combination of both factors (Finby & Archibald, 1963; Lubin et al, 1990).

Even though Turner's syndrome is characterized by short stature, ovarian dysgenesis and possibly also a perturbed GH profile, no one has investigated the secondary effects of the possible hormonal perturbations and reduced stature on muscle size and function. As well there has been no quantification of total body bone mineralization in relation to dietary intake or physical activity levels in this population. This is why the cross-sectional study was carried out.

Given the possible role of GH and sex steroids in the etiology of somatic growth delay, and the common place usage of GH and estrogen therapy in the clinical management of this disorder, it was important to determine the secondary effect

of such treatment on muscle and bone development and muscle function in TS girls. This was the objective of the longitudinal study.

PART I: CROSS-SECTIONAL STUDY

A. ANTHROPOMETRY

The TS girls were, as expected, much shorter than the control girls despite being older. Their mean height was below the 3rd percentile for their age based on values from Tanner and Davies (1985) and the Canada Fitness Survey (1985). The mean height for these patients was slightly higher compared to the mean height for TS females cited by Ranke (1983). This is probably due to the wide range of heights reported for TS girls. The control girls were at the 50th height percentile for their age.

The TS girls were at the 5th percentile for body mass and the control girls, being heavier, were at the 77th percentile for age. However, the TS girls were within the normal range for body mass reported for other TS girls of a similar age range (Ranke et al, 1983).

The measure of BMI indicated that the TS girls (17.6) were of normal weight for height. This conflicts with Becker's (1990) report that TS girls were overweight for height with a mean BMI of 21.3. The lower BMI in the present study reflects the fact that our TS girls were taller and lighter than Becker's TS females. Our control girls had a higher than

normal BMI, being above the 75th percentile for their age. This was due primarily to a greater than normal body mass, since they were at the 50th percentile for height.

There were no significant differences between the TS and control girls, for sums of skinfolds, percent body fat and fat mass: however, there was a distinct trend toward lower fat values in the TS girls. The lack of significance probably reflects the large variability of scores within the groups. This finding of leaner TS girls is contrary to reports in the literature where obesity is a more common clinical observation (Lippe, 1987; Raiti et al, 1986; Rosenfeld et al, 1990). This discrepancy may be due to the uniqueness of this small patient group or the wide range of physical characteristics in TS females.

B. MUSCLE SIZE & MORPHOLOGY

The cross-sectional area measurements of bone, fat and EF area for the control girls are within the range reported by Blimkie and colleagues (1989b) for normal healthy boys within the same age range (mean age 11.1 $\pm$ 1.6 years). Lean cross-sectional area (bone and muscle) for the lower leg in the control girls appeared to be similar to the results displayed in graphic form by Davies and colleagues (1983) for their 11 year old girls. There were no comparative data available for upper leg CSA.

In keeping with the finding that the TS girls are smaller, they also had reduced total limb CSA, as determined by computerized axial tomography, for all 3 limbs. This is explained by their smaller bones and muscles.

It was expected that the TS girls might have lower muscle density values since GH and estrogen both influence protein and fat synthesis and these hormones are frequently abnormal in TS patients. Yet statistically the muscle densities were comparable between the groups. The values for the KE density in our girls were similar to those reported by Bulcke et al (1979) for children 10-19 years old, yet our values for the plantar flexors were much higher than those measured in Bulcke's lab (1979; Termote et al, 1980). It is unclear why there are differences in PF density but due to the sparsity of normative data for muscle density scores, comparisons could not be made with other sources. Hopefully future studies will provide more information about muscle morphology and the effects of conditions such as TS on muscle growth and density characteristics.

C. ISOMETRIC STRENGTH & MOTOR UNIT ACTIVATION

Electrically evoked peak isometric twitch torque (TT) is a useful measure of the intrinsic force-producing capacity of muscle (McDonagh et al, 1983) since it is independent of skill, motivation and neural activation. There

were no statistically significant differences in TT between the groups for the EF's or PF's at any joint angle. The lack of significance probably reflects the small sample size and large variability since the TS girls were consistently weaker than the controls. The involuntary EF and PF strength values for the control girls were within the ranges reported by Davies et al (1983) and Blimkie et al (1989a) for girls and boys of comparable ages.

Evoked TT for the KE's was significantly lower in the TS girls at all joint angles when compared to the control girls. These lower values in the TS patients do not appear to be due to qualitative differences such as muscle packing density since muscle density values were similar for the TS and control girls. Yet when corrected for other factors also known to influence force production, eg. muscle CSA (Close, 1972) and lever length, the involuntary specific tension was similar between the groups for each muscle group. Collectively, these results suggest that when size was taken into account there were no differences in evoked strength between the TS and control girls. Davies et al (1983) made similar conclusions when they found that absolute differences in muscle strength in healthy children are a function of muscle mass. This also appears to be true for differences between normal healthy girls and girls with Turner's syndrome.

Absolute maximal voluntary torque was significantly

lower in the TS girls for the EF's, PF's and KE's at all joint angles. This finding could not be explained by a difference in motivation or neural activation since both groups had comparable and very high levels of motor unit activation for all muscle groups. Interestingly, the high level of MUA for both groups of girls for all three muscle groups is similar to or even higher than that seen in young boys (Blimkie et al, 1989a; Ramsay, 1989) and older untrained men (Belanger & McComas, 1981).

The absolute EF and KE MVC values for the control girls were comparable to those reported by Blimkie et al (1989a & b) and Hosking and colleagues (1978) for normal children. Strength scores for the control girls' KE MVC were at the lower end of the range reported by Davies et al (1983). Yet their girls were 2 years older which is a considerable difference at this stage of growth and development.

When these MVC strength measures were corrected for differences in body size, the relative measures of voluntary specific tension were comparable between TS girls and the controls. Therefore the decreased absolute strength (TT & MVC) appears to reflect the smaller size of the TS patients and does not appear to be due to a difference in motivation, neural activation or muscle density.

Absolute strength values for the TS girls are lower than those reported by Parker (1990) for her 6 TS patients.

Her girls were taller however, than girls in the present study, and when strength was normalized for height, this discrepancy between studies was reduced but not totally eliminated. Had a further correction been made for muscle size, perhaps there would have been no difference in strength scores for the TS girls in these two studies. As well Parker's girls were slightly older than our TS patients.

D. CONTRACTILE PROPERTIES

The values for the control girls' time-related contractile properties are similar to those reported by other investigators (McDonagh et al, 1983; Blimkie et al, 1989a & b). The slower time-to-peak torque in the EF's of the TS girls is the only difference in time-related contractile properties between the groups. This suggests that there may be dissimilarities in the mechanisms regulating the rate of muscle contraction in the TS girls. This possibility deserves further study. Nevertheless, the dissimilarities appear to have no affect on the magnitude of peak force production in TS when normalized for differences in muscle size and mechanical advantage. Thus it seems unlikely that this reflects a defect in the contractile apparatus of the TS patients.

E. BONE MINERALIZATION

At present, skeletal investigations involving the

determination of bone mineral content and density in TS patients have been limited to single sites such as the distal portion of the radius (Brown et al, 1974: Smith et al, 1982), the os calcis (Risch et al, 1976) and the lumbar spine (Stepan et al, 1989). These studies have all found that bone mineralization (content and density) is significantly lower in TS girls compared to control values.

Total body bone mineral content for the Turner's girls measured in this study with dual photon absorptiometry, was significantly lower when compared to the control girls. The largest difference between the groups was at the legs which probably reflects
the shorter and more narrow leg bones in the TS girls. The lack of a significant difference between the groups at the head, spine and pelvis is surprising since TS girls in general, have smaller skulls (Lubin et al, 1990) and there are also reports of spinal abnormalities (Rubin et al, 1990; Massa & Vanderschueren-Lodeweyckx, 1989). However segmental measures of bone mineralization must be interpreted cautiously since the measurement error is greater than that for the whole body technique.

Total body BMC normalized for body mass (g/kg) was comparable between the TS and control girls. The slightly lower leg value and higher head BMC (g/kg) was an unexpected finding. This probably reflects the measurement error

associated with segmental analysis since there is no biological explanation. Total body and segmental bone density, measures of bone quality, were not significantly different between the groups. Therefore the lower values for BMC reflect the smaller body size in the TS girls. Lubin and coworkers (1990) also found quantitative differences in TS bones' with a reduced height of the proliferative and hypertrophic cell columns, yet qualitatively their bones were normal for their age.

Dawson-Hughes and colleagues (1987) noted the importance of body mass in determining bone mineralization. This was also found for the girls in the present study and confirmed by multiple regression analysis which showed that body mass rather than age, physical activity profile, nutritional intake or strength, was the major determinant of bone mineralization in this study. These results also demonstrated that the TS girls were on a similar bone development curve and their position at the lower end of the curve merely reflects their reduced body size as opposed to a qualitatively abnormal bone mineralization.

Thus prior to puberty, there appears to be no qualitative difference such as an abnormal skeletal mineralization or osteoporosis, as previous reports have indicated. Rubin and colleagues (1990) noted that mean lumbar density in TS is not statistically different from age matched

controls until age 14 and beyond. Perhaps after this age normal BMD cannot be maintained throughout adulthood in TS women without estrogen supplementation. This concept requires further study.

Since there is a void in the literature of normal bone mineral values in children for total body and segmental BMC and BMD, the control girls were compared to other healthy girls measured in our lab at McMaster University Medical Centre (Webber & Meyer, 1990 unpublished data). Total body values for BMC were similar to those for the control girls. However the bone density values for the control girls were slightly lower than the previously measured values for Webber and Meyer's matched control girls. At present, there is no obvious explanation for this difference.

F. PHYSICAL ACTIVITY

A physical activity questionnaire was used because it is the most practical and widely used approach for the assessment of physical activity in epidemiologic research (Washburn & Montoye, 1986). The combination of a personal interview and a self-administered questionnaire was employed to overcome the limitations inherent in each method. It is possible that the similar physical activity scores for the TS and control girls reflects the fact that all of these girls were moderately active. On the other hand, the physical

activity questionnaire used in this study may not have been sensitive enough to differentiate between the groups. A motion sensor might have given a more precise measure of physical activity patterns. Yet this was impractical with some of the patients coming from out-of-town. Nevertheless, accepting the limitations of this methodology, it appears that differences in physical activity appear not to be an important factor in differentiating strength performances between TS and control girls in this study.

G. DIET

There were no significant differences between the TS and control girls on any of the nutritional indices. This may reflect the large daily variations in food intakes or the fact that these girls were of a similar socioeconomic class so that they all had adequate food availability.

When these values were compared with the recommended nutrient intake (RNI) from the Ministry of Health and Welfare (1990), the values met or exceeded the range of the RNI. The carbohydrate content was slightly lower than recommended, and fat and protein intake was much higher than the RNI, as is common in the North American diet. All of the girls had near normal calcium intakes.

Total calories consumed per day were within the accepted range for age for almost all the girls. However, when caloric intake was expressed per kilogram of body mass the TS girls consumed more calories than the control girls yet this did not reach statistical significance. This trend may reflect a lower metabolic efficiency in the control girls since they had a greater lean body mass. On the other hand, it could be indicative of a higher basal metabolic rate in the TS girls. It may also be that the control girls chose to give "socially desirable" answers (Gibson, 1987). As a group they had excess fat and may have realized that they should be taking in fewer calories and hence chose to report or consume fewer calories. The patients had often complied with physician's orders, so they may have given a truer diet history since the procedure was explained to them by a doctor.

Since the diets were not significantly different between the TS and control girls, it is not likely that dietary factors contributed to any of the differences in muscle size and function or bone development.

SUMMARY

From the cross-sectional data it appears that for these 7 TS girls, their lower absolute strength and bone mineral content merely reflects their reduced body size (short stature, smaller bones and muscles) since relative measures of

strength and bone mineralization are comparable to those of the control girls. It is still unclear what causes this size difference but neither the genetic nor the hormonal hypothesis can be ruled out at this point.

PART II: LONGITUDINAL STUDY

Growth hormone has been administered to increase linear growth in pituitary dwarfs since 1951 (Escamilla & Bennett). With the advent of synthetic human growth hormone preparations, shGH has been used in numerous clinical trials to increase height in children with short stature (Aceto et al, 1972; Ferrandez et al, 1970; Frasier et al, 1981; Raben et al, 1958).

The early studies of hormonal therapy in Turner's patients had problems such as variability in patient age, GH dosage, length of therapy and presence or absence of concomitant sex steroid treatment, which made the results difficult to interpret (Rosenfeld et al, 1990). More recently, carefully designed and well controlled multicentre trials have been initiated. Despite the reports of skeletal abnormalities, reduced bone mineralization and a possible GH perturbation in TS females, there is surprisingly little known about the anabolic effects of GH therapy on the muscles and bones of these patients.

In addition to growth hormone, patients, especially those approaching the normal age of puberty are often treated with estrogen to induce secondary sexual characteristics. Like growth hormone, estrogen too has been shown to exert significant independent effects on skeletal development and somatic growth. Despite its common usage in treatment of TS girls, surprisingly little is known about the influence that estrogen has on muscle development and function and bone development in this population.

A. ANTHROPOMETRY, MUSCLE SIZE AND MORPHOLOGY

(i) growth hormone effects

Growth hormone therapy had a dramatic effect on the height in the TS patient (ST_{gh}) as seen by the increase in absolute height and the dramatic improvement in height velocity. The improvement in height velocity exceeded the normal values for TS girls on hGH therapy (Ranke et al, 1983) and was even higher than that of our control girl entering puberty. It is unclear why $ST_{(gh)}$ grew so much faster than the typical TS patient. Some investigators have found that increases in height velocity and height, were greatest in younger patients (Vanderscheuren-Lodewckx et al, 1990) but $ST_{(gh)}$ was already 12.5 years old at the onset of GH therapy. However, according to Brook's hypothesis (page 201, 1988) her low pretreatment height velocity of 3.4 cm/year could account

for this large spurt since "the more slowly a child grows before treatment....the greater will be the augmentation in height velocity".

The dramatic decrease in fat mass and the increase in lean mass during hGH therapy in $ST_{(gh)}$ is in agreement with other reports in the literature (Rudman et al, 1990; Jorgensen et al, 1989). This is due to the fact that GH enhances fat oxidation and stimulates protein synthesis (Ponting et al, 1988). The increase in whole body lean mass (based on the sum of 4 skinfolds) was confirmed at the regional level by increases in muscle area (using computed tomography scans) in all 3 muscle groups. This increase was most apparent at the EF's and KE's. This may reflect a specific proximal effect of GH therapy on muscle size. This will be discussed below with the strength data.

A. ANTHROPOMETRY, MUSCLE SIZE & MORPHOLOGY
(ii) estrogen effects

As $JV_{(e2)}$ had reached her 13th birthday, she was too old for the GH trial so low dose estrogen was prescribed to induce development of secondary sexual characteristics and initiate menstruation. Estrogen treatment did not result in a major gain in height or height velocity. This was expected since it has been shown that response to estrogen therapy is better in younger TS patients (Kastrup et al, 1986).

The fat mass increase in $JV_{(e2)}$ was larger than that of the control girl, reflecting the estrogen induced deposition of subcutaneous fat (Guyton, 1981). Muscle area increased for all 3 muscle groups which probably reflects the laying down of new muscle proteins as a result of estrogen therapy (Lorenz, 1954: Trenkel, 1976).

A. ANTHROPOMETRY, MUSCLE SIZE & MORPHOLOGY
(iii) control effects

The control girl $SB_{(c)}$ had a substantial increase in height and her height velocity was above the 97th percentile for her age, indicating that she is probably an early maturer, entering puberty. She also had an increase in fat mass (from the four site skinfold technique: Moritani & DeVries, 1980) and fat area (from CAT scan analysis) which is characteristic for females as they enter womanhood. However when using the 2 site technique to estimate percent body fat, subcutaneous fat was unchanged. The discrepancy between these measures of adiposity reflects the fact that the latter measure is merely a combination of fat measurements from two sites and the former indices estimate total body adiposity from a greater number of sites. Therefore the lack of change in percent body fat (from the 2 site method) is a result of an increase in subscapular fat and a concomitant decrease in triceps fat. Since $SB_{(c)}$ had such a high percent body fat, total body

measures of fat provide a more reliable estimate of body fatness.

Total limb cross-sectional area was increased at all 3 sites for $SB_{(c)}$ due to the substantial increase in fat area. However, the only change in muscle area for $SB_{(c)}$ was a minimal increase in lower leg area. Muscle density values were reduced from the pre test scores at all sites, which reflects an increase in intramuscular fat.

B. ISOMETRIC STRENGTH & MOTOR UNIT ACTIVATION.
(i) growth hormone effects

Despite consistent increases in muscle CSA for all three muscle groups, strength increased only for the EF's for $ST_{(gh)}$. This is surprising given the known close association between muscle size and strength both during normal growth (Chapman et al, 1984: Close, 1972) and with weight training in adults (MacDougall, 1986: Sale et al, 1987). This dissociation between changes in muscle size and strength appears not to be related to changes in muscle quality, since there was only a slight increase in PF muscle density.

The slight increase in PF muscle density is probably due to an intramuscular fat loss similar to the increase reported after gastroplasty (surgery to reduce body fat) in morbidly obese patients (Newham et al, 1988). However these investigators found that leg strength was unchanged despite an

increase in density, and we found a decrease in all measures of PF strength. Perhaps our 13% increase in PF density should be interpreted cautiously since decreases in muscle volume could reflect changes in fluid retention or connective tissue mass, independent of changes in contractile protein (Jorgensen et al, 1989). An alteration in non contractile material might help to explain the apparent dissociation between density and strength changes. As well the change could be within the measurement error of this technique (Jones et al, 1983).

The decrease in KE strength (TT and MVC) cannot be explained by a dramatic change in KE muscle density or motor unit activation. Perhaps the decrease in leg strength values in $ST_{(gh)}$ reflects a detraining effect from the pre to post test as a result of cessation of her skating training program (as mentioned by the patient's mother).

The dramatic increases in arm strength and reduced leg strength (PF & KE) may be due to the greater sensitivity of the smaller proximal arm muscles to GH therapy. This is substantiated by the much larger increase in EF TT and MVC in $ST_{(gh)}$, compared to $JV_{(e2)}$ or $SB_{(c)}$. Bigland and Jehring (1952) noted that GH therapy in rats exerted a stronger influence on the proximal muscles observed by proximal muscle hypertrophy. As well, they found that an increase in muscle size was accompanied by a reduction in strength, indicative of the laying down of a non-contractile substance. However our

results were not in complete agreement with these earlier findings since we observed an increase in both proximal EF muscle area and strength but a decrease in KE strength despite an increase in KE CSA. This apparent discrepancy remains unresolved.

The larger increase in EF evoked versus voluntary strength probably reflects the passive stretch of the muscle as a result of the rapid bone growth (Vandenburgh & Kaufman, 1979). As well, the smaller increase in voluntary strength may reflect a delay in the co-ordination of the neurological pathways serving the enlarged muscles.

B. ISOMETRIC STRENGTH & MOTOR UNIT ACTIVATION.
(ii) estrogen effects

The increase in strength for $JV_{(e2)}$ at all muscle groups, is probably the result of the increase in muscle area for all 3 muscle groups. This is because MUA was essentially unchanged and muscle density was, with the exception of only a slight increase in PF density, also virtually unchanged. It is interesting to note that the only major increases in muscle density occurred at the PF's for both of the TS girls. Yet it is unclear why this occurred.

The only increase in twitch strength, relative to muscle area, occurred at the EF's which may reflect a greater capacity for change in the relatively untrained arm muscles.

Absolute and relative PF maximal voluntary strength increased dramatically more than strength at the EF's or KE's. Even though PF motor unit activation was unchanged the increase in PF strength could have been due to an improved motor unit firing rate (MUFR) since there is an optimal MUFR needed for maximal force development and a change in MUFR could occur without a change in MUA (Sale, 1987).

B. ISOMETRIC STRENGTH & MOTOR UNIT ACTIVATION.
(iii) control effects

Even though muscle cross-sectional area was not substantially increased and muscle density was reduced, absolute measures of strength (TT & MVC) increased for all muscle groups, except the PF (twitch) score, which remained the same. This is contrary to reports in the literature in which reduced muscle density, due to fatty infiltration and muscle atrophy, resulted in lower strength values (Horber et al, 1985). Thus the increase in intramuscular fat in the control girl did not impair her voluntary or evoked strength. The improved strength scores were not due to dramatic improvements in MUA since the values remained relatively the same. The increases in strength may, at least in part, be due to maturational changes in neurological development during the follow-up period. This may have resulted in improvements in neuromuscular coordination of prime movers and improved

coordination of agonist and antagonist muscle. As well, an increase in motor unit firing rate without a significant change in motor unit activation (Sale, 1987) could partly explain the noted strength increases in this subject.

The increase in all specific tension measures, except the PF twitch measure, without an increase in muscle area confirms the previous hypothesis that factors other than an increase in muscle size (e.g. improved coordination or motor unit firing) may have contributed to the enhanced strength.

C. CONTRACTILE PROPERTIES
(all three girls)

The changes in total contraction time were within ± 10% of their original values for all girls, and all muscles, and changes in TPT and HRT were highly variable and unpredictable. Given the magnitude and nature of these changes it is difficult to state conclusively, the effects of the various hormone therapies on the contractile properties of the girls in this study. Additionally, it is known that test-retest reliability of these measures is not high (Ramsay, 1989), thus it would be imprudent to extend the interpretation of these data.

D. BONE MINERALIZATION

Total body BMC (absolute and relative to body mass)

and BMD appear to have remained unchanged from pre test values for the two Turner's patients; this is due to the balance of increases and decreases in segmental bone mineralization.

Regional bone mineralization was measured in adults in our lab with a reproducibility of 4% (Ormerod et al, 1990). Thus when repeat segmental measurements were made for the longitudinal study for this group of 3 small children it's limitations must be kept in mind since the segments are very small and the error could be quite significant.

(i) growth hormone effects

The largest increase in $ST_{(gh)}$'s bone mineralization occurred for leg BMC. This probably reflects the increase in leg bone length resulting from GH therapy. Even though she had an increased bone growth reflected by the increase in bone area, leg bone density was preserved. On the other hand, GH therapy did not result in such a dramatic change at pelvis, trunk and spine BMC. This is in agreement with the known direct actions of GH on development of limb bones.

Bone mineral content and density at the head were decreased. This decreased mineralization of the head could reflect a failure of GH to influence the cranial bones or it could be a characteristic unique to $ST_{(gh)}$ since neither $JV_{(e2)}$ nor $SB_{(c)}$ had any change in head BMD. Bone mineral density at the spine remained constant which is a positive indication that there was no spinal demineralization. The pelvis and

trunk BMD were dramatically reduced in $ST_{(gh)}$. It is unclear why BMD at the pelvis was so dramatically reduced for $ST_{(gh)}$ as well as for $JV_{(e2)}$. A longer observation period for the TS patients and control girl might provide more insight into this finding.

D. BONE MINERALIZATION
(ii) estrogen effects

There was a slight increase in leg BMC for $JV_{(e2)}$ but this was much less than that observed for $ST_{(gh)}$. This is probably due to the fact that estrogen speeds up closure of the epiphyseal plates (Ranke et al, 1986; Kastrup et al, 1986) and prevents the larger increase in height that is seen in $ST_{(gh)}$, which was accompanied by a large gain in BMC. Nonetheless the slight increase in leg BMC with estrogen therapy is a good sign of normal bone mineralization at this stage in her life. The small increase in spine BMC and decrease in spine BMD do not appear to be of significance. The main point here is that estrogen did not result in any dramatic changes in spine mineralization.

The lack of a dramatic effect of E2 on bone mineralization during such a brief follow-up period is consistent with previous reports where short term discontinuous estrogen therapy failed to improve bone mineralization in TS females (Shore et al, 1981; Smith et al,

1982; Rubin & Dawlski unpublished observations in Rubin et al, 1990). Perhaps the anticipated positive effects of E2 on bone mineralization might only be evident over a longer time course than was used in this study.

D. BONE MINERALIZATION
(iii) control effects

It is unclear why $SB_{(c)}$ did not have a major gain in leg bone mineralization when she did have a substantial increase in height. However she did manifest greater increases in trunk, pelvis and spine BMC due to normal growth and development as compared to the TS girls. This may be because the duration of the treatment phase was not long enough for the TS girls to manifest a large gain in bone mineralization, a finding which has been noted for TS girls on either GH and estrogen therapy (Kirkland et al, 1990; Rubin et al, 1990). The control girl also had the largest increase in body mass which is an important factor since it has a profound influence on bone mineralization (Dawson-Hughes et al, 1987). As well, a genetic predisposition to well mineralized bones cannot be ruled out since this factor could not be isolated in the present study.

In order to evaluate bone mineralization segmentally, repeated scans every three months would probably be more helpful. As well a longer follow-up period of a few years

would provide more information about the long term process of bone mineralization under the influence of hormonal therapy.

E. DIET
(i) growth hormone effects

The TS patient receiving GH maintained a constant energy intake which led to her minimal weight gain. This is surprising because during this period of rapid growth one would expect an increased caloric requirement. Since this was not the case, some of this extra energy required was obviously mobilized from fat stores by circulating growth hormone. As well she may have been able to maintain her body weight since her meals were carefully planned by her mother (personal communication) which was reflected by the proportional loss of fat mass to the gain in lean mass.

E. DIET
(ii) estrogen effects

The TS patient receiving estrogen therapy had a decrease in energy intake. Thus her 9.5 kg increase in body mass may be partly due to the effects of estrogen, which promotes fat deposition and weight gain. She also had a slightly lower score on the parental assessment of physical activity, resulting in a reduced caloric requirement.

E. DIET
(iii) control effects

The control subject $SB_{(c)}$ had a 7.9 kg increase in body mass, an increase in all limb cross-sectional areas and increases in absolute strength, yet she reported a major decrease in energy consumed. The reduced caloric intake may be partly due to the report of a substantial decrease in physical activity reflecting a reduced energy requirement or she may have underestimated the number of calories she consumed.

SUMMARY

As expected, growth hormone therapy did increase height, height velocity and lean mass, as well as reducing adiposity. Measures of absolute and relative arm strength (EF) increased dramatically during growth hormone therapy. In most cases the increases were larger than those seen with estrogen therapy or in the control girl during normal growth and development. Yet all measures of leg strength (PF & KE) were reduced compared to the substantial gains seen in absolute strength for $JV_{(e2)}$ and $SB_{(c)}$. These large decreases cannot be explained by changes in anthropometry, muscle density or motor unit activation. These differences may be due to the action of growth hormone on the leg muscles or a strength detraining effect resulting from $ST_{(gh)}$'s withdrawal from her skating program.

The gain in voluntary and evoked strength in $JV_{(e2)}$ appears to be due to the increased muscle area as a result of estrogen therapy. However this increase in strength is still less than that observed for the control girl, who had increases in voluntary strength without increases in muscle area. The latter is probably due to better co-ordination and control of agonist and antagonist muscles or an enhanced motor unit firing rate and synchronization since muscle density was decreased at all sites and motor unit activation remained unchanged.

Growth hormone therapy resulted in an increase in leg, pelvis, spine and trunk BMC. Bone density was maintained for the legs and spine, whereas BMC of the head and BMD of the pelvis, trunk and head were reduced, even with GH therapy. On the other hand, estrogen therapy resulted in a slightly higher leg and spine bone mineral content. Estrogen maintained BMC in the pelvis, trunk and head but could not prevent the decreases in BMD for the pelvis and spine. During 5 months of normal growth and development, the control girl had substantial increases in BMC for the trunk, pelvis and spine. Bone mineral density in $SB_{(c)}$ was dramatically increased for the trunk and spine and it was maintained or slightly increased for the pelvis, legs and head. These increases are probably due to the increased body mass and normal growth in $SB_{(c)}$.

The changes in musculoskeletal parameters were not due to differences in dietary intake or physical activity patterns as these measures were comparable for the TS patients and the control girls.

Many of the musculo-skeletal changes in response to different hormonal therapies were as expected; however not all of the changes could be accounted for physiologically. Measurement error associated with the various techniques used in this research probably accounts for many of the unanticipated and unexplained results. Measurement error would be expected to take on an even more prominent role with the longitudinal component of this research.

Further studies are needed with larger subject groups of varying ages and longer treatment periods in order to make conclusive statements about the effects of hormonal therapy on the musculoskeletal system.

Chapter V

SUMMARY

Skeletal muscle development, function and bone mineralization were examined in a cross-sectional study of young girls with TS and healthy controls. The rationale underlying the cross-sectional study was that TS patients have an abnormal hormonal milieu which could impair normal musculo-skeletal development and function. Using case studies, and a prospective design, the effects of GH and E2 therapy on musculo-skeletal parameters were evaluated in two girls with TS and one healthy control girl.

The major findings of this study were:

Cross-Sectional Study

1. The TS girls were shorter, lighter and leaner than the controls.
2. Absolute measures of strength (TT and MVC) were lower, but the lower strength scores for the TS girls could not be accounted for by differences in muscle density, contractile properties, MUA, diet or level of physical activity.

3. Evoked and absolute strength measures, corrected for differences in body size (strength per cross-sectional area of muscle and lever length), were not significantly different between the TS and control girls.

These results represent new findings and indicate that both voluntary and evoked strength of TS girls is reduced compared to normal girls, and that the reduction in strength is due solely to quantitative differences in muscle size.

4. As expected absolute total body and segmental (leg, and trunk) BMC were lower in the TS girls compared to the controls.

5. Contrary to most observations, total body bone mineral content normalized for body mass (g/kg) or bone density were comparable between the TS and control girls. Prior to puberty these TS girls were on the same bone mineral growth line as the control girls; hence, their lower absolute BMC values merely reflected their smaller size and not a qualitative abnormality in bone mineralization.

Prospective Study

Growth Hormone Effects

1. As expected growth hormone therapy resulted in an increase in height, height velocity and lean mass, as well as a reduction in adiposity.

2. All measures of arm strength increased but leg strength (PF & KE) was reduced.

3. These decreases cannot be explained by changes in anthropometry, muscle density or motor unit activation. They may reflect the lack of GH effects on the leg muscle or possibly a detraining effect from the subject's withdrawal from a skating program.

4. There was an increase in BMC at the legs and BMD was reduced at the head, pelvis and trunk during GH therapy. This may reflect the lag time between bone growth and the subsequent increase in bone mineralization.

Estrogen Effects

1. Estrogen therapy did not result in a major gain in height but there were increases in muscle area and fat mass.

2. There were significant gains in strength at all 3 muscle groups, probably reflecting the laying down of muscle proteins as a result of estrogen therapy.

3. There was a lack of dramatic changes in BMC and BMD, probably reflecting the short duration of the follow-up period.

Control Effects

1. The control girl had a large gain in height which was probably due to the early onset of her growth spurt.

2. Percent body fat increased and muscle density decreased due to the accumulation of intramuscular fat.

3. Almost all measures of strength improved despite a lack of change in muscle area. It appears that the increases in strength reflect maturational changes.

4. The increase in trunk, pelvis and spine BMC may be due to the control girl's increased body mass combined with normal growth and development. Bone density was increased at the trunk and spine indicating that bone mineralization kept pace with bone growth, at these sites.

These results notwithstanding, there were several limitations to this study which limit the generalizability of the conclusions. These include the small sample size and bias in subject selection, the short duration of the follow-up period and the difficulty in finding an appropriate control group. However, this study was unique in that new information was provided about habitual physical activity levels, dietary intake and musculo-skeletal parameters in young Turner's patients. Further studies are required with larger numbers, for longer treatment periods in order make conclusive statements about the effects of hormonal therapy on muscle development, function and bone mineralization in Turner's patients.

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APPENDIX A. Tanner's pubic hair stage rating.

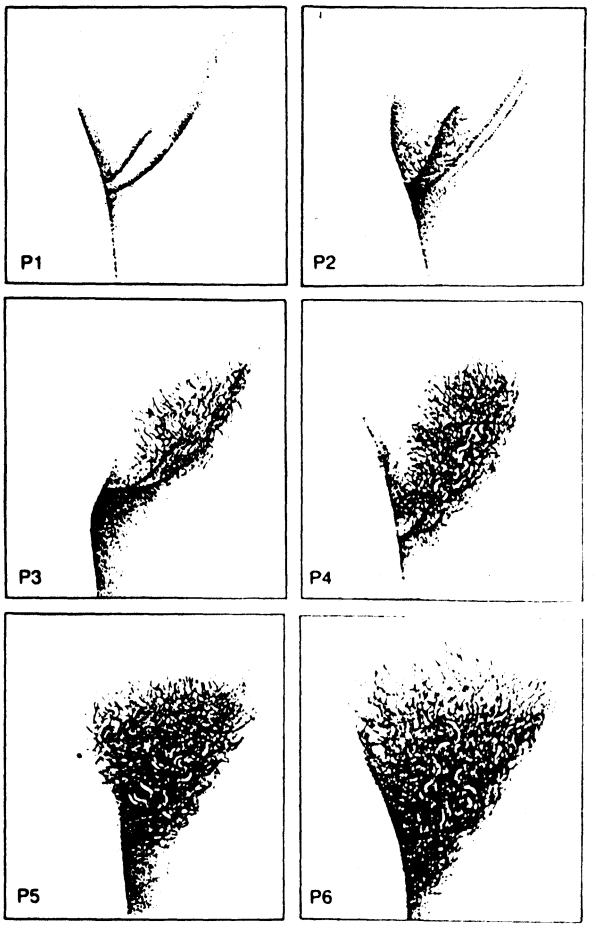
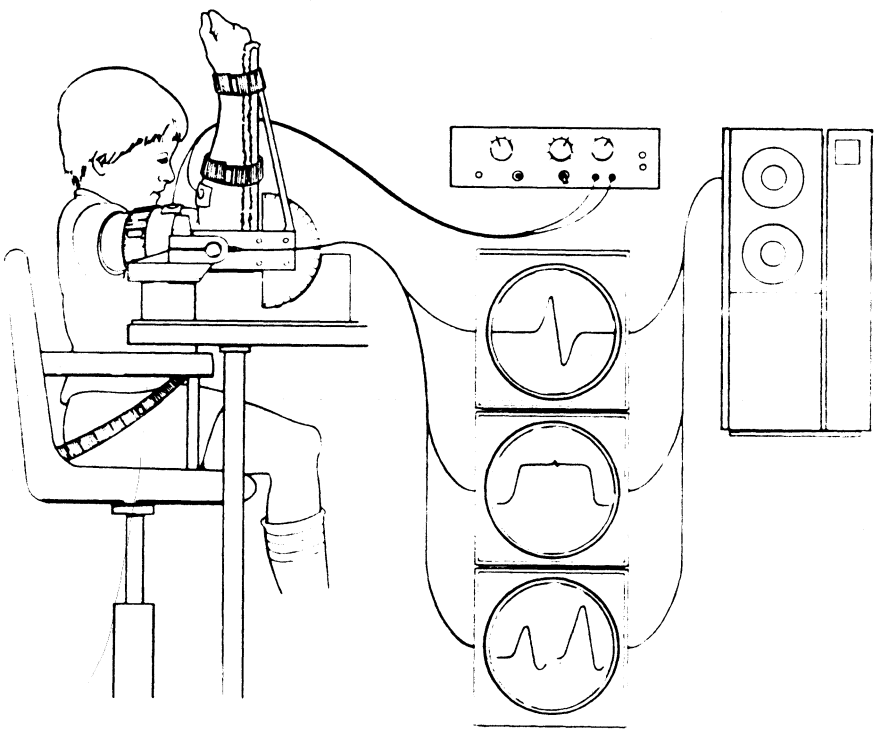


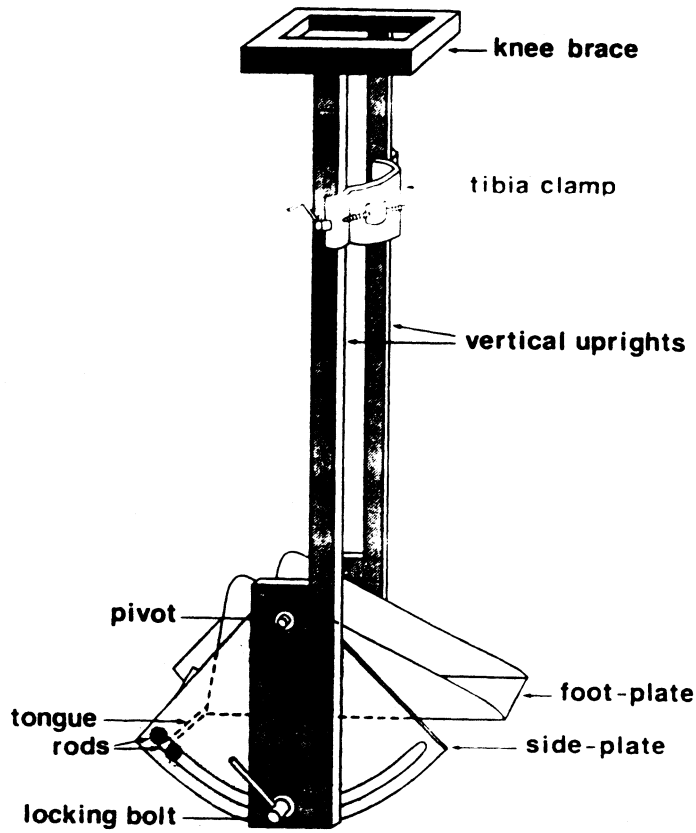
Fig. 6.22 Stages in pubic hair development in girls. In the development of pubic hair, six stages can be distinguished:
P1 — no growth of pubic hair.
P2 — initial, scarcely pigmented hair, especially along the labia (not visible on black-white photographs).
P3 — sparse dark, visibly pigmented, curly pubic hair on labia.
P4 — hair 'adult' in type, but not in extent.
P5 — lateral spreading; type and spread of hair — adult.
P6 — further extension laterally, upwards, or disperse (apparently occurs in only 10% of women).

Redrawn, with permission, from *Growth Diagrams 1965*, by J.C. Van Wieringen, F. Wafelbakker, H.P. Verbrugge, J.H. De Haas, Nederlands Instituut voor Praeventieve Gezondheidszorg TNO. Wolters-Noordhoff Publishing, Groningen, 1971.

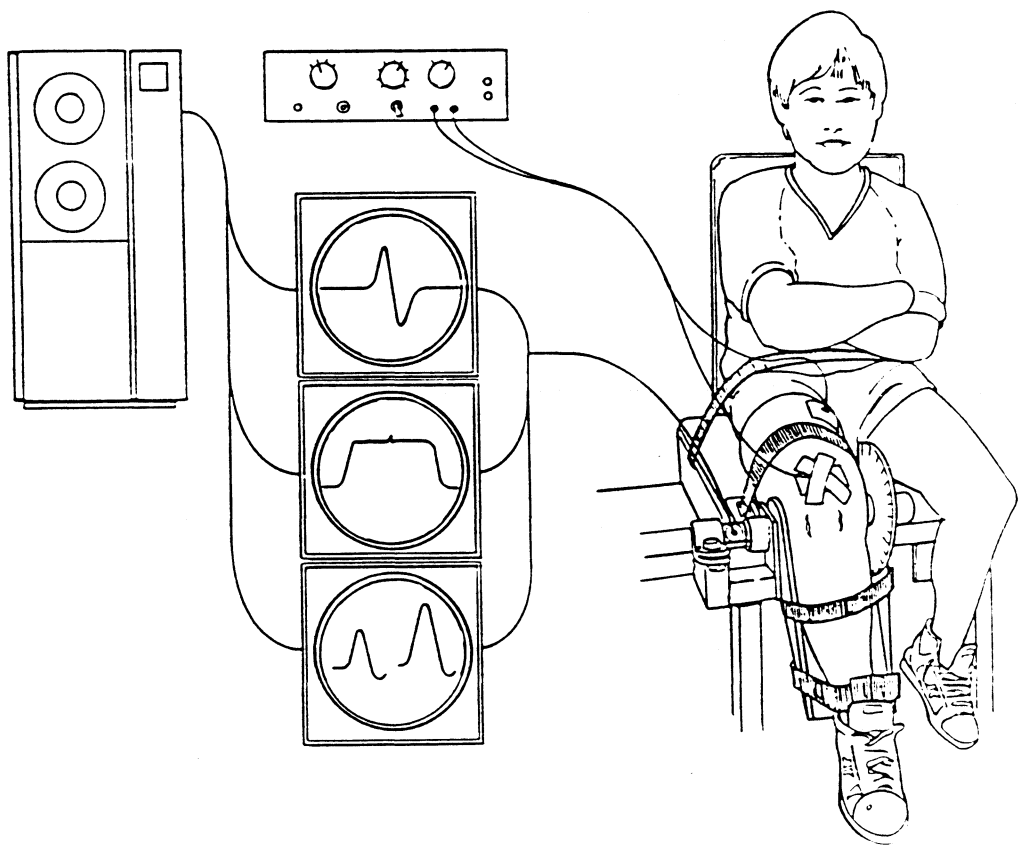
APPENDIX B. Elbow flexor testing apparatus.



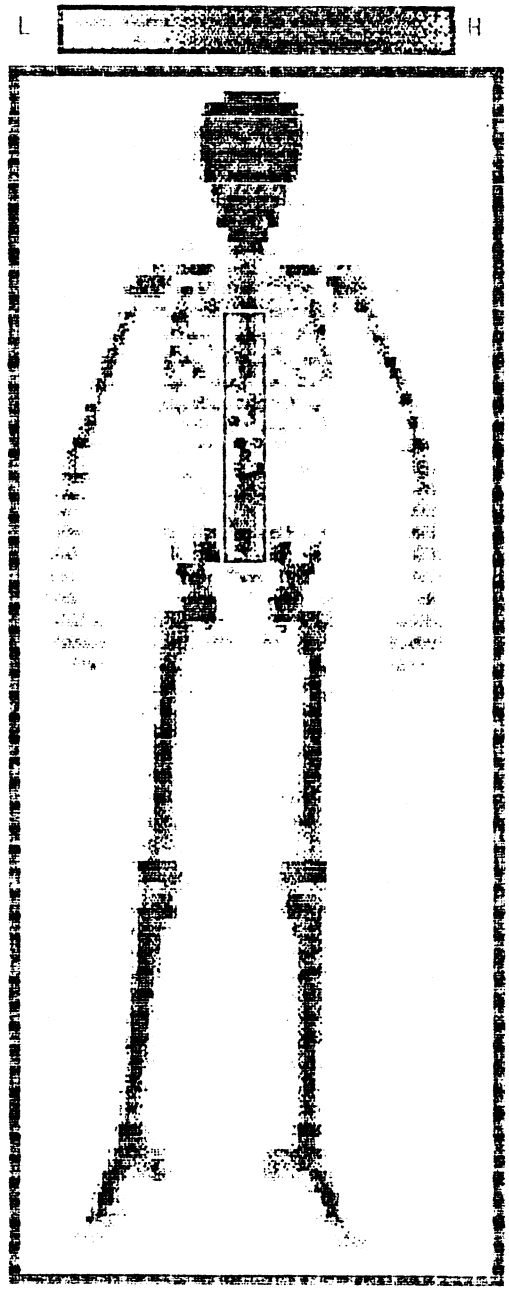
APPENDIX C. Plantar flexor testing apparatus.



APPENDIX D. Knee extensor testing apparatus.



APPENDIX E. Bone scan from the dual photon absorptiometry technique to assess bone mineralization.



APPENDIX F. Parental physical activity questionnaire and children's survey.

QUESTIONS REGARDING CHILD'S ACTIVITY

Circle the number corresponding to the most appropriate answer.

1. Would you say that your child moves _____ other children of the same age and sex?
   - (0) much less than
   - (1) somewhat less than
   - (2) about the same as
   - (3) somewhat more than
   - (4) much more than

2. Would you say that your child moves _____ his/her:

siblings of the same sex?	siblings of the opposite sex?
(0) much less than	(0) much less than
(1) somewhat less than	(1) somewhat less than
(2) about the same as	(2) about the same as
(3) somewhat more than	(3) somewhat more than
(4) much more than	(4) much more than

3. In general, would you say your child prefers to be _____.
   - (1) usually indoors
   - (2) equally indoors and outdoors
   - (3) usually outdoors

4. In general, when your child plays, he/she _____.
   - (0) never gets out of breath nor sweats
   - (1) rarely gets out of breath or sweats
   - (2) sometimes gets out of breath and/or sweats
   - (3) often gets out of breath and/or sweats
   - (4) always gets out of breath and/or sweats

5. How does your child usually get to school?
   - (0) bus/car
   - (1) walk Distance _____ mile(s) (12 blocks/mile)
   - (2) bicycle Distance _____ mile(s)
   - (3) other Please specify _____

6. Do you consider your child to be active?
   - (1) no
   - (2) yes

Fill in the blanks.

7. What are your child's most common indoor activities?

   1. _____

   2. _____

   3. _____

8. What are your child's most common outdoor activities?

   1. _____

   2. _____

   3. _____

9. What is the average number of hours/day that your child spends outdoors in the winter? _____ hrs/day

10. What is the average number of hours/day that your child spends outdoors in the summer? _____ hrs/day

11. Choose one of the responses (0-4) regarding the level of physical activity for each time frame (a-f) of a typical day for your child.

    _____a. before school
    _____b. after school/before dinner
    _____c. after dinner/before bed
    _____d. on a weekend morning
    _____e. on a weekend afternoon
    _____f. on a weekend evening

Responses
(0) Inactive
(1) Light
(2) Moderate
(3) Hard
(4) Very hard

12. What is the average number of hours/night that your child sleeps on week nights? _____ hrs/night
    on weekends? _____ hrs/night

13. What is the average number of hours/day that your child spends watching T.V. on weekdays? _____ hrs/day
    on weekends? _____ hrs/day

14. List any games, activities, or sports that your child frequently participates in during each season of the year. Use the activity list (page - as a guideline, but you also may include other activities (be specific). Please consult your child when necessary.

ACTIVITY	Frequency (times/wk)	Duration (min/ session)	SEASON(S) (Summer, Fall, Winter, Spring)

TO SCORE PHYSICAL ACTIVITY QUESTIONNAIRES: ADD TOGETHER THE NUMBERS IN PARENTHESES THAT WERE CIRCLED. THEN SUBTRACT THE SUM OF QUESTIONS 12 & 13. TO SCORE THE ACTIVITIES IN QUESTIONS 7,8 & 14 REFER TO THE ACTIVITY CHART ON PAGE 136.

INACTIVE(0)	LIGHT(1)	MODERATE(2)	HARD(3)
TV/VIDEOS	BUILDING	SLEDDING	WAR(OUTDOORS)
BOARDGAMES	(i.e.,LEGO'S)	BASEBALL	CLIMBING
READING	PLAYING WITH:	GYMNASTICS	(TREES,ETC.)
DRAWING/ART	CARS	SWIMMING	SOCCER
SLEEPING	DOLLS	SKIING	BASKETBALL
EATING	TOYS	SNOWBALL FIGHTS	FOOTBALL
COMPUTER	PETS	SNOWMEN(BUILD)	TAG-ALL TYPES
WRITING	PLAYING HOUSE/	SNOWFORTS(BUILD)	RUN
PLAYING	SCHOOL	BIKING	HOCKEY
PIANO	SANDBOX PLAY	HIKING	SHOVELING
PLAYING	HIDE-N-GO-SEEK	SWINGING/	HOPSCOTCH/
CARDS	HOUSEWORK W/	PLAYGROUND	JUMPING
TALKING	PARENT	DANCING(GENERAL)	GAMES
LISTENING	COOKING	GENERAL RUN-	JUMP ROPE
TO MUSIC	SNOWMOBILE	AROUND	WRESTLING
PUZZLES	RIDING		
	GENERAL QUIET		
	PLAY		

*NO VERY HARD ACTIVITIES WERE CLASSIFIED.

PHYSICAL ACTIVITY INTERVIEW

Child's Name_____ ID#_____

Age_____ Sex_____ Grade_____ School_____

Interviewer_____ Date ____/____/____ Time_____ am pm

1. Would you say you move _____ other boys/girls your age?
   (0) lots less than
   (1) a little less than
   (2) about the same as
   (3) a little more than
   (4) lots more than

2. Would you say you move _____ than your brothers and/or sisters?
   (0) lots less than
   (1) a little less than
   (2) about the same as
   (3) a little more than
   (4) lots more than

3. When you play, do you sweat or breathe hard? _____
   (0) never
   (1) hardly ever
   (2) sometimes
   (3) lots
   (4) always

4. Do you belong to any community or school based sports clubs or leagues?
   (1) no
   (2) yes - - If yes, which ones? (show activity list)

   _____ _____
   _____ _____
   _____ _____

5. Do you play sports during recess or after school (not organized through the school)?
   (1) no
   (2) yes - - If yes, which ones? (show activity list)

   _____ _____
   _____ _____
   _____ _____

6. Does your school have physical education?
   (1) no
   (2) yes - - If yes, how many days per week? _____

7. Do you like physical education?
   (1) no
   (2) yes

8. Do you know what grade you got in physical education?
   (1) no
   (2) yes - - If yes, what was it?
   A D
   B F
   C other:_____

9. How do you get to school?
   (0) bus/car
   (1) walk How far?_____mile(s) (12 bocks=1 mile)
   (2) bicycle How far?_____mile(s)
   (3) other _____

TO SCORE PHYSICAL ACTIVITY QUESTIONNAIRES: ADD TOGETHER THE NUMBERS IN PARENTHESES THAT WERE CIRCLED.

APPENDIX G. Three day food record for dietary analysis.

HOW TO KEEP A FOOD DIARY

1. Record all of the food and beverages you eat or drink over a three day period, including two weekdays, and one weekend day.

2. It's a good idea to record immediately after eating so you won't forget any item.

3. List every food on a different line.

4. Give the amount of food you eat in standard quantities, that is:
   -teaspoons or tablespoons (level or heaping)
   -cup or ounces or milliliters for liquids
   -slices or ounces or dimensions for meat or fish, etc.

5. Give method of preparation, that is:
   -broiled or boiled, roasted or fried, etc.

6. Give brand name if applicable, for example:
   -1 Oreo cookie - NOT 1 chocolate cookie

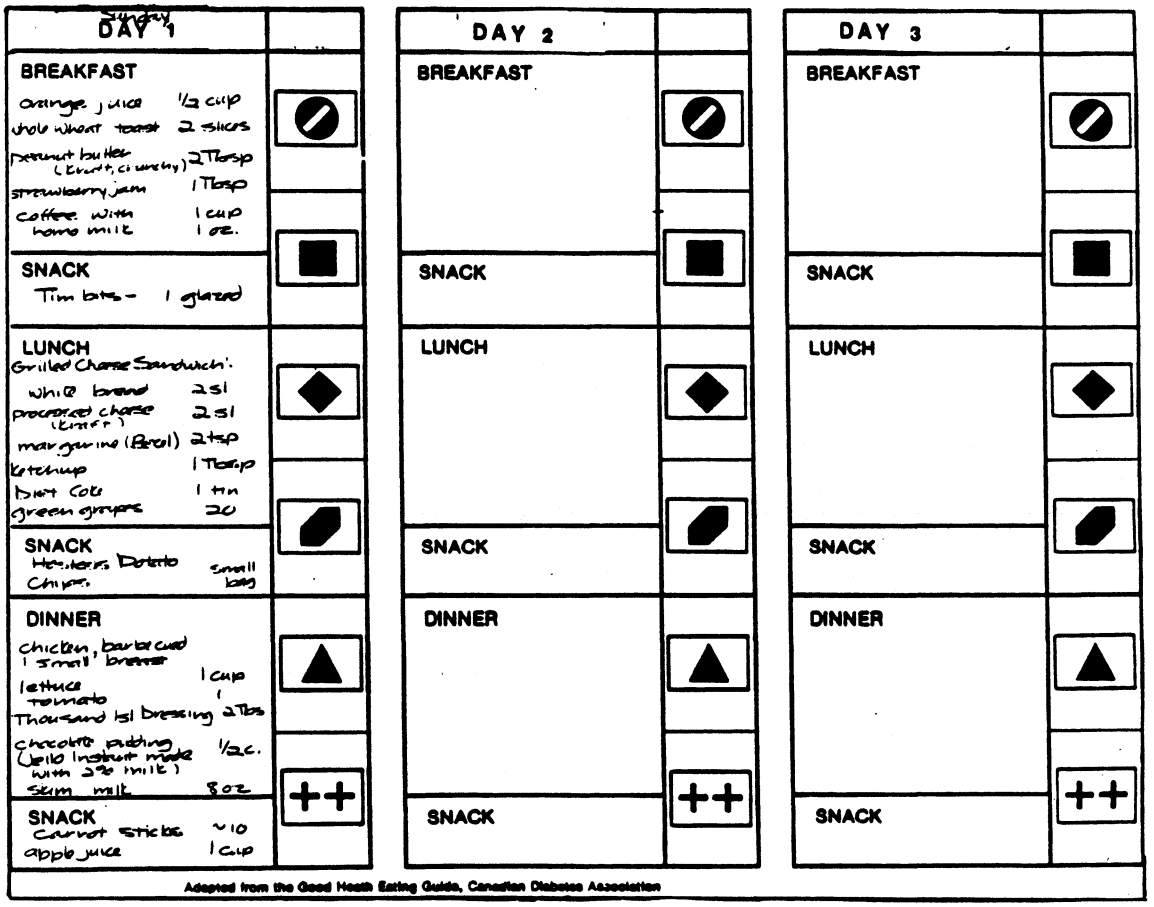
7. Specify anything added to food or beverage, for example:
   -1 half grapefruit with 1 teaspoon sugar
   or
   -1 cup coffee with 1 ounce table cream

8. For foods made at home, such as casseroles, sandwiches, record the main ingredients and approximate amounts of each, for example:

   Egg salad sandwich:
   -2 slices bread
   -2 teaspoons butter
   -1 egg
   -1 tablespoon Miracle Whip salad dressing

9. Include all meals, snacks and beverages

10. If you eat out, indicate the restaurant name when describing what you ate, for example:
    -1 McDonald's quarter pounder with cheese
    -1 single Baskin and Robbins Pralines 'n Cream Ice Cream in a sugar cone


DAY 1
BREAKFAST
orange juice 1/2 cup
whole wheat toast 2 slices
peanut butter (crunchy) 2 Tbsp
strawberry jam 1 Tbsp
coffee with 1 cup
home milk 1 oz.
SNACK
Tim bits - 1 glazed
LUNCH
Grilled Cheese Sandwich:
white bread 2 sl
processed cheese (Kraft) 2 sl
margarine (Becel) 2 tsp
ketchup 1 Tbsp
Diet Coke 1 tin
green grapes 20
SNACK
Hamburg Potato small bag
Chips
DINNER
chicken, barbecued 1 small breast
lettuce 1 cup
tomato 1
Thousand Isl dressing 2 Tbs
chocolate pudding 1/2 c.
(Jello Instant made with 2% milk)
skim milk 8 oz
SNACK
Carrot sticks x 10
apple juice 1 cup
DAY 2
BREAKFAST
SNACK
LUNCH
SNACK
DINNER
SNACK
DAY 3
BREAKFAST
SNACK
LUNCH
SNACK
DINNER
SNACK
Adapted from the Good Health Eating Guide, Canadian Diabetes Association

APPENDIX H. Analysis of variance tables.

PT ARM

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	2.258	1	2.258	3.860	.062
ERROR	10.526	18	.585		
WITHIN BLOCKS/SUBJECTS					
JT ANG	1.041	2	.521	14.472	<.001
GROUP JT ANG	.150	2	.075	2.083	.137
ERROR	1.310	36	.036		

PT CALVE

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	33.282	1	33.282	1.969	.174
ERROR	304.249	18	16.903		
WITHIN BLOCKS/SUBJECTS					
JT ANG	3.685	1	3.685	.887	
GROUP JT ANG	.808	1	.808	.195	
ERROR	74.749	18	4.153		

PT QUADS

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	245.305	1	245.305	9.380	.006
ERROR	470.717	18	26.151		
WITHIN BLOCKS/SUBJECTS					
JT ANG	23.785	1	23.785	10.772	.004
GROUP JT ANG	.771	1	.771	.349	
ERROR	39.740	18	2.208		

SPECIF TEN ARM TWITCH

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	8.49378645E-05	.000 1	0.000		
ERROR	.124	18	.007		
WITHIN BLOCKS/SUBJECTS					
JT ANG	.018	2	.009	9.000	<.001
GROUP JT ANG	.004	2	.002	2.000	.148
ERROR	.019	36	.001		

SPECIFIC TENSION CALVES

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	.003	1	.003	1.500	.235
ERROR	.042	18	.002		
WITHIN BLOCKS/SUBJECTS					
JT SNL	.000	1	.001	.500	
GROUP JT SNL	4.36152338E-04	.000 1	0.000		
ERROR	.031	18	.002		

SPECIFIC TENSION QUADS

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	6.06860209E-05	.000 1	0.000		
ERROR	.136	18	.008		
WITHIN BLOCKS/SUBJECTS					
JT ANG	.011	1	.011	11.000	.004
GROUP JT ANG	.000	1	.001	1.000	
ERROR	.026	18	.001		

MVC ARM

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	394.607	1	394.607	7.943	.011
ERROR	894.246	18	49.680		
WITHIN BLOCKS/SUBJECTS					
JT ANG	459.743	2	229.871	82.687	<.001
GROUP JT ANG	28.034	2	14.017	5.042	.011
ERROR	100.070	36	2.780		

MVC CALVES

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	1405.982	1	1405.982	4.917	.037
ERROR	5146.989	18	285.944		
WITHIN BLOCKS/SUBJECTS					
JT ANG	.868	1	.868	.019	
GROUP JT ANG	.759	1	.759	.017	
ERROR	822.368	18	45.687		

MVC QUADS

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	7047.864	1	7047.864	8.753	.008
ERROR	14493.056	18	805.170		
WITHIN BLOCKS/SUBJECTS					
JOINT	441.473	1	441.473	9.486	.006
GROUP JOINT	3.851	1	3.851	.083	
ERROR	837.710	18	46.539		

SPECIF TENION MVC ARM

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	.374	1	.374	1.133	.301
ERROR	5.945	18	.330		
WITHIN BLOCKS/SUBJECTS					
JT ANG	5.939	2	2.969	48.672	<.001
GROUP JT ANG	.117	2	.058	.951	
ERROR	2.206	36	.061		

SPECI TENSION MVC CALVES

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	.032	1	.032	.360	
ERROR	1.607	18	.089		
WITHIN BLOCKS/SUBJECTS					
JT ANG	.000	1	.001	.063	
GROUP JT ANG	2.17630435E-04.000	1	0.000		
ERROR	.293	18	.016		

SPECIF TENSION MVC QUADS

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	.053	1	.053	.272	
ERROR	3.511	18	.195		
WITHIN BLOCKS/SUBJECTS					
JT ANG	.197	1	.197	9.850	.005
GROUP JT ANG	.005	1	.005	.250	
ERROR	.365	18	.020		

TPT ARM

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	1605.773	1	1605.773	4.907	.037
ERROR	5890.003	18	327.222		
WITHIN BLOCKS/SUBJECTS					
JT ANG	36.833	2	18.417	.330	
GROUP JT ANG	281.400	2	140.700	2.522	.092
ERROR	2008.724	36	55.798		

HRT ARM

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	245.513	1	245.513	.479	
ERROR	9234.067	18	513.004		
WITHIN BLOCKS/SUBJECTS					
JT ANG	1908.647	2	954.323	6.589	.003
GROUP JT ANG	408.967	2	204.483	1.412	.256
ERROR	5213.995	36	144.833		

TCT ARM

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	2788.859	1	2788.859	3.498	.074
ERROR	14349.713	18	797.206		
WITHIN BLOCKS/SUBJECTS					
JT ANG	1925.318	2	962.659	4.959	.012
GROUP JT ANG	1681.837	2	840.918	4.332	.020
ERROR	6988.414	36	194.123		

TPT CALVE

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	.031	1	.031	3.77630164E-05	.000
ERROR	14776.363	18	820.909		
WITHIN BLOCKS/SUBJECTS					
JT ANG	45.956	1	45.956	.373	
GROUP JT ANG	65.157	1	65.157	.528	
ERROR	2219.319	18	123.295		

HRT CALVE

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	800.977	1	800.977	1.077	.313
ERROR	13380.741	18	743.375		
WITHIN BLOCKS/SUBJECTS					
JT ANG	88.476	1	88.476	.620	
GROUP JT ANG	25.977	1	25.977	.182	
ERROR	2566.742	18	142.597		

TCT CALVE

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	877.298	1	877.298	.332	
ERROR	47526.684	18	2640.371		
WITHIN BLOCKS/SUBJECTS					
JT ANG	202.336	1	202.336	.463	
GROUP JT ANG	125.618	1	125.618	.288	
ERROR	7861.922	18	436.773		

TPT QUADS

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	240.172	1	240.172	.845	
ERROR	5117.793	18	284.322		
WITHIN BLOCKS/SUBJECTS					
JT ANG	4.431	1	4.431	.020	
GROUP JT ANG	310.431	1	310.431	1.420	.247
ERROR	3935.534	18	218.641		

HRT QUADS

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	.100	1	.100	2.34639336E-04	.000
ERROR	7671.352	18	426.186		
WITHIN BLOCKS/SUBJECTS					
JT ANG	1097.912	1	1097.912	8.794	.008
GROUP JT ANG	165.902	1	165.902	1.329	.263
ERROR	2247.240	18	124.847		

TCT QUADS

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	9.710	1	9.710	.026	
ERROR	6785.380	18	376.966		
WITHIN BLOCKS/SUBJECTS					
JT ANG	367.125	1	367.125	1.452	.242
GROUP JT ANG	183.824	1	183.824	.727	
ERROR	4549.564	18	252.754		

BMC

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	83459.152	1	83459.152	6.321	.021
ERROR	224456.805	17	13203.341		
WITHIN BLOCKS/SUBJECTS					
BONE S	565164.437	4	141291.109	96.512	<.001
GROUP BONE S	26458.657	4	6614.664	4.518	.003
ERROR	99550.263	68	1463.974		

BMD

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	.009	1	.009	.225	
ERROR	.684	17	.040		
WITHIN BLOCKS/SUBJECTS					
BONE S	13.688	4	3.422	85.550	<.001
GROUP BONE S	.035	4	.009	.225	
ERROR	2.701	68	.040		

G/KG BONE MINERAL

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	.046	1	.046	.034	
ERROR	23.219	17	1.366		
WITHIN BLOCKS/SUBJECTS					
BONE S	604.161	4	151.040	171.247	<.001
GROUP BONE S	19.511	4	4.878	5.531	<.001
ERROR	60.000	68	.882		

APPENDIX I. Descriptive data of Turner's patients and control girls.

	TURNERS n=7	CONTROLS n=13	
SUM 2SF mm	22.7 ± 2.69	28.0 ± 4.16	
SUM 4SF mm	42.1 ± 4.45	52.1 ± 7.64	
%BF sum 2SF	21.2 ± 1.77	23.8 ± 2.52	
%BF sum 4SF	24.0 ± 2.15	26.0 ± 2.97	
FAT MASS sum 2SF kg	6.0 ± 0.80	9.2 ± 1.56	
FAT MASS sum 4SF kg	6.8 ± 0.95	10.1 ± 1.80	
LBM sum 2SF kg	21.3 ± 1.45	27.0 ± 1.26	$p<0.05$*
LBM sum 4SF kg	20.6 ± 1.28	26.1 ± 1.07	$p<0.05$

Values are mean ± SE

* indicates a significant difference between TS and controls

SF skinfolds
LBM lean body mass

APPENDIX J. Limb cross-sectional area and muscle morphology of Turner's patients and controls as determined by computed axial tomography.

	TURNERS n=7	CONTROLS n=13	
TOTAL AREA (cm^2)			
Upper Arm	34.7 ± 3.56	44.4 ± 3.76	
Lower Leg	52.2 ± 4.96	69.1 ± 5.00	p<0.05*
Upper Leg	114.6 ± 13.7	153.6 ± 11.6	
BONE AREA (cm^2)			
Humerus	2.00 ± 0.07	2.49 ± 0.12	p<0.05
Tibia	3.99 ± 0.28	5.40 ± 0.34	p<0.05
Fibula	0.58 ± 0.04	0.88 ± 0.07	p<0.01
Femur	3.10 ± 0.22	4.00 ± 0.18	p<0.01
MUSCLE AREA (cm^2)			
+ EF	5.2 ± 0.31	7.3 ± 0.45	
++ LL	30.9 ± 2.55	40.7 ± 2.47	p<0.05
+++ KE	30.7 ± 2.72	45.0 ± 3.47	p<0.05
SUBCUTANEOUS FAT AREA (cm^2)			
Upper Arm	18.5 ± 2.61	22.9 ± 2.99	
Lower Leg	17.1 ± 2.69	22.2 ± 2.39	
Upper Leg	51.6 ± 8.46	64.6 ± 7.22	
MUSCLE DENSITY (Hu)			
+ EF	63.5 ± 0.79	62.1 ± 2.33	
! PF	61.6 ± 2.88	65.5 ± 1.70	
+++KE	60.1 ± 1.80	59.0 ± 1.37	

Values are mean ± SE
* indicates a significant difference between TS & controls
+ Elbow Flexors (EF); biceps brachii, brachialis,
++ Lower Leg (LL); soleus, gastrocnemius, tibialis anterior, peroneus longus, extensor digitorum longus, extensor hallucis longus, tibialis posterior, flexor digitorum longus
+++ Knee Extensors (KE); rectus femoris, vastus lateralis, vastus intermedius, vastus medialis
! Plantar Flexors; soleus, gastrocnemius

APPENDIX K. Electrically evoked strength measurements in Turner's patients and control girls.

PEAK TWITCH TORQUE Nm

	TURNERS n=7	CONTROLS n=13	
EF			
80°	1.13 ± 0.13	1.40 ± 0.14	
110°	1.02 ± 0.17	1.44 ± 0.14	
150°	0.69 ± 0.12	1.22 ± 0.14	
PF			
70°	7.69 ± 2.25	9.30 ± 0.88	
90°	6.75 ± 1.48	8.96 ± 0.72	
KE			
90°	9.06 ± 1.92	14.45 ± 1.04	$p<0.01$*
120°	7.73 ± 1.25	12.63 ± 0.88	$p<0.01$

TWITCH SPECIFIC TENSION Nm x cm^{-2} x m^{-1}

EF			
80°	0.16 ± 0.03	0.14 ± 0.01	
110°	0.14 ± 0.03	0.15 ± 0.01	
150°	0.10 ± 0.02	0.12 ± 0.01	
PF			
70°	0.19 ± 0.02	0.16 ± 0.01	
90°	0.17 ± 0.02	0.16 ± 0.01	
KE			
90°	0.23 ± 0.04	0.24 ± 0.01	
120°	0.20 ± 0.03	0.19 ± 0.02	

Values are mean + SE
* indicates a significant difference between TS and controls

APPENDIX L. Maximal voluntary isometric strength measurements in Turner's patients and control girls.

MAXIMAL VOLUNTARY STRENGTH Nm

	TURNERS n=7	CONTROLS n=13	
EF			
80°	10.9 ± 1.64	17.0 ± 1.29	p<0.05*
110°	8.9 ± 1.61	15.6 ± 1.41	p<0.01
150°	5.4 ± 0.75	8.8 ± 1.00	p<0.05
PF			
70°	34.8 ± 4.51	47.5 ± 3.87	p<0.05
90°	34.7 ± 4.55	46.6 ± 3.51	p<0.05
KE			
90°	43.2 ± 6.17	70.4 ± 6.72	p<0.05
120°	35.6 ± 6.00	64.1 ± 5.76	p<0.01

VOLUNTARY SPECIFIC TENSION Nm x cm-2 x m-1

EF			
80°	1.51 ± 0.26	1.73 ± 0.11	
110°	1.32 ± 0.17	1.57 ± 0.09	
150°	0.83 ± 0.08	0.87 ± 0.06	
PF			
70°	0.91 ± 0.08	0.85 ± 0.05	
90°	0.92 ± 0.11	0.86 ± 0.07	
KE			
90°	1.12 ± 0.13	1.18 ± 0.09	
120°	0.95 ± 0.16	1.05 ± 0.07	

Values are mean ± SE.
* indicates a significant difference between TS and controls

APPENDIX M. Percent motor unit activation in Turner's patients and control girls.

		TURNERS n=7	CONTROLS n=13
EF	110°	96 ± 2.7	99 ± 0.4
PF	90°	94 ± 2.4	93 ± 2.2
KE	90°	95 ± 1.6	96 ± 1.2

Values are mean ± SE

APPENDIX N. Contractile properties of Turner's patients and control girls.

		TURNERS n=7	CONTROLS n=13	
TIME TO PEAK TORQUE ms				
EF	80°	69.6 ± 3.08	56.5 ± 3.25	
	110°	71.1 ± 7.79	56.2 ± 2.05	p<0.05*
	150°	67.3 ± 5.91	62.8 ± 2.76	
PF	70°	107.9 ± 4.49	110.6 ± 5.37	
	90°	108.3 ± 11.2	105.7 ± 6.39	
KE	90°	72.9 ± 5.91	72.2 ± 2.40	
	120°	77.7 ± 3.12	78.7 ± 3.69	
HALF RELAXATION TIME ms				
EF	80°	86.0 ± 8.79	76.6 ± 3.60	
	110°	95.4 ± 5.98	87.9 ± 4.59	
	150°	93.4 ± 4.97	96.8 ± 4.60	
PF	70°	112.0 ± 3.27	104.3 ± 5.84	
	90°	110.6 ± 8.92	99.5 ± 6.62	
KE	90°	77.1 ± 3.01	1.3 ± 6.26	
	120°	70.4 ± 4.70	66.1 ± 4.01	
TOTAL CONTRACTION TIME ms				
EF	80°	155.6 ± 6.76	133.9 ± 4.78	p<0.05
	110°	166.3 ± 8.01	143.7 ± 5.25	p<0.05
	150°	158.1 ± 10.2	159.5 ± 5.37	
PF	70°	219.9 ± 6.91	213.7 ± 9.69	
	90°	218.9 ± 19.3	205.3 ± 12.2	
KE	90°	150.0 ± 4.86	153.5 ± 7.47	
	120°	148.1 ± 3.25	142.6 ± 2.78	

Values are mean ± SE
* indicates a significant difference between TS and controls
ms milliseconds

APPENDIX O. Physical activity scores for Turner's patients and control girls from personal interviews and parental assessments.

	TURNERS n=7	CONTROLS n=13
Child interview	11.4 ± 0.78	11.5 ± 0.50
Parent's assessment	38.2 ± 3.63	44.6 ± 3.03

Values are mean ± SE.

APPENDIX P. Dietary intake of Turner's patients and control girls for the cross-sectional study.

	TURNERS n=5	CONTROLS n=13
Energy (kcal/day)	2028.8 ± 132.2	2188.3 ± 116.5
Energy (kcal/kg/day)	80.6 ± 13.7	63.3 ± 5.7
Protein (g/day)	67.6 ± 5.4	76.8 ± 4.7
Carbohydrate (g/day)	267.6 ± 28.8	284.5 ± 18.0
Fat (g/day)	89.9 ± 12.2	93.0 ± 7.6
Calcium (mg/day)	1091.6 ± 109.7	1078.9 ± 98.2
Vitamin D (mcg/day)	4.8 ± 1.54	5.0 ± 0.62
Phosphorus (mg/day)	1571.6 ± 128.5	1510.9 ± 113.3
Zinc (mg/day)	8.7 ± 0.38	9.5 ± 0.65
Iron (mg/day)	14.2 ± 0.91	13.3 ± 0.88

Values are means ± SE determined from 3 day food records.

kcal= kilocalories
g= grams
mg= milligrams
mcg=micrograms

APPENDIX Q. Changes in anthropometric data during hormonal therapy.

	PRE	POST	% CHANGE
AGE yrs			
$ST_{(gh)}$	12.5	13.1	4.8
$JV_{(e2)}$	13.3	13.8	3.8
$SB_{(c)}$	9.4	10.0	6.4
HEIGHT cm			
$ST_{(gh)}$	125.8	132.5	5.3
$JV_{(e2)}$	128.7	131.5	2.2
$SB_{(c)}$	146.7	151.8	3.5
HT VELOCITY cm/yr			
$ST_{(gh)}$	3.4	10.3	202.9
$JV_{(e2)}$	4.4	5.0	13.6
$SB_{(c)}$	-	9.2*	-
BODY MASS kg			
$ST_{(gh)}$	33.9	34.5	1.7
$JV_{(e2)}$	29.5	32.3	9.5
$SB_{(c)}$	44.4	52.3	17.8
BODY FAT % (Sum 2 SF)			
$ST_{(gh)}$	27.8	17.6	-36.7
$JV_{(e2)}$	24.8	26.0	4.8
$SB_{(c)}$	33.2	32.6	-1.8
LEAN MASS kg (Sum 2 SF)			
$ST_{(gh)}$	24.5	28.4	15.9
$JV_{(e2)}$	22.2	23.9	7.7
$SB_{(c)}$	29.7	35.3	18.9
FAT MASS kg (Sum 2 SF)			
$ST_{(gh)}$	9.4	6.1	-35.1
$JV_{(e2)}$	7.3	8.4	15.1
$SB_{(c)}$	14.7	17.0	15.6
MUSCLE DENSITY Hu			
EF			
$ST_{(gh)}$	64.2	65.5	2.0
$JV_{(e2)}$	62.6	62.3	-0.5
$SB_{(c)}$	72.4	60.4	-16.6
PF			
$ST_{(gh)}$	57.7	65.2	13.0
$JV_{(e2)}$	60.1	65.1	8.3
$SB_{(c)}$	70.1	62.4	-11.0
KE			
$ST_{(gh)}$	53.7	53.9	0.4
$JV_{(e2)}$	57.8	59.9	3.6
$SB_{(c)}$	57.3	49.5	-13.6

* above the 97th percentile for her age

APPENDIX R. Limb girth and fat cross-sectional area, changes during hormonal therapy.

	PRE	POST	% CHANGE
TOTAL LIMB AREA (cm^2)			
Upper Arm			
$ST_{(gh)}$	48.4	34.4	-28.9
$JV_{(e2)}$	34.4	43.2	25.6
$SB_{(c)}$	50.8	59.8	17.7
Lower Leg			
$ST_{(gh)}$	67.6	58.7	-13.2
$JV_{(e2)}$	56.0	62.5	11.6
$SB_{(c)}$	71.1	79.8	12.2
Thigh			
$ST_{(gh)}$	177.3	165.8	-6.5
$JV_{(e2)}$	96.9	108.1	11.6
$SB_{(c)}$	189.9	202.2	6.5
SUBCUTANEOUS FAT AREA (cm^2)			
Arm			
$ST_{(gh)}$	30.0	10.3	-65.7
$JV_{(e2)}$	19.3	21.8	13.0
$SB_{(c)}$	26.5	33.7	27.2
L Leg			
$ST_{(gh)}$	29.9	18.5	-38.1
$JV_{(e2)}$	18.4	20.0	8.7
$SB_{(c)}$	21.8	26.6	22.0
U Leg			
$ST_{(gh)}$	92.4	67.5	-26.9
$JV_{(e2)}$	39.1	43.5	11.3
$SB_{(c)}$	85.0	97.7	14.9

APPENDIX S. Limb bone and muscle cross-sectional area, changes during hormonal therapy.

	PRE	POST	% CHANGE
BONE AREA (cm^2)			
Humerus			
$ST_{(gh)}$	2.11	2.19	3.8
$JV_{(e2)}$	2.30	2.58	12.2
$SB_{(c)}$	3.06	3.39	10.8
Tibia			
$ST_{(gh)}$	4.87	5.20	6.8
$JV_{(e2)}$	4.70	4.99	6.2
$SB_{(c)}$	5.61	6.20	10.5
Fibula			
$ST_{(gh)}$	0.59	0.62	5.1
$JV_{(e2)}$	0.70	0.72	2.9
$SB_{(c)}$	1.15	1.20	4.3
Femur			
$ST_{(gh)}$	3.84	4.28	11.5
$JV_{(e2)}$	3.23	3.53	9.3
$SB_{(c)}$	5.59	6.01	7.5
MUSCLE AREA (cm^2)			
+ Elbow Flexors			
$ST_{(gh)}$	4.92	5.99	21.7
$JV_{(e2)}$	5.62	6.7	19.2
$SB_{(c)}$	8.51	8.67	1.9
++ Lower Leg			
$ST_{(gh)}$	33.1	34.8	5.1
$JV_{(e2)}$	32.9	36.8	11.9
$SB_{(c)}$	42.6	45.9	7.7
+++ Knee Extensors			
$ST_{(gh)}$	42.2	47.7	13.0
$JV_{(e2)}$	28.3	31.9	12.7
$SB_{(c)}$	44.9	44.8	-0.2

+ Elbow Flexors; biceps brachii, brachialis,
++ Lower Leg; soleus, gastrocnemius, tibialis anterior, peroneus longus, extensor digitorum longus, extensor hallucis longus, tibialis posterior, flexor digitorum longus
+++ Knee Extensors; rectus femoris, vastus lateralis, vastus intermedius, vastus medialis

APPENDIX T. Evoked twitch strength measurements during hormonal therapy.

	PRE	POST	% CHANGE
PEAK TWITCH TORQUE Nm			
EF			
$ST_{(gh)}$	0.72	1.64	127.8
$JV_{(e2)}$	1.37	2.00	46.0
$SB_{(c)}$	1.40	2.28	62.9
PF			
$ST_{(gh)}$	7.83	6.98	-10.9
$JV_{(e2)}$	9.35	10.30	10.2
$SB_{(c)}$	11.51	11.50	-0.1
KE			
$ST_{(gh)}$	12.11	10.70	-11.6
$JV_{(e2)}$	11.13	12.91	16.2
$SB_{(c)}$	14.62	17.12	17.1
TWITCH SPECIFIC TENSION Nm x cm^{-2} x m^{-2}			
EF			
$ST_{(gh)}$	0.12	0.21	75.0
$JV_{(e2)}$	0.18	0.23	27.8
$SB_{(c)}$	0.11	0.17	54.5
PF			
$ST_{(gh)}$	0.19	0.16	-15.8
$JV_{(e2)}$	0.22	0.21	-4.5
$SB_{(c)}$	0.19	0.17	-10.5
KE			
$ST_{(gh)}$	0.23	0.17	-26.1
$JV_{(e2)}$	0.31	0.31	0
$SB_{(c)}$	0.22	0.26	18.2

APPENDIX U. Maximal voluntary strength measurements during hormonal therapy.

	PRE	POST	% CHANGE
MAXIMAL VOLUNTARY TORQUE Nm			
EF			
$ST_{(gh)}$	6.1	10.0	63.9
$JV_{(e2)}$	12.5	14.4	15.2
$SB_{(c)}$	17.0	23.1	35.9
PF			
$ST_{(gh)}$	23.2	18.6	-19.8
$JV_{(e2)}$	44.3	60.1	35.7
$SB_{(c)}$	72.0	96.8	34.4
KE			
$ST_{(gh)}$	81.5	64.0	-21.5
$JV_{(e2)}$	58.0	64.3	10.9
$SB_{(c)}$	95.4	120.4	26.2
VOLUNTARY SPECIFIC TENSION Nm x cm^{-2} x m^{-2}			
EF			
$ST_{(gh)}$	0.99	1.26	27.3
$JV_{(e2)}$	1.61	1.63	1.2
$SB_{(c)}$	1.36	1.76	29.4
PF			
$ST_{(gh)}$	0.56	0.41	-26.8
$JV_{(e2)}$	1.04	1.22	17.3
$SB_{(c)}$	1.05	1.40	33.3
KE			
$ST_{(gh)}$	1.54	1.01	-34.4
$JV_{(e2)}$	1.59	1.53	-3.8
$SB_{(c)}$	0.67	0.81	20.9

APPENDIX V. Percent motor unit activation during hormonal therapy.

	PRE	POST	% CHANGE
EF			
$ST_{(gh)}$	96	100	4.2
$JV_{(e2)}$	99	99	0
$SB_{(c)}$	94	99	5.3
PF			
$ST_{(gh)}$	85	85	0
$JV_{(e2)}$	97	99	2.1
$SB_{(c)}$	98	100	2.0
KE			
$ST_{(gh)}$	89	100	12.4
$JV_{(e2)}$	94	100	6.4
$SB_{(c)}$	94	100	6.4

APPENDIX W. Electrically evoked contractile properties during hormonal therapy.

	PRE	POST	% CHANGE
TIME TO PEAK TORQUE ms			
EF			
$ST_{(gh)}$	58.7	37.3	-36.5
$JV_{(e2)}$	91.7	44.0	-52.0
$SB_{(c)}$	63.0	49.4	-21.6
PF			
$ST_{(gh)}$	100.0	88.0	-12.0
$JV_{(e2)}$	142.0	135.9	-4.3
$SB_{(c)}$	115.5	128.0	10.8
KE			
$ST_{(gh)}$	73.0	70.0	-4.1
$JV_{(e2)}$	81.0	76.5	-5.6
$SB_{(c)}$	72.0	79.0	10.4
HALF RELAXATION TIME ms			
EF			
$ST_{(gh)}$	74.0	105.3	42.3
$JV_{(e2)}$	86.3	130.6	51.3
$SB_{(c)}$	74.7	91.1	22.0
PF			
$ST_{(gh)}$	108.0	141.0	30.6
$JV_{(e2)}$	135.5	119.2	-12.0
$SB_{(c)}$	130.5	112.5	-13.8
KE			
$ST_{(gh)}$	69.0	73.5	6.5
$JV_{(e2)}$	67.0	70.0	5.2
$SB_{(c)}$	66.5	55.5	16.5
TOTAL CONTRACTION TIME ms			
EF			
$ST_{(gh)}$	132.7	142.7	7.8
$JV_{(e2)}$	178.0	174.6	-1.9
$SB_{(c)}$	137.7	140.3	-1.9
PF			
$ST_{(gh)}$	208.0	228.0	10.1
$JV_{(e2)}$	277.5	255.0	-8.1
$SB_{(c)}$	246.0	240.5	-2.2
KE			
$ST_{(gh)}$	142.0	143.5	1.1
$JV_{(e2)}$	148.0	147.0	-0.7
$SB_{(c)}$	138.5	135.0	-2.5

ms millliseconds

APPENDIX X. Bone mineral content during hormonal therapy.

BMC g		PRE	POST	% CHANGE
total	$ST_{(gh)}$	1093.8	1123.7	2.7
	$JV_{(e2)}$	1001.6	1038.5	3.7
	$SB_{(c)}$	1583.9	1754.2	10.8
head	$ST_{(gh)}$	327.3	274.0	-16.3
	$JV_{(e2)}$	254.4	242.2	-4.8
	$SB_{(c)}$	377.2	350.8	-7.0
trunk	$ST_{(gh)}$	214.7	233.0	8.5
	$JV_{(e2)}$	191.3	97.7	3.3
	$SB_{(c)}$	306.9	356.7	16.2
pelvis	$ST_{(gh)}$	177.7	198.3	11.6
	$JV_{(e2)}$	170.7	173.3	1.5
	$SB_{(c)}$	233.1	295.4	26.7
legs	$ST_{(gh)}$	162.3	264.3	62.8
	$JV_{(e2)}$	241.3	274.4	13.7
	$SB_{(c)}$	451.9	422.5	-6.5
spine	$ST_{(gh)}$	74.2	78.4	5.6
	$JV_{(e2)}$	72.6	77.9	7.3
	$SB_{(c)}$	116.9	157.1	53.0

APPENDIX Y. Bone mineral density during hormonal therapy.

		PRE	POST	% CHANGE
BMD g/cm^2				
total	$ST_{(gh)}$	0.748	0.681	-8.5
	$JV_{(e2)}$	0.633	0.653	3.2
	$SB_{(c)}$	0.796	0.841	5.7
head	$ST_{(gh)}$	1.54	1.26	-18.2
	$JV_{(e2)}$	1.26	1.26	0
	$SB_{(c)}$	1.65	1.64	-0.6
trunk	$ST_{(gh)}$	0.37	0.33	-10.8
	$JV_{(e2)}$	0.31	0.30	-3.2
	$SB_{(c)}$	0.35	0.42	20.0
pelvis	$ST_{(gh)}$	1.19	0.86	-27.7
	$JV_{(e2)}$	1.00	0.74	-26.0
	$SB_{(c)}$	0.79	0.83	5.1
legs	$ST_{(gh)}$	1.01	1.00	-1.0
	$JV_{(e2)}$	0.95	0.95	0
	$SB_{(c)}$	1.15	1.19	3.5
spine	$ST_{(gh)}$	0.52	0.51	-1.9
	$JV_{(e2)}$	0.52	0.48	-7.7
	$SB_{(c)}$	0.50	0.59	18.0

APPENDIX Z. Physical activity scores during hormonal therapy.

	PRE	POST	% CHANGE
CHILD'S SCORE			
$ST_{(gh)}$	11	11	0
$JV_{(e2)}$	11	11	0
$SB_{(c)}$	10	9	-10
PARENT'S ASSESSMENT			
$ST_{(gh)}$	28.0	30.0	7.1
$JV_{(e2)}$	42.0	38.5	-8.3
$SB_{(c)}$	35.5	20.5	-42.3

APPENDIX AA. Dietary intake during hormonal therapy.

	PRE	POST	% CHANGE
Energy (kcal/day)			
$ST_{(gh)}$	1922.6	1969.0	2.4
$JV_{(e2)}$	1662.6	1006.6	-39.5
$SB_{(c)}$	2764.5	1495.7	-46.0
Energy (kcal/kg/day)			
$ST_{(gh)}$	56.7	57.1	0.7
$JV_{(e2)}$	56.4	31.2	-80.9
$SB_{(c)}$	62.3	28.6	-54.1
Phosphorus (mg/day)			
$ST_{(gh)}$	1680.6	1353.1	-19.5
$JV_{(e2)}$	1297.8	739.7	-43.0
$SB_{(c)}$	2089.2	959.0	-54.1
Protein (g/day)			
$ST_{(gh)}$	74.2	73.9	-0.4
$JV_{(e2)}$	50.7	38.0	-25.0
$SB_{(c)}$	110.6	51.7	-53.3
Calcium (mg/day)			
$ST_{(gh)}$	1366.5	997.3	-27.0
$JV_{(e2)}$	916.2	508.9	-44.5
$SB_{(c)}$	1224.1	653.7	-46.6
Vitamin D (mcg/day)			
$ST_{(gh)}$	7.5	5.1	-32.0
$JV_{(e2)}$	3.1	1.6	-48.4
$SB_{(c)}$	5.3	3.7	-30.2
Zinc (mg/day)			
$ST_{(gh)}$	9.9	9.5	-4.0
$JV_{(e2)}$	7.7	5.5	-28.6
$SB_{(c)}$	12.8	6.8	-46.9
Iron (mg/day)			
$ST_{(gh)}$	13.7	14.1	2.9
$JV_{(e2)}$	11.1	9.8	-11.7
$SB_{(c)}$	16.3	12.7	-22.1
Carbohydrate (g/day)			
$ST_{(gh)}$	244.2	271.0	11.0
$JV_{(e2)}$	221.4	141.6	-36.0
$SB_{(c)}$	276.9	188.3	-32.0
Fat (g/day)			
$ST_{(gh)}$	80.7	68.9	-14.6
$JV_{(e2)}$	69.3	36.7	-47.0
$SB_{(c)}$	137.7	60.0	-56.4