EXERCISE AND BONE MINERAL DENSITY

EFFECTS OF RESISTANCE TRAINING ON BONE MASS AND BODY COMPOSITION IN YOUNG WOMEN

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Foreword

This thesis has been written in a format suitable for publication. The review of literature section entitled "Exercise and Bone Mineral Density" was written in the format of Sports Medicine. The two papers; "Effects of Resistance Training on Bone Mass and Body Composition in Young Women" and "Reproducibility of Bone Mass and Body Composition Measurements by X-Ray and Gamma-Ray Dual Photon Absorptiometry" were written in the format of Bone and Mineral.

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INTRODUCTION

Osteoporosis is a growing problem in today's society, with osteoporotic fractures increasing faster than the increase in age (Martin et al., 1991). Although numerous factors affect bone mineral density, which determines bone strength (Erickson et al., 1989; Carter, 1976), an increasingly sedentary lifestyle is blamed for the decreasing bone density of our population (Martin et al., 1991). Bone mineral density is higher in young (Kanders et al., 1988) and elderly (Cheng et al., 1991) women who engage in greater amounts of physical activity. Osteoporotic fractures are associated with a less physically active lifestyle (Cooper et al., 1988). Exercise is recommended as a treatment for increasing bone density after menopause (Kaplan, 1987) and as a preventative measure before menopause, since substantial trabecular bone loss occurs pre-menopausally (Buchanan et al., 1986; Riggs et al., 1982; Riggs et al., 1986). Exercise, as a treatment, is associated with fewer side effects than traditional osteoporosis treatments, such as oestrogen supplementation, which increases the risk of endometrial malignancy and hypertension (Gambrell, 1982).

The purpose of this review is to identify the physical and mechanical changes that occur in bone, as a result of exercise stress, to review which exercise regimes are most effective in increasing bone density and to review the possible mechanisms by which strain is detected and transformed into a biochemical signal to activate bone formation. The first part of this review will

examine studies using animal models which show how the physical and mechanical properties of bone change with exercise and which strain regimes are most effective in forming new bone. Conclusions from these studies will then be compared to cross - sectional and longitudinal studies involving humans. The third part of this review will discuss the normal bone remodelling cycle and how it may be affected by mechanical loading. The final part will review proposed mechanisms by which strain is detected and transformed into biochemical signals for new bone formation.

I. STUDIES OF ANIMALS

Physical Changes in Stressed Bone

When certain species of animals undergo treadmill exercise training (Raab et al., 1990), have artificial external loads applied to bone through implants (Meade et al., 1984; Rubin & Lanyon, 1985; O'Connor & Lanyon, 1982), or have increased loads imposed on radii, by the removal of ulnae (Lanyon et al., 1982), increases in new bone formation on loaded bone surfaces are evident, on posthumous examination. Tetracyline, injected into exercising animals, allows labelling of areas of mineral deposition and new bone formation. The periosteum, which is the thick fibrous membrane covering the entire bone surface, usually shows the greatest increase in bone following loading, due to the muscular attachments which induce greater strains (Lanyon et al., 1982). The endosteum, the layer of cells lining the inner surface of bone gives rise to smaller amounts of new bone in response to loading. Total volume, and the dry, ash, calcium and fat free weights of stressed bone are greater, following exercise training (Woo et al., 1981; Raab et al., 1990).

Mechanical Changes in Stressed Bone

Bending tests show that bone taken from exercised animals is stronger, in that there is more energy stored (a greater area under the load-deformation curve) and higher maximal loads are reached,

before breaking (Woo et al., 1981). The force that bone is able to resist at yield (bending) and ultimate (breaking) points is higher in exercised than control animals (Raab et al., 1990). Thus, loading regimes result in increased bone formation, and enhanced structural and mechanical properties.

Optimal Strain Characteristics for Bone Formation

Strain Magnitude. Using implanted strain gauges in rooster ulnae, it was shown that bone subjected to greater magnitudes of strain, through external artificial loading, shows a greater amount of new bone formation (Rubin & Lanyon, 1985). With increasing levels of strain magnitude, a greater amount of bone is laid down, resulting in a stronger bone. Frost (1987) proposes a theory in which a minimum effective strain is necessary for bone formation to occur. Once this strain is detected by bone cells, new bone is formed, strengthening the bone and reducing the strain response to a given stress. The newly reduced strain is then ineffective as a stimulus and bone formation ceases. Thus, a feed-back mechanism is present in bone to reduce strain levels (Frost, 1983). This feedback mechanism is evident in in vivo experiments, in which pigs have the ulna from one limb removed, resulting in increased strain (measured by implanted strain gauges) in the remaining radius, during functional loading (Goodship et al., 1979). New bone is laid down in the radius, making it stronger and reducing the strain. The newly reduced strain is no longer effective in stimulating bone formation and formation ceases, through the

feedback mechanism. A minimum effective strain is needed, as shown in loading experiments on long bones, where new bone only forms on the compression side of the bone (where strain is greatest) and little or no bone forms on the tensile side (where there is less strain) [Lanyon & Baggot, 1976; Lanyon et al., 1982]. Further evidence of increased bone formation resulting from higher strain magnitudes is shown in studies which demonstrate that immature bone shows greater increases in formation than mature bone, given a fixed level of stress (Rahn, 1982). Immature bone is much more compliant, resulting in greater strain magnitudes than those which occur in stiffer mature bone (Currey, 1975).

Strain Rate. By applying intermittent loads to sheep radius, in vivo, through implants, it was found that most bone is formed in response to the highest physiological strain rates (O'Connor & Lanyon, 1982) and that strains of high magnitude are inadequate in stimulating new bone formation, unless imposed at high strain rates (Lanyon et al., 1982). Substantial changes in bone are observed after only six weeks, with one hour of artificial loading per day. High strain rates applied over a short period of time are therefore effective in stimulating new bone formation.

Strain Distribution. When strain distribution is altered in sheep radius by removal of the ulna, new bone is deposited to compensate for the structural loss (Lanyon et al., 1982). Following 50 weeks, strain levels are actually lower than normal, suggesting that peak strain levels are less responsible for stimulating the adaptive response, than the changes in strain

distribution. Bone formation can be stimulated by submaximal strains if strain distribution is abnormal.

Static Versus Dynamic Strain. It was shown that continuously applied stress (static strain) is ineffective in stimulating bone formation, whereas intermittent loading (dynamic strain) is effective. When remodelling activity in turkey ulnae is assessed under conditions of disuse and disuse interrupted by short daily periods of either static or dynamic compressive loads of similar magnitude, non-loaded and statically loaded bone demonstrate losses in bone, while dynamically loaded bone shows increases in formation (Lanyon & Rubin, 1984). Similar results are found in the rabbit tibia, which does not respond to continuous stress (Hert et al., 1969), but shows new bone formation following intermittent loading. Stress must be dynamic in nature in order for bone formation to be stimulated.

Strain Cycles. By altering the number of strain cycles imposed on rooster ulnae in situ, and keeping strain magnitude and rate constant, it is found that only a small number of loading cycles per day is required for gains in bone mineral content (Rubin & Lanyon, 1984). When mathematical modelling, relating bone density to daily stress histories, is applied to running studies, it is found that stress magnitude has a greater influence on bone mass than the number of loading cycles (Whalen & Carter, 1988). If strains are dynamic, high in magnitude and rate and of abnormal distribution, a substantial bone formation response can be achieved

after remarkably few loading cycles.

Implications for Designing a Therapeutic Exercise Program. From the results of these experiments, exercise regimes designed to increase bone mass and strength should involve loads of high magnitude and rate, should be dynamic in nature and involve varied and diverse patterns of stress. Relatively few cycles of loading per day would be required, so the exercise would not have to be long in duration.

Weight training offers loading of high magnitude and varied patterns of stress, through lifting exercises that offer strain distributions which are different from those encountered in normal daily activities. Disadvantages are that loads are more static in nature and applied at relatively low rates. Running or aerobic dance programs involve dynamic, high impact loading at high strain rates. Disadvantages are that peak strain magnitudes are not that high and strain distribution is not altered to a great degree. The advantages of one exercise program over the other can be elucidated from an examination of human exercise studies.

II. STUDIES WITH HUMANS

Cross - Sectional Studies

Athletic Groups. Cross-sectional studies of athletic groups show that strength-trained athletes have higher bone mineral density (BMD) than endurance trained athletes and controls. Lumbar spine, distal femur, patella and distal radius BMD is higher in young strength trained females than orienteers, cyclists, cross country skiers and controls (Heinonen et al., 1992) and femoral neck and distal radius BMD is higher in young female weight lifters than swimmers, runners and controls (Heinrich et al., 1990). Young weight trained males similarly have greater femoral BMD than runners and swimmers (Nilsson & Westlin, 1971), but the confounding effects of anabolic steroid use in the weight lifters may have affected the results of this study. A retrospective study of young male world-class power lifters found that lumbar vertebral BMD is highly correlated ($r=0.82$) with the total poundage lifted over the past training year (Grauled et al., 1987). The vertebral bone mineral content (BMC) of the powerlifters was 36% higher than age matched controls. Mathematical modelling found that the loads on the lumbar spine during maximal dead lifts exceed those found at the maximal ultimate strength of experimentally tested vertebrae. Thus, the heavy loading of bone is associated with an elevated BMC, inferring a bone formation response to high strain magnitudes.

Weight training may stimulate bone formation through the direct action of muscle pulling on bone.

Studies of endurance athletes show that young female runners have lumbar BMD similar to controls (Heirich et al., 1990; Buchanan et al., 1988), while older male and female life-long runners have greater BMC in the lumbar spine (Lane et al., 1986). Bone may take longer to respond to running stresses or perhaps the response of bone to running is age-specific, with older individuals responding better than young. It is found that lumbar BMD is significantly reduced in young male runners when compared to controls (Bilanin et al., 1989). It is hypothesized that the high weekly mileage (an average of 92 km) of this running group may result in lowered testosterone and higher cortisol levels, having a catabolic effect on bone. There may be a threshold level of exercise that stimulates bone formation, with higher levels having a reducing effect. Male runners (age 20-45 years) who run 15 to 20 miles per week have significantly greater lower leg BMD than runners of five to 10 miles per week and controls (MacDougall et al., 1992). With mileage greater than 20 miles per week, BMD tends to decrease. Serum testosterone levels were not different between groups in this study. Cortisol levels were not measured. A hormonal mechanism may also be responsible for reduced BMD in female endurance athletes who engage in a high volume of training (Drinkwater et al., 1984), where estradiol and prolactin levels may fall. Proponents of impact loading endurance-type training hypothesize that the best stimulus for bone formation is the effect of gravity plus increased

body weight upon bone, instead of muscle pull. A finding that contradicts this theory is that male swimmers have greater lumbar spine and radial BMD than non-exercising males (Orwall et al., 1989). Swimming is a non-weight bearing activity, so higher bone densities would have to be attributed to the effects of muscle pull. While studies of athletic groups favour strength training over endurance training for improving BMD, cross-sectional studies must be interpreted with caution, as individuals who start off with stronger bones may be more likely to participate in weight training.

Strength, Muscle Mass, Maximal Oxygen Uptake and BMD. To eliminate this potential bias of participation in sports by individuals with stronger bones, several investigators have looked at the relationship between muscle strength, muscle mass, or maximal oxygen uptake and BMD in normal populations. If strength training builds bone through local effects, there should be a relationship between the strength of a muscle and the bone it is attached to. Moderate, but significant correlations are found between hip adductor strength and hip BMD (r=0.42) in young females (Snowharter et al., 1990), back extensor strength and spine BMD (r=0.34-0.46) in older males (Bevier et al., 1989) and females (Sinaki et al., 1988), and grip strength and radius BMD (r=0.37-0.47) in older males and females (Bevier et al., 1989) and young females (Snowharter et al., 1990). The strength of some muscle

groups correlates with densities of bone far from their sites of attachments. Biceps and grip strength are two of the best predictors (r=0.45) of lumbar spine BMD in older (Revieu et al., 1989) and young (Snowharter et al., 1990; Pockock et al., 1989) females. It is possible that biceps and forearm strength are good indicators of overall strength or that arm activity is linked to the simultaneous contraction of trunk stabilizing muscles, that exert forces on the spine. Also, the length of the lever arm between arm muscles and the spine is greater than that between the back extensors and the spine, so that loads on the spine generated by arm activity are greater than those generated by back extensors. Arm strength may therefore be an indicator of spine bone strength.

The lack of strong correlations between many strength measures and bone densities may be due to the fact that strength is not only dependent on the size of the muscle that attaches to bone, but also on the neural drive to the muscle (Sale et al., 1983). This weakens the relationship between strength and bone. Studies which have measured the size of specific muscles have found good correlations with the density of the bones to which they are attached. The weight of the left psoas muscle, obtained from 47 cadavers, correlates well (r=0.72) with the ash weight of the third lumbar vertebra (Doyle et al., 1970). Leg lean muscle mass and leg BMD, measured by dual energy x-ray absorbtiometry (DEXA) in young females, are significantly correlated (r=0.59) [Nichols et al., 1992]. Thus, muscle size tends to be a better indicator of BMD than strength. When maximal oxygen uptake, an indicator of aerobic

power, is measured, significant correlations are found with lumbar spine BMD in males (r=0.41) [Bevier et al., 1989] and females (r=0.54) and with femoral neck BMD (r=0.6) in females (Pocock et al., 1986). From these findings it is difficult to draw conclusions on which type of training is more beneficial in increasing BMD.

Longitudinal Studies

Many longitudinal studies investigating the effects of training on BMD have been flawed by lack of control in subject selection, imprecise measuring instruments, poor compliance, or too short a training duration to allow for changes in bone to take place. Subjects should be screened for outside factors which have effects on BMD, such as smoking (Daniell 1976), oral contraceptive use (Linsay et al., 1986), oestrogen replacement therapy (Recker et al., 1977), menstrual cycle irregularities (Cann et al., 1984) and certain drugs (Kaplan, 1987). Exercise and control subjects should have similar age, height, weight, menopausal status, and nutritional status, since these are also related to BMD (Angus et al., 1988; Buchanan et al., 1988; Picard et al., 1988; Heaney et al. 1978). Devices used to measure bone must have high precision (coefficients of variation < 2%), since bone changes over time are small. The bone remodelling cycle lasts from four to six months, therefore it is recommended that training studies be continued for at least one year to ensure that the training effect is measured in an equilibrium period (Dalsky, 1987). Few longitudinal studies

have met these criteria, therefore it is difficult to accept some of their conclusions.

Strength Training Studies. Four recent training studies have employed whole body strength training in an attempt to increase BMD, with the hypothesis that training may have effects either systemically, through the direct attachment of muscle on bone, or through stabilizing musculature used during different lifts. Three showed small increases, while one showed a significant decrease in BMD with training. Premenopausal females showed small increases of 0.81% in lumbar spine BMD, measured by dual photon absorptiomery (DPA), following a 12 month exercise program, during which they trained 30 minutes per day, three days per week (Gleeson et al., 1990). These changes were only significant when compared to the 0.5% decrease of the control group. Smoking and oral contraceptive use were not controlled for. The relatively light resistance used (two sets of 20 repetitions per exercise) may not have resulted in sufficient peak strain magnitudes to stimulate bone formation.

Postmenopausal females showed an increase of 1.6% in DPA-measured lumbar spine BMD, which was significant compared to the 3.6% decline for control group subjects, following nine months of strength training for one hour, three times per week (Pruitt et al., 1992). Compliance was good, showing that a moderate strength training program is feasible in middle-aged women and has the beneficial effect of preventing postmenopausal bone loss in the spine. Longer durations of strength training (18 months) have beneficial effects on DEXA-measured femoral trochanter (+2.6%), as

well as lumbar spine (+1.1%) BMD, when compared to control group changes (0% and -0.5%), in premenopausal women (Lohman et al., 1992). The proximal femur, having a higher percentage of cortical bone (which is less metabolically active than trabecular bone) than the lumbar spine, may require longer training durations to produce changes and the higher precision of DEXA to detect changes. In contrast to these studies, premenopausal females demonstrated significant decreases (-3.96%) in DEXA-measured lumbar spine BMD, following nine months of training, 45 minutes, twice a week (Rockwell et al., 1990). Measurement of parathyroid hormone indicated acute and significant increases in concentration following training. Parathyroid hormone is released in response to low serum calcium levels and causes bone resorption. It is hypothesized that during exercise, serum calcium levels increase due to hemoconcentration and lactic acidosis (Aloia et al., 1985) and, after peak exercise, fall to levels below baseline, causing a release of parathyroid hormone (Ljunghall et al., 1984). This type of exercise may indirectly increase bone resorption through the release of parathyroid hormone.

To determine if muscle may have direct effects on bone, through tension caused by its attachment, several studies have investigated the effects of training specific muscle groups on the density of the bones on which they pull. Training of one (Smidt et al., 1992) and two (Sinaki et al., 1989) years, using back extensions, situps and leg lift exercises, failed to change DPA-measured lumbar spine BMD in a group of postmenopausal women.

Exercises were done at home, without supervision and therefore compliance may have been low. The study by Sinaki et al (1989) failed to screen subjects for estrogen use, therefore outside factors may have influenced results. Postmenopausal females did show a 16% increase in lumbar spine BMD, as measured by lateral DEXA lumbar scanning, with no change in BMD, as measured by conventional anterior-posterior scanning, following six months of back extension exercise performed once per week (Pollock et al., 1992). Lateral spine scanning is a relatively new technique, which is supposed to provide increased sensitivity, compared to anterior-posterior scanning, because with elimination of the dense posterior arch portion of the lumbar vertebrae, lateral scanning enables measurement of mainly trabecular bone. While these results are impressive, lateral examinations have proven to be difficult to reproduce due to the fact that only small changes in the positioning of subjects can result in large variations in the measured area of bone (Diamond et al., 1991). The accuracy of lateral scanning is questionable, since L2 is often blocked by the rib cage and L4 obscured by the ilium (Delmas, 1991). Until this type of scanning can be perfected, conclusions from studies should be interpreted with caution.

Elderly women showed significant increases (3.4%) in exercised and non-significant increases (1.9%) in control arm single photon absorptiometry (SPA)- measured BMD, following six weeks of forearm exercise (tennis ball squeezing), for 30 seconds a day (Beverly et al., 1989). Six months of detraining resulted in a 2.6% decrease

in BMD. Thus, small amounts of training are effective if done at sufficient strain magnitudes. The detraining results give evidence of the dynamic nature of bone. When forearms of elderly women were loaded in tension, bending, compression and torsion (ensuring abnormal strain distributions), at high strain rates, three times a week for five months, compton scattering-measured BMD increased 3.8% (Simkin et al., 1987). Compton scattering measures bone volume, allowing expression of BMD in actual density units (g/cm^3), instead of areal density units (g/cm^2), as done with SPA, allowing the inclusion of more trabecular bone, which allows the detection of greater change. Site specific training appears to be effective in increasing forearm BMD, whereas whole body strength training is more effective in increasing lumbar BMD, suggesting that stabilizing contractions of trunk musculature or the effects of gravitational loading are more effective than specific exercises that involve muscle attached to the spine.

Endurance Training Studies. Endurance training may be beneficial in increasing bone mass or density, by imposing stresses through repetitive impact loading. Lack of weight bearing, imposed by long term bed rest results in marked loss of bone (LeBlanc et al., 1990; Issekutz et al., 1966). If bed rest is interrupted by three hours of standing per day, bone loss is slowed, but cycling or sitting has no effect (Issekutz et al., 1966). Gravity is therefore an important factor in stressing bone and maintaining bone balance. Enhancing this effect through high impact loading may allow bone balance to become more positive. Nine months of

weight bearing exercise imposed by walking, jogging and stair climbing for 50 to 60 minutes, three times a week, resulted in a 5.2% increase in DPA-measured lumbar spine BMD of postmenopausal females (with a control group decrease of 1.4%). An additional 13 months of training results in a 6.1% BMD increase above baseline, with a decrease to 1.1% above baseline with 13 months of detraining (Dalsky et al., 1988). This is a good demonstration of the dynamic behaviour of bone to loading and unloading. Increases in BMD however, cannot be attributed solely to the weight bearing activity, since 15-20 minutes of cycling, rowing and bench press exercises were included in each session to alleviate boredom. Following long term (four years) exercise training by aerobic dance and light upper body weight lifting exercises for 45 minutes a day, three days per week, trained postmenopausal women showed significantly smaller ulna and radius BMD and BMC losses (-0.043 and -0.65%) than a control group (-1.38 and -1.67%) [Smith et al., 1989]. Thus, light to moderate training slows the rate of long term bone loss in postmenopausal women. Once again results are difficult to attribute to the aerobic program alone and results may have been affected by the fact that the exercise group consumed significantly higher amounts of calcium and magnesium over the study. It is unfortunate that only forearm bone was measured, as it probably was not the site of greatest stress imposed by the aerobics program. When changes in lumbar spine BMC were measured by DPA, significant increases (+3.5%) were observed in comparison to control group declines (-2.7%), in middle aged women, following

eight months of strictly aerobic training (walking, running and calisthenics) done one hour a day, twice a week (Krolner et al., 1983). Subjects' diets were not monitored, oestrogen supplementation was allowed and even menopausal status varied among subjects, so it is difficult to attribute BMC changes to the exercise program alone. The results of these studies show that bone can be affected by endurance-type impact loading as well as strength training. Future studies will have to involve better control in screening of appropriate subjects and strict adherence to one type of exercise, to determine which mode of exercise is most effective.

Studies of Strength Versus Endurance Training. In an attempt to find which type of training is most effective, several investigators have formed two training groups, each performing either endurance exercise, or endurance exercise combined with strength exercise, within one study. Studies involving postmenopausal women that either trained aerobically, or combined aerobic and strength training, 30 to 45 minutes a day, three times per week for 10 to 12 months, showed small increases in upper thigh and trunk calcium bone index, as measured by neutron activation analysis (Chow et al., 1987), and SPA-measured distal radius BMD (Rikle et al., 1990) but no change in DPA-measured hip and spine BMD (Peterson et al., 1991), when compared to control groups. Differences between training groups were not found. By combining strength and aerobic training on the same day, strength development may be impeded (Sale et al., 1990). Thus, the effects of strength

training alone cannot really be compared to the effects of combined strength and endurance training on BMD. Compliance in the study of Peterson et al. (1991) is suspect, as weight training was done unsupervised, at home. Rikle et al. (1990) did not screen out smoking or oestrogen usage among their subjects and the neutron activation analysis technique used by Chow et al. (1987) lacks sufficient precision, due to the problem of site relocation, when part-body measurements are performed (Fogelman & Ryan, 1992). Future studies of this type will have to have strictly endurance-trained and strength-trained groups to determine which type of loading is best for bone formation.

In general, longitudinal studies show increases in bone with exercise, but not nearly as much as suggested by cross-sectional studies of athletes. Future studies will have to involve better control and should be directed at finding an optimal exercise prescription for bone mass gains.

III. EFFECTS OF LOADING ON THE REMODELLING CYCLE OF BONE

Bone is continually turning over (remodelling), with specialized cells causing bone resorption (osteoclasts) and bone formation (osteoblasts). This rate of turnover is determined by hormonal and local factors.

Parathyroid hormone is released in response to a low serum calcium level and stimulates the resorption of calcium from bone (Guyton, 1990). Calcitonin has the opposite effect and inhibits osteoclastic resorption (Guyton, 1990). Oestrogen increases bone formation by stimulating osteoblastic activity, while an adequate level of vitamin D is necessary for proper calcification of bone (Pan & Price, 1984).

Local factors affecting rate of turnover involve mechanical loading, which has beneficial effects on bone formation. Lanyon (1984) hypothesized that any functional level of bone mass results from the balance between mechanical drive towards formation and net hormonal drive towards resorption. The remodelling cycle is made up of five stages: quiescence, activation, resorption, reversal and formation (Parfitt, 1984), and usually takes four to six months to complete in the normal adult (Epstein, 1988). Mechanical loading can increase net bone formation by affecting the various stages of this cycle.

Quiescence to Activation

Osteoclast activation is mainly under hormonal (parathyroid hormone) influence. Activation involves the recruitment of osteoclast precursors (haematopoietic stem cells) from bone marrow (Parfitt ,1984). These precursor cells are mononuclear and display phagocytotic recognition of bone mineral particles (Chambers, 1981). Once attached to the surface of bone, they fuse to form multinucleated osteoclasts (Chambers, 1980).

Resorption

Once activated on the surface of bone, osteoclasts dissolve mineral, while mononuclear cells, which fail to fuse, digest collagen, forming a characteristic cavity within the bone surface (Parfitt, 1984).

Reversal

At a certain depth, resorption is halted, possibly due to signals from osteocytes (bone cells within the matrix; derived from osteoblasts) or lining cells (Chambers, 1980). Reversal involves the possible release of a coupling factor (human skeletal growth factor) (Farley et al., 1982), which stimulates recruitment and proliferation of preosteoblasts from the bone marrow.

Formation

Osteoblasts secrete alkaline phosphatase, type 1 collagen and bone Gla-protein (osteocalcin) (Wright & LeBlond, 1981; Hauschka et

al., 1975; Price et al., 1976). Secreted collagen monomers polymerize, forming collagen fibres (Guyton,1990). Bone Gla-protein is required for binding of calcium into calcium salts called hydroxylapatite (Lian et al., 1978), while alkaline phosphatase induces collagen fibres to deposit hydroxylapatite (Guyton, 1990). Serum alkaline phosphatase and bone Gla-protein levels are often used as measures of bone formation (Riggs et al., 1986). The cavity formed by resorption is gradually filled by formation and bone then returns to the quiescent stage. Osteoblasts either disappear, settle into the bone matrix and become osteocytes or flatten out and become lining cells, losing their ability to synthesize collagen (Parfitt, 1984).

Modifications of the Remodelling Cycle with Loading

Without load bearing, resorption exceeds formation with a ratio of 20 parts bone resorbed for every 19 parts formed (Frost, 1987). With load bearing, one of two situations may arise:

1) The remodelling cycle may be shifted in favour of formation, with either depression of osteoclasts or enhanced stimulation of osteoblasts.

2) Bone formation may be activated from the quiescent stage without intervening resorption.

Evidence that the remodelling cycle is shifted in favour of formation is that dogs subjected to loading exercise for two years (by wearing weighted jackets) [Martin et al., 1981] and postmenopausal women trained by eight weeks of muscular endurance

exercise (Snow-Harter, 1987), show elevated serum alkaline phosphatase levels. Nine months of weight-bearing exercise in postmenopausal women results in elevated levels of serum Gla-protein (Dalsky et al., 1988), while cross sectional studies show weight lifters to have higher serum bone Gla-protein levels than controls (Bell et al., 1988; Fiore et al., 1991). Elevations of both of these parameters indicate increased osteoblastic activity and enhanced bone formation over resorption. The use of serum alkaline phosphatase levels have been criticized since they correlate poorly with bone formation rates as determined by tetracycline labelling (Shifrin, 1970). Serum levels of alkaline phosphatase could be difficult to interpret because multiple isoenzymes exist, derived from the small intestine, kidneys and other sources (McComb et al., 1979; Posen et al., 1977).

In response to loading, bone may be transformed directly from the quiescent stage to formation. Osteocytes are probably the cells most suited for detection of strain changes, since they are located throughout the entire bone matrix, in a three-dimensional interconnecting network (Pead et al., 1988). Osteocytes have gap junctions with osteoblasts and lining cells and may be involved in transmitting proliferating or differentiation factors or other regulatory proteins to these cells (El Haj et al., 1990). Both <u>in vitro</u> and <u>in vivo</u> studies show that within hours following strain, RNA or DNA synthesis is increased in osteocytes and osteoblasts, indicating that protein synthesis and cell proliferation are activated, without preceding resorption (Pead et al., 1988; Pead &

Lanyon, 1989; El Haj et al., 1987; El Haj et al., 1990; Skerry et al., 1989; Nuland et al., 1987; Buckley et al., 1988; Raisz & Kream, 1983; Hasegawa et al., 1985). Radio-labelled uridine uptake indicates increases in RNA synthesis in osteocytes, following loading in vitro (El Haj et al., 1990) and in vivo (El Haj et al.,1987; Pead et al., 1988). Measurement of increases in glucose-6-phosphate dehydrogenase (G6PD) and decreases in aldolase and glyceraldehyde-3-phosphate dehydrogenase (GA3PD) activities also indicate increased DNA and RNA production (El Haj et al., 1987; El Haj et al., 1990; Skerry et al., 1989), through the pentose phosphate pathway (also known as the hexose monophosphate shunt).

While the primary catabolic pathway for glucose is through glycolysis, there exists several other minor pathways, such as the pentose phosphate pathway, specialized for certain purposes, such as synthesis of precursors for RNA and DNA. The first reaction in this pathway is catalyzed by G6PD. A rise in the activity of this enzyme, along with a lack of aldolase and GA3PD activity (both of which are involved in glycolysis) indicates that glucose has been shunted to the pentose phosphate pathway. One of the products of this pathway is ribose-5-phosphate, which can be converted to phosphoribosylpyrophosphate (PRPP). PRPP acts as an activated sugar involved in the synthesis of purines and pyrimidines necessary for DNA and RNA synthesis (Lehninger, 1982). Increased RNA synthesis in osteocytes, signifies that protein synthesis has increased. Proteins could include proliferation or differentiation factors which could have direct effects on osteoblasts or lining

cells, when released (El Haj et al., 1990). DNA and RNA synthesis increase in osteoblasts, following loading in vitro (Hasegawa et al., 1985; Nuland et al., 1987; Buckley et al., 1988), as measured by incorporation of radio-labelled thymidine and uridine, signifying increases in osteoblast proliferation and differentiation. Production of collagenous and non-collagenous protein show increases, as measured by incorporation of radio-labelled proline or leucine (Nuland et al., 1987; Hasegawa et al., 1985; Raisz & Kream, 1983). Non-collagenous protein production includes osteonectin, which is involved in the induction of calcium phosphate deposition on type 1 collagen (Hasegawa et al., 1985; Raisz & Kream, 1983). The immediate increases in DNA and RNA activity in osteocytes and osteoblasts indicate that formation may be directly activated in response to loading, bypassing the other stages in the remodelling cycle.

IV. MECHANISMS FOR TRANSFORMATION OF MECHANICAL STIMULI TO BIOCHEMICAL SIGNALS FOR NEW BONE FORMATION

A number of mechanisms have been proposed for the transformation of mechanical stimuli into biochemical signals for bone formation. These include prostaglandin release, piezoelectric and streaming potentials, increased bone blood flow, microdamage and hormonally mediated mechanisms. It is possible that more than one mechanism is involved, depending on the loading situation.

Prostaglandin Release

Prostaglandin release is implicated as a necessary stage in bone formation with loading. When external in vivo loading is imposed on rooster ulnae with half the roosters receiving indomethacin, (a prostaglandin inhibitor), the indomethacin group has a significantly lower amount of activated osteoblasts than the regular loaded group, when examined posthumously (Pead & Lanyon, 1989). Since loading was short term (one day), prostaglandin release is implicated in the early response of bone formation to loading.

Prostaglandins of the E series (PGE2) administered to rats (Mori el al., 1990) and dogs (Li et al., 1990) for 30 days resulted in increased mineral apposition rates, increased trabecular and cortical bone formation (as evidenced by fluorescent labelling upon sacrifice) and increased serum levels of bone Gla-protein and alkaline phosphatase, indicating osteoblast activation and bone

formation. Furthermore, PGE2 added to rat bone tissue culture increases radio-labelled thymidine incorporation into DNA and radio-labelled proline incorporation into collagen over a time period of 96 hours (Chyun & Raisz, 1984). These results mirror the effects of loading and indirectly imply that PGE2 could be a mediator of bone formation with loading.

Following physical deformation of in vitro cultured bone cells, PGE2, cAMP, G6PD and radio labelled thymidine incorporation into DNA all increase, but are blocked when indomethacin is administered (El Haj et al., 1990; Somjen et al., 1980). When PGE2 is added to cultures without deformation, the stress induced rise in cAMP and radio-labelled thymidine incorporation is mimicked (Somjen et al., 1980). This indicates that PGE2 may act to increase DNA levels though cAMP regulation, when bones are subject to strain. PGE2 may act to enhance adenylate cyclase activity or inhibit phosphodiesterase activity, to increase cAMP levels. cAMP may then act as a second messenger to increase DNA levels and bone cell proliferation.

Stress-induced increases in PGE2 and cAMP are abolished when antiphospholipid antibodies are administered to bone cell cultures (Binderman et al., 1988). Antiphospholipid antibodies inhibit the reaction between cell membrane phospholipids and phospholipase A2. It is proposed that membrane phospholipids, when exposed to phospholipase A2, release arachidonic acid, which is a precursor to PGE2 synthesis. Addition of arachidonic acid or PGE2 to the cultures in the presence of antiphospholipid antibodies stimulate

cAMP formation. From these results, a mechanism is proposed for PGE2-mediated transformation of strain into bone formation (Binderman et al., 1988):

Strain causes stretching of bone cell membranes. This may either cause membrane phospholipid exposure to phopholipase A2 or increased calcium influx, which could increase the activity of membrane phospholipase A2. This could cause the release of arachidonic acid from membrane phospholipid, which then acts as a precursor for PGE2 synthesis. PGE2 activates adenylate cyclase or inhibits phophodiesterase, resulting in increased intracellular levels of cAMP. cAMP would then act as a second messenger to increase DNA or RNA synthesis, resulting in bone cell differentiation and proliferation, and the formation of new bone.

While prostaglandin release is implicated as a necessary stage in the formation of bone, some studies have paradoxally found that prostaglandins added to bone tissue in organ culture actually stimulate osteoclasts (Klein & Raisz, 1970; Tashjianish et al., 1973). One explanation involves the osteoblastic control of osteoclasts:

It is hypothesized that osteoblasts can either inhibit osteoclasts, through prostaglandin release (Chambers, 1985) or stimulate osteoclasts through mineral exposure (Chambers, 1980). When prostaglandins reach bone from an external source (as occurs with prostaglandin addition to organ cultures), homeostasis is disturbed and osteoblasts stimulate osteoclasts in an attempt to preserve homeostasis (Chambers, 1985).

Piezoelectric and Streaming Potentials

Organic crystals, which lack a centre of symmetry, display the generation of an electrical potential (separation of opposite charges) when deformed (Bassett, 1967). Bone may be a piezoelectric substance, since collagen and hydroxylapatite exist in a crystalline state, and the production of electrical potentials upon the application of loads may be a mechanism which stimulates new bone formation.

Upon the application of stress to long bones in vitro, electrical fields are observed, with the compression side of the bone displaying a negative charge and the tensile side, a positive charge (Bassett & Becker, 1962; Shamos & Lavine, 1964, 1967). The amplitude of electrical potentials are dependent on rate and magnitude of strain (Bassett & Becker, 1962). When strain gauges and electrodes are implanted in sheep radius to record strain and electrical potentials in vivo, strain magnitude and strain rate are related to the amplitude of recorded electrical potentials, during fast locomotion (Lanyon & Hartman, 1977).

The piezoelectric effect is maximal with shear stresses and minimal when compressive or tensile stress is imposed (Shamos & Lavine, 1967). Shearing force, acting on collagen fibres may cause them to slide past one another, resulting in distortion of cross-linking bonds (most likely hydrogen bonds) and production of electrical charge separation. Electrical charge separation may also occur with the bending of hyaluronic acid, a mucopolysaccharide, found in the ground substance of bone (Bassett,

1965). Alternatively, charge separation could result from bending at the junction of collagen and hydroxylapatite (calcium salt) crystals. When hydroxylapatite is removed from bone, electrical potentials are greatly reduced (Bassett, 1965).

Most experiments displaying piezoelectrical effects have used dry bone. When bone exists in a physiologically moist condition, fully hydrated collagen may lose its piezoelectric property due to the structured water it contains, making the collagen molecule more symmetrical (Anderson & Eriksson, 1970). Electrical charge separation within stressed bone may be due to "streaming potentials". This occurs when strain causes the movement of ions in liquid, within bone canals, past ions of opposite charge, which remain in a fixed position. Ions of one sign are attracted to the channel walls, leaving the current rich in ions of opposite sign (Anderson & Eriksson, 1970; Gross & Williams, 1982). When experiments are performed in physiologically moist conditions, in which the ionic composition of the fluid forced through bone under stress is changed, a change in the voltage measured across bone is produced, indicating that streaming potentials cause electrical charge separation (Gross & Williams, 1982). Streaming potentials are affected by the velocity of fluid flow. In experiments in which the viscosity of fluid flowing through bone under stress is altered (resulting in decreased velocity), the observed electrical potential is reduced (Gross & Williams, 1982). These two experiments show that electrical potentials are most likely due to streaming potentials instead of a piezoelectric effect. Thus,

streaming potentials appear to be the dominant mechanism by which electric fields are produced across stressed bone.

In vivo studies, in which electrical stimulation is applied to bone, result in bone formation. When electrical potentials are generated in dog femur by the implantation of battery packs, bone formation is observed to occur around the negative electrode, when bone is inspected following sacrifice (Basset, 1965). When mechanical stress or electrical stimulation is applied to canine teeth, in vivo, staining intensity for osteoblasts is increased following mechanical loading and osteoblastic production of cAMP, cGMP and PGE2 are increased following electrical stimulation, indirectly implying that electrical potential generation in response to stress may be involved in osteoblastic activation and bone formation (Davidovitch, 1984).

Several mechanisms are proposed by which electrical potential generation in stressed bone may cause formation of new bone. Based on the observation that a negative charge usually occurs on the bone surface under compressive strain, and that this surface usually shows the greatest amount of new bone formation, it is proposed that free positively charged calcium ions may be attracted to the negatively charged surface and deposited there (Shamos & Lavine, 1964). Another proposed mechanism is that osteocytes, which influence proliferation and differentiation of osteoblasts and lining cells, through interconnections, may depend on nourishment, through fluid flow, to be activated. Osteocytes are situated far from blood vessels within bone and are usually

undernourished. Deformations, causing alternating electrical signals, may act to pump fluid, full of ions and charged molecules, through bone and allow delivery of nutrients to osteocytes (Bassett, 1965). Strain induced flow of charged fluid may cause reorientation of proteoglycans (Skerry et al., 1988). The core proteins of proteoglycans are either attached to, or penetrate cell membranes. Reorientation of proteoglycans may alter membrane permeability, leading to altered influx of ions, such as calcium. Calcium may activate enzymes, such as phospholipase A2, resulting in the cascade of phospholipid release of arachidonic acid, PGE2 production, increased adenylate synthase activity, increased cAMP production, which could then act as a second messenger to increase DNA or RNA content, resulting in proliferation and differentiation of the bone cell (Davidovitch et al., 1984).

Bone Blood Flow

Increased bone blood flow with exercise (Tondevold & Burlow, 1983; Kiiskinen & Suominen, 1975) is proposed as a mechanism by which bone formation may increase. A significant correlation is found between bone blood flow and endosteal new bone formation (McInnis et al., 1980). Increased blood flow could be in response to increased metabolic demand within bone, and results in an increased surface area for diffusion, allowing a greater amount of nutrients to be delivered to bone cells (osteocytes) responsible for release of growth and proliferative factors (McKinnis et al., 1980).

Bone Formation in Response to Microdamage

With repetitive loading, microcracks may appear within bone (Martin & Burr, 1982). It is observed that osteons (central canals within bone, surrounded by concentric lamellae) arrest and trap microcracks produced by cyclic loading. The changes produced within the canal wall adjacent to the crack initiate the production of a new secondary osteon (stimulation of remodelling). Osteoclasts remove damaged material so osteoblasts can deposit matrix and mineral along the paths of imposed stress (Carter, 1981). The repair of damage by secondary osteons may lead to the formation of new cortical bone (Martin & Burr, 1982). When damage is gradual, bone mass increases. With a high rate of damage (from continuous repetitions), bone formation may not keep up and fracture may occur. When military recruits were subjected to 14 weeks of strenuous training (eight hours a day, six days a week), BMD of the distal tibia increased by 7.5%, as measured by Compton scattering, with almost one half of the recruits suffering stress fractures (Leichter et al., 1989), lending support to this mechanism.

The major argument against this microdamage theory is that functional adaptation can be produced in bone in which strain magnitudes and cycles are too low to produce microdamage (Lanyon, 1987). New bone formation via microdamage repair may take place only at moderately high strains and may act together with other bone forming mechanisms to lay down new bone.

Hormonally Mediated Mechanisms

Although local strain related factors are most likely to influence new bone formation, there is some evidence that altered hormone levels, as a result of heavy resistance exercise training, may effect bone formation.

Various weight training protocols result in increased endogenous testosterone production, as measured from blood serum samples (Kraemer et al., 1990). Human osteoblast cells, in vitro, show increased levels of radio-labelled thymidine incorporation into DNA, when dihydrotestosterone is added to the medium (Kasperk et al., 1989). Osteoblasts may have androgen receptors and may respond to resistance training-induced testosterone increases by increasing the rate of proliferation and formation of new bone.

In a cross-sectional study, weight trained males had greater levels of serum vitamin D than controls (Bell et al, 1988). The vitamin D endocrine system may undergo some sort of modification in response to resistance training. Vitamin D stimulates osteoblastic production of bone-Gla protein (Pan & Price, 1986), which is required for the binding of calcium to hydroxylapatite within bone (Lian et al., 1978), resulting in bone formation. Osteoblasts may have vitamin D, as well as testosterone receptors, and may respond to exercised-induced increases in these indices by laying down new bone.

An argument against a systemic mechanism arises when looking at cross-sectional unilateral limb studies. The playing arm of tennis players has significantly greater bone mineral than the non-

playing arm (Huddleston et al., 1980; Jacobson et al., 1984) and this difference is greater than the difference between dominant and non-dominant arms in normal populations (Calder et al., 1992), suggesting that strain acts through local effects.

SUMMARY

Studies of humans and animals give evidence of increased bone formation with exercise. Studies of animals show that bone has enhanced physical and mechanical properties following periods of increased stress. Strains which are high in rate and magnitude and of abnormal distribution, but not necessarily long in duration, are best for inducing new bone formation, resulting in the strengthening of bone by increased density. Cross-sectional studies show that athletes, especially those who are strength trained, have greater bone mineral densities than controls and that strength, muscle mass and maximal oxygen uptake correlate with bone density. Longitudinal training studies indicate that strength training and high impact endurance training increase bone density.

Strain induction may cause a greater level of formation and an inhibition of resorption within the normal remodelling cycle of bone, or it may cause direct activation of osteoblastic bone formation from the quiescent state.

Various mechanisms have been proposed for the transformation of mechanical strain into biochemical stimuli to enhance bone formation. These include prostaglandin release, piezoelectric and streaming potentials, increased bone blood flow, microdamage and hormonally mediated mechanisms. These mechanisms may act on their own or in concert, depending on the loading situation and the characteristics of the bone.

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Effects of Resistance Training on Bone Mass and Body Composition in Young Women

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Summary

Twenty young women (20.3 ± 1.0 y) took part in a strength training program involving five sets of four upper body and three lower body exercises, performed twice per week, for 24 weeks, while 10 women (20.2 ± 0.4 y) served as controls. Scans of the whole body and proximal femur (neck, trochanter and Ward's triangle) were made using X-ray based dual photon absorptiometry (DPX) to determine bone mass and body composition, pre- and post-training. Strength changes were measured by tests for 1-repetition maximum of bench press, leg press and biceps curl. Significant group by time interactions were found for thoracic spine ($p \leq 0.046$) and left arm ($p \leq 0.029$) bone mineral density (BMD), from segmental analyses of whole body scans, with the exercise group demonstrating increases of 0.5% and 0.2% and the control group decreases of 1.7% and 2.3% in left arm and thoracic spine BMD. Group by time interactions approached significance for right arm ($p=0.056$) and right ribs ($p=0.172$) bone mineral content (BMC), with the exercise group demonstrating increases of 0.9% and 1.5% and the control group demonstrating decreases of 2.3% and 4.6% in right arm and right ribs BMC. When subjects who used oral contraceptives thoughout the study were excluded from the analysis (7 exercisers and 4 controls), the group by time interaction remained significant ($p \leq 0.016$) for left arm BMD, with the remaining exercisers

increasing by 1.0% and the remaining controls decreasing by 2.8%. The group by time interaction for thoracic spine BMD did not remain significant, with the remaining exercisers decreasing by 0.7% and remaining controls decreasing by 2.3%. No changes were observed in whole body or femur bone mass. Significant increases in lean mass and strength were demonstrated by the exercise group, for all measures, with no changes in the control group. Strength training in young women results in substantial lean mass and strength gains, with only small, site specific gains in bone mass.

Key words: Strength training; Women; Bone mass; body composition; x-ray based dual photon absorptiometry

Introduction

Osteoporosis is a growing problem in today's society, with osteoporotic fractures increasing at a rate which cannot be explained entirely by the ever increasing proportion of elderly subjects in society [1]. Exercise has been suggested as a protective measure against osteoporosis [2], since physically active individuals generally have greater bone mass [3,4] and a decreased incidence of osteoporotic fracture [5].

Strength training may be the best mode of exercise to promote gains in bone mass, since cross-sectional studies show that male and female strength-trained athletes have larger, denser bones than endurance trained individuals and sedentary controls [6-8]. Longitudinal strength training studies have generated mixed results. Some demonstrate small increases [9-13], some no change [14-16] and one actually shows a decrease in bone mass [17].

Most longitudinal studies have concentrated on training in postmenopausal [10,13-16] or late premenopausal [9,12,17] women, because it is generally accepted that this is the age of greatest bone loss due to the decrease in endogenous estrogen production. While this is true in cortical bone, and has been demonstrated in trabecular bone in some cross-sectional studies (18,19), others show that substantial trabecular bone loss occurs from the spine and proximal femur well before menopause. Some authorities believe that this bone loss is linear from early in the third decade of

life, with no increase in the rate at menopause [20,21]. For example, one longitudinal study, which followed 139 females (age 20-88 yrs) over 0.8 to 3.4 years, showed that significant lumbar spine bone loss occurred before (-1.32%/year) as well as after (-0.97%/year) menopause, with no difference between the rates [22]. It is predicted that trabecular bone mass reaches its peak between the midpoint of the second decade and the early part of the third decade of life [20]. If strength training can increase the peak bone mass reached at this time, it would serve as a good preventative measure against the development of osteoporosis later in life.

The purpose of this study was to investigate the effects of a strength training program on the bone mass of females in their early twenties. Bone mass and body composition change were measured by x-ray based dual photon absorptiometry (DPX), which has been shown to have better precision than gama-ray dual photon absorptiometry [23-25], the method most commonly used in training studies. It is concluded that while strength and lean muscle mass show substantial increases with training, gains in bone mass are very small and site specific.

Methods and Materials

Subjects

Thirty young women without previous exposure to serious strength training exercise served as subjects and were screened for menstrual cycle irregularities, smoking and diseases affecting calcium or bone metabolism. Twenty women were assigned to a training group and ten comprised a control group. Groups were matched for age, height, weight and physical activity patterns. Age, height, weight and BMI of the training group subjects were (mean ± SD) 20.3±1.0 y, 166.6±5.5 cm, 61.1±7.2 kg, 22.0±2.2 kg/m^2. The corresponding values for the control group of 20.2±0.4 y, 165.8 ±6.5 cm, 61.9±7.7 kg and 22.5±1.9 kg/m^2 were not significantly different. Seven subjects in the training group and four in the control group used oral contraceptives throughout the study. Subjects were fully informed of the procedures and signed a consent form prior to experimentation. The study was performed with the approval of the McMaster University Ethics Committee.

Training Program

There were two 12 week training periods, divided by a two week Christmas recess. Training was done on "universal" type weight machines. The upper body exercises consisted of: 1) bench press 2) "lat" pulldown (shoulder adduction and elbow flexion), 3) arm "curl" (elbow flexion), and 4) triceps extension (elbow extension).

The lower body exercises consisted of 1)leg press (combined hip and knee extension), 2) knee extension and 3) knee flexion. Each exercise was done twice per week. The upper body exercises were done for five sets of six to 10 repetitions to concentric failure (i.e., 6-10 RM), in each training session. The lower body exercises were done for five sets of 10-12 RM. The alternate set system was used; that is, two exercises employing opposing muscle groups were alternated until all sets had been completed. The next pair of exercises was then performed, and so on. Exercises that were paired were the bench press and lat pulldown, arm curl and triceps extension and the knee extension and knee flexion. There were two minute rest periods between pairs of sets and between sets of the leg press. Exercise sessions typically required one and a half to two hours to complete. Each exercise session was supervised and resistance, sets and repetitions entered into training logs. A subject's resistance was increased if she displayed proper form in completing six to 10 RM on the upper body exercises and 10 to 12 RM on the lower body exercises.

Measurements

All measurements, except the nutritional assessment, were done on two occasions prior to training and after the 24 week training period.

Bone Mass and Body Composition. Bone mass of the whole body and left hip (trochanter, Ward's triangle and femoral neck) and body composition were measured by DPX, on a Hologic 1000 W

densitometer.

For the hip scans, subjects were placed in a supine position, with the left foot rotated slightly inwards. The left foot and leg were stabililzed by a leg brace, incorporating velcro and nylon straps. The operator was responsible for marking a point lateral to the greater trochanter, from which the densitometer began the scan. During the analysis of scans, the operator was responsible for defining the region of interest around the hip joint and the area of the various bone sites, to be analyzed. A "compare" feature, incorporated into the Hologic software allows analysis of duplicate scans by comparison to the original scan made on a subject.

For the whole body scans, the subjects lay in a supine position, within specific markings upon the scan table, while the densitometer scanned from head to toe. Analysis of the original scan involved the defining of specific subregions of the body, by the operator. Duplicate scans were analyzed by the Hologic software, using the "compare" feature.

Voluntary Strength. Weight lifting performance was measured for the bench press, arm curl and leg press, as the one repetition maximum (1 RM); that is, the heaviest weight that can be lifted only once. A standard protocol was used [26].

Nutritional Assessment. Nutritional status was assessed midway through the study, by having subjects keep food diaries over two weekdays and one weekend day. Subjects were given verbal and written instructions on how to record the amount of food they

consumed. Dietary records were assessed by the Nutritionist III food analysis program (N-Squared Computing Company, Silverton, Oregon). Training and control groups were compared for their intake of calcium, iron , magnesium, zinc, vitamin D, phosphorous, alcohol, caffeine, protein and total energy.

Statistical Analyses. A two factor (group, time) analysis of variance, with repeated measures on one factor (time), was used to analyze the bone and body composition data. A training response would be indicated by a significant group by time interaction. Tukey post-hoc tests, to compare mean values, were performed if significant interactions were found. A one factor (between group) analysis of variance was used to analyze the strength (1 RM) and nutritional data. Strength gains were expressed in relative terms (% increase), since training was done on three different bench press and leg press machines (an equal number of subjects were trained and tested on each machine). Significance was set at $p \leq 0.05$.

Results

All subjects completed the study, with attendance to exercise sessions exceeding 90%. All results are expressed as mean ± SD.

Bone Mass

Whole body and hip bone mass values of subjects were within expected normal values for females of their age [27,28].

Changes in bone mineral density (BMD) were very small. From segmental analyses of the whole body scans, significant group by time interactions were found for left arm ($p \leq 0.029$) and thoracic spine ($p \leq 0.046$) BMD. For left arm BMD, the training group had a greater value post-training (Fig.1), while for thoracic spine BMD, the control group had a greater value pre-training (Fig.2). Exercisers increased by 0.5%, from 0.813 ± 0.037 to 0.817 ± 0.04 g/cm² and by 0.2%, from 0.942 ± 0.090 to 0.944 ± 0.098 g/cm² for left arm and thoracic spine BMD measures, while the control group decreased by 1.7%, from 0.810 ± 0.029 to 0.796 ± 0.023 g/cm² and by 2.3%, from 0.958 ± 0.119 to 0.935 ± 0.111 g/cm² for the two measures (Fig.1 and 2). Precision (method error, expressed as a coefficient of variation) of the left arm and thoracic spine BMD measures was 1.8% and 2.9%, respectively. From segmental analyses of whole body scans, group by time interactons approached significance for right arm and right ribs bone mineral content (BMC) [Table 1]. Precision of arm and ribs BMC measures was 5.9% and 8.6%, respectively. The poorer precision of segmental BMC

measures may have made it difficult to detect BMC changes. Changes in whole body and hip bone mass meausures were not significant (Table 2). Precision of whole body and hip BMD measures was 1.1% and that of whole body BMC was 1.7%.

When the seven exercise and four control subjects who were on oral contraceptives were excluded from the analyses, the group by time interaction for left arm BMD improved, while the group by time interaction for thoracic spine BMD failed to reach significance (table 3).

Body Composition

The group by time interaction for whole body lean mass was significant ($p<0.001$), with the exercise group increasing by 3.7%, from 40.86 $\pm$ 4.31 to 42.39 $\pm$ 4.66 kg and the control group decreasing by 0.5%, from 41.74 $\pm$ 3.49 to 41.52 $\pm$ 3.46 kg (figure 3). Precision of this measure was 1.4%. Segmental analyses of legs, arms and trunk lean mass from whole body scans also showed significant group by time interactions for each body segment. Further analyses by repeated measures ANOVA revealed that post-training lean mass values were significantly greater than pre-training values, for the whole body and for each segment, in the exercise group (Table 4). The greatest changes were seen in the arms. Control group pre- and post-training values were not significantly different.

The group by time interaction for total body fat mass approached significance ($p\leq0.058$), with the exercise group decreasing from 13.45 $\pm$ 3.00 to 13.01 $\pm$ 2.89 kg and the control

group increasing from 13.36 ± 4.42 to 13.77 ± 4.29 kg. Precision of this measure was 1.8%. Analyses of body segments from the whole body scans failed to show significant group by time interactions. A significant group by time interaction ($p \leq 0.001$) was found for changes in percent body fat, with the exercise group decreasing from 23.3 ± 2.9 to 22.2 ± 2.7% and the control group increasing from 22.7 ± 4.4 to 23.3 ± 4.2%.

Strength

Pre-training strength measures did not differ significantly between exercise and control groups. The exercise group showed greater strength gains than the control group, in all measurements, with the greatest gains occurring in the upper body measurements (Table 5).

Nutritional Data

Dietary records showed that there were no significant differences in the average daily intake of major nutrients between the exercise and control groups (Table 6). The majority of subjects' nutrient intakes met the Canadian recommended nutrient intakes (RNIs), with the exception of zinc and iron. Of the 20 exercise and 10 control group subjects, 11 exercisers and 8 controls failed to meet the zinc RNI (8 mg), 17 exercisers and seven controls failed to meet the iron RNI (14 mg), while 10 exercisers and three controls failed to meet the calcium RNI (700 mg).

Discussion

The small site specific gains in BMD found with training in our study agree with results of other strength training studies [9-13]. Small increases of 0.8% and 1.6% have been demonstrated in lumbar spine BMD of pre and post menopausal women, following nine to 12 months of strength training [9,10]. Similar to our findings, these gains are only significant when compared to the small losses of BMD in control groups. Eighteen weeks of strength training in premenopausal women significantly increased trunk BMD, relative to controls, by 0.9% [12]. This is similar to our finding of a 0.2% increase in thoracic spine BMD and a 1.5% increase in right ribs BMC, from segmental analyses of whole body scans. Spinal BMD responds better than BMD at other sites due to its higher percentage of trabecular bone and correspondingly higher rate of bone turnover. Another site of high trabecular bone content, the distal radius, responds well to short durations (six weeks) of resistance training, with increases of 3.4%, as measured by single photon absorptiometry, in post-menopausal women [13]. This is in agreement with the small increase in left arm BMD and right arm BMC found in our subjects. The lack of changes in hip BMD in our study reflect the findings of other strength training studies done in premenopausal women over nine months [17] and postmenopausal women over nine [10] and 12 months [16]. The training duration of these studies, as well as ours, may not have been long enough to show

changes at the hip, as the hip has a higher proportion of cortical bone when compared to the spine. The femoral trochanter does show significant increases of 2.6% when premenopausal females are strength trained over one and a half years [11]. It is suggested that as bone takes four to six months to remodel, a training study should last two to three times that period to ensure that the training effect is measured in an equilibrium period [29].

The significance of our group by time interactions in thoracic spine and left arm BMD were mainly due to a decrease in BMD of our control group (Figures 1 and 2). This is especially true with the spine measures, which differed pre-training, with the control group having the higher value. BMD for the control group then decreased to levels similar to the exercise group, indicating that the effect of exercise may have been to maintain BMD at the spine site. Decreases of BMD in premenopausal females are not unusual, as cross-sectional and longitudinal studies show decreases of 0.73 and 1.32% per year, respectively, in lumbar spine BMD [20,22]. Seasonal variations may also have caused our control group BMD to decrease. Lumbar spine BMD is 1.4% higher in the months of August to November than February through May, in postmenopausal women [30] and 1.7% higher in the months of July to September than January to March, in pre and postmenopausal women [31]. Our pre-training measurements were performed in early October, while our post-training measurements were made in late March; therefore, our subjects' BMD could have been subjected to a seasonal variation, causing slight decreases in BMD over the length of the study.

Seasonal change in daily activity pattern [30] or vitamin D metabolism [32,33] may cause BMD to vary.

Although the intake of nutrients was similar between our exercise and control groups (Table 6), the fact that half of our exercise subjects failed to meet the calcium RNI may have had a negative effect on their BMD [34]. It may have been useful to supplement our subjects' calcium intake, as has been done in other training studies [11,17].

It is difficult to say if oral contraceptive use by any of the subjects had an effect on the results. When subjects on oral contraceptives were excluded from the analyses, the group by time interaction for left arm BMD improved, but failed to reach significance for thoracic spine BMD (table 3).

Cross-sectional studies show that strength [28,35-37] and muscle mass [38,39] correlate significantly with the BMD at the sites of strength and muscle mass measurements. This is reflected to a degree in our study, since the greatest changes in BMD occurred at the same sites as the greatest changes in strength and muscle mass, specifically the upper body.

DPX is a relatively new technique for measuring body composition. Precision (expressed as coefficients of variation) is reported to be 0.96 and 0.4% for total fat and lean mass [40], similar to the precision results we found. DPX body composition measurements in young women show good correlations (r=0.92) with the often-used method of under water weighing [41]. Our finding of substantial gains in lean muscle mass in strength trained

premenopausal females agrees with one other study that employed the DPX technique [42]. Our finding that the group by time interaction for fat mass approached significance was unexpected, since strength training is generally thought to have effects on building muscle, but not decreasing body fat. Magnetic resonance imaging, however, has shown decreases in subcutaneous mid-thigh fat in response to leg strength training in elderly males [43]. Strength training may have a stimulating effect on resting metabolic rate, causing increased post-exercise energy expenditure and a decrease in body fat [44].

In conclusion, although strength training favourably effects body composition measures in young females, increasing lean mass and decreasing percent body fat, only small changes are seen in bone. Training of longer duration may be needed to increase bone formation, due to the length of the remodelling cycle of bone.

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Table 1

Bone mineral content (g) changes in right arm and right ribs (from segmental analyses of whole body scans)

	Pre-trainig	Post-training	% Change	Group by Time interaction
Right Arm				
Exercise	162.9±21.8	164.3±22.6	+0.9	p=0.056
Control	160.5±19.5	156.8±19.9	-2.3	
Right Ribs				
Exercise	114.2±19.8	115.9±17.7	+1.5	p=0.172
Control	127.8±27.6	121.9±21.1	-4.6	

Table 2

Whole body and hip bone mass changes in exercise and control groups

	Pre-training	Post-trainig	% Change
Whole body BMD			
Exercise	1.099±0.056	1.104±0.053	+0.5
Control	1.099±0.070	1.104±0.066	+0.5
Whole body BMC			
Exercise	2347.6±245.5	2341.6±254.9	-0.3
Control	2350.1±338.9	2333.0±322.4	-0.7
Total Hip BMD			
Exercise	1.022±0.125	1.022±0.129	0
Control	1.031±0.098	1.028±0.098	-0.3

BMC=Bone Mineral Content (g)

BMD=Bone Mineral Density (g/cm^2)

Table 3

Bone mineral density (g/cm^2) changes in left arm and thoracic spine (from segmental analyses of whole body scans), in subjects not using oral contraceptives

	Pre-Training	Post-Training	% Change	Group by Time Interaction
<u>Left Arm</u>				
Exercise (n=13)	0.814±0.041	0.822±0.046	+1.0	$p \leq 0.016$
Control (n=6)	0.815±0.036	0.792±0.026	-2.8	
<u>Thoracic Spine</u>				
Exericse (n=13)	0.949±0.096	0.942±0.106	-0.7	NS
Control (n=6)	0.923±0.088	0.902±0.107	-2.3	

Table 4

Lean mass (g) changes with strength training in 20 young women

Site	Pre	Post	% Increase	p
Whole Body	40861 ± 4306	42391 ± 4660	3.7	<0.001
Left Arm	1945 ± 232	2167 ± 314	11.4	<0.001
Right Arm	2082 ± 315	2248 ± 313	7.9	≤0.003
Trunk	22040 ± 2581	22701 ± 2712	3.0	≤0.001
Left Leg	7375 ± 784	7599 ± 894	3.0	≤0.005
Right Leg	7418 ± 790	7677 ± 887	3.5	≤0.001

All values are means ± SD

Table 5

1-Repetition Maximum test improvements between pre and post training

Exercise	Improvement (%Change)	Group Difference
Arm Curl		
Exercise	+73.0	p<0.001
Control	-7.8	
Bench Press		
Exercise	+32.6	p<0.001
Control	-0.9	
Leg Press		
Exercise	+22.7	p<0.001
Control	+3.6	

Table 6

Average daily nutrient intake of exercise (n=20) versus control (n=10) groups

Nutrient	Exercise	Control
Calcium (mg)	1002 ± 552	899 ± 285
Vit D (IU)	176 ± 147	112 ± 60
Iron (mg)	10.2 ± 2.5	10.9 ± 3.6
Magnesium (mg)	262 ± 131	217 ± 47
Zinc (mg)	7.6 ± 3.3	6.6 ± 2.8
Protein (g)	73.5 ± 28.0	75.1 ± 31.9
Caffeine (mg)	62.7 ± 79.8	93.5 ± 58.0
Alcohol (g)	35.7 ± 86.8	0

All values are means ± SD daily intake, from three day food diaries. There were no differences between groups in intake.

Figure 1

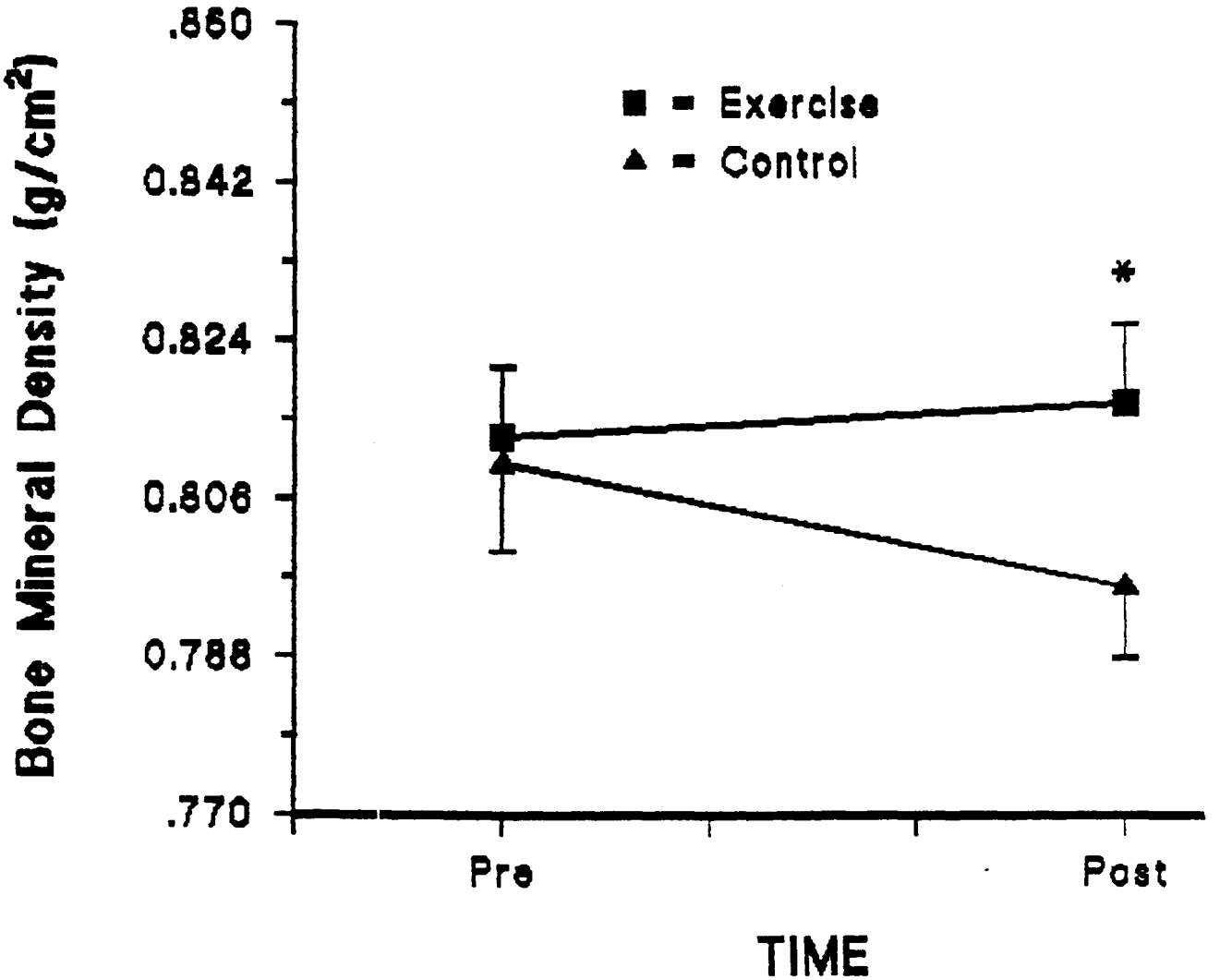
.860
0.842
0.824
0.806
0.788
.770
Bone Mineral Density (g/cm²)
■ = Exercise
▲ = Control
*
Pre
Post
TIME

Figure 2

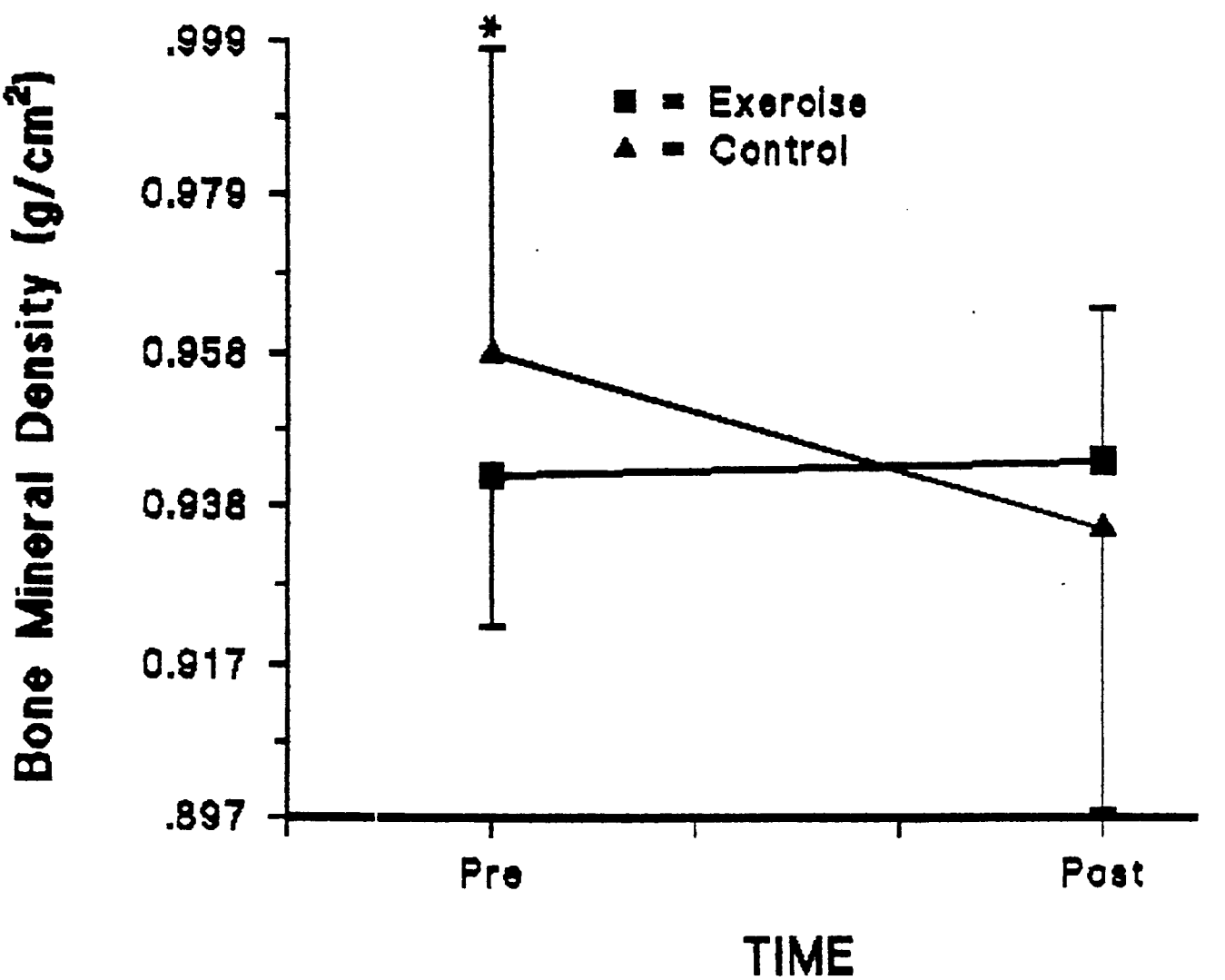
.999
0.979
0.958
0.938
0.917
.897
Bone Mineral Density (g/cm²)
*
■ = Exercise
▲ = Control
Pre
Post
TIME

Figure 3

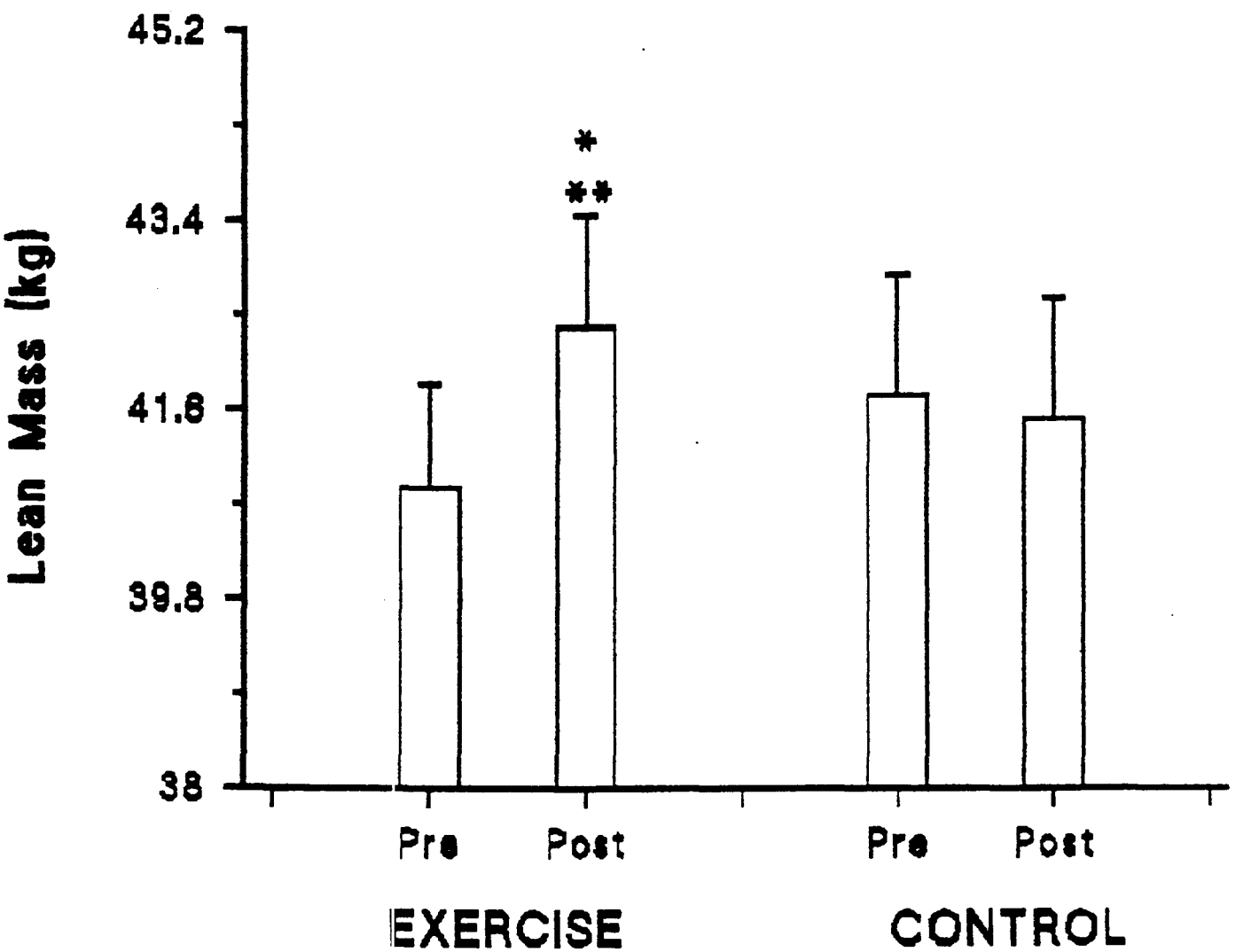
45.2
43.4
41.6
39.8
38
Lean Mass (kg)
*
**
Pre
Post
Pre
Post
EXERCISE
CONTROL

Figure Legends

Figure 1: Pre and post training values for exercise and control left arm bone mineral density (BMD). Group by time interaction is shown. *:$p<0.05$, represents the difference between post measures.

Figure 2: Pre and post training values for exercise and control thoracic spine bone mineral density (BMD). Group by time interaction is shown. *:$p<0.05$, represents the difference between pre measures.

Figure 3: Pre and post training values for exercise and control whole body lean mass. *:$p<0.001$, represents the interaction, where exercise post is greater than control post. **:$p<0.001$, represents the difference between pre and post measures in the exercise group.

REPRODUCIBILITY OF BONE MASS AND BODY COMPOSITION MEASUREMENTS BY X-RAY AND GAMMA RAY DUAL PHOTON ABSORPTIOMETRY

by

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Running Head: BONE DENSITY AND BODY COMPOSITION REPRODUCIBILITY

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Summary

Bone mineral content (BMC) of the spine and femoral neck, and spine and hip (femoral neck, trochanter, and Ward's triangle) bone mineral density (BMD), measured by gama ray dual photon absorptiometry (DPA), were measured on two separate occasions (1-2 weeks apart) in ten men (22±1y) and women (21±0.4y). In a separate group of 21 women (20.9±1.6 y), left hip and whole body BMD, femoral neck and whole body BMC and body composition were measured on two separate occasions (1-2 weeks apart) by x-ray dual photon absorptiometry (DPX). The method error (ME) of duplicate measurements, expressed as a percentage of combined (test 1 and test 2) mean values ranged from 4.7 - 6.2% and for DPA hip BMD and from 1.0 - 2.5% for DPX hip BMD. The MEs for DPA spine BMC and BMD were 2.4 and 3.4%. For DPX whole body BMD, BMC, fat and lean tissue mass the reproducibilities were 1.1, 1.7, 1.8 and 1.4%, respectively. No effect on BMD MEs was observed due to subject size. When the DPA densitometer Gd-153 source was changed, MEs showed a non-significant increase (p=0.059). Some DPA bone measurements may not be precise enough for usage in longitudinal studies. DPX bone, fat and lean tissue mass measurements are more precise than measurements by DPA and are preferred for the detection of small changes expected in exercise studies.

Key words: Bone mineral density, body composition, reproducibility, x-ray dual photon absorptiometry, gama ray dual photon absorptiometry

Introduction

Studies investigating the effects of exercise training on bone mass and body composition measurements demand methods of high reproducibility, since the expected bone mass (1,2) and body composition (3,4) changes are small. The purpose of the present study was to compare the reproducibility (precision) of various bone and body composition measurements made by gama ray dual photon absorptiometry (DPA) using a 153 Gd source and x-ray dual photon absorptiometry (DPX).

Two separate reproducibility studies were conducted. In the first, spine and femoral neck bone mineral content (BMC) and spine and hip BMD reproducibility was determined from DPA measurements made on two separate occasions in 10 young men and 10 young women. In the second study, BMD for the hip and whole body as well as femoral neck BMC, whole body BMC, lean mascle and fat mass reproducibility was obtained by DPX measurements made on two separate occasions in 21 young women.

Reproducibility studies from the literature show that DPX-measured spine (5-10), hip (5,7,9,11) and whole body BMD (9,12) is more precise than DPA BMD measurements (9,10,13-21) and that DPX body composition measurement (22,23) is more precise than body composition by DPA (24). Although most reports in the literature express reproducibility as a coefficient of variation, few define exactly how their coefficients are calculated, making comparisons

between studies difficult.

Our results show that DPX measurements are more precise than measurements by DPA, and are preferred for use in longitudinal exercise training studies.

Materials and Methods

Subjects

Ten young men and 10 young women participated in the first study, in which bone measurements were done by DPA. Their characteristics are shown in Table 1.

Twenty-one young women participated in the second study in which bone and body composition measurements were taken by DPX. Their characteristics are shown in Table 2. Subjects were fully informed of the procedures and signed a consent form prior to experimentation. Experimentation was performed with the approval of the McMaster University Ethics Committee.

Measurements

All measurements were made on two separate occasions, one to two weeks apart.

DPA Bone mineral content and density. Bone mineral content of the lumbar spine (L2-4) and femoral neck and bone mineral density of the left hip (femoral neck, trochanter and Ward's triangle sites) and lumbar spine (L2-4) were measured by gama ray dual-photon absorptiometry (22), on a Norland 2600 dichromatic bone densitometer, incorporating "Bonestar" software revision 3.4.1.

For the spine bone scans, subjects were placed in a supine position, with knees and hips flexed at 90°. The operator was responsible for marking a soft tissue baseline point on the abdomen and the two points (1-2 cm below the xiphoid process and 1-2 cm

below the anterior iliac crest), which defined the region to be scanned by the computer. The raw data were analyzed by a single technician, who was unaware of the order of the scans. This analysis involved repositioning of baseline and bone edges, which the technician felt were incorrectly positioned by software, and defining of the individual (L2-4) vertebrae.

For the hip scans, subjects were placed in a supine position, with feet rotated slightly inwards and supported by a trapezoid-shaped block, with velcro straps. The operator was responsible for marking a soft-tissue baseline point on the thigh and a point at the centre of the femoral neck, for the start of a scout scan, which produced a rough image of the hip region. From this rough image, the operator marked a soft tissue baseline point 1 cm from the femoral neck and two points, which when connected, form a straight line parallel to and bisecting the femoral neck. The correct positioning of these two points is necessary for the vertical placement of the femoral neck on the final scan image. During analysis, the software automatically placed two horizontal lines, 1 cm apart, over the narrowest region of the vertical image, defining the areas from which BMD readings were calculated. All hip analyses were done by a single technician, who was unaware of the order of the scans.

Half way through the study, the DPA Gd-153 radiation source was changed. This resulted in half of the subjects having their second set of scans performed with a different Gd-153 radiation source.

DPX bone mineral density, bone mineral content and body composition. BMD of the left hip (femoral neck, trochanter, and Ward's triangle sites), BMC of the femoral neck and whole body BMD, BMC, fat and lean tissue mass were measured by DPX on a Hologic 1000 W densitometer.

For the hip scans, subjects were placed in a supine position, with the left foot rotated slightly inwards. The left foot and leg were stabilized by a leg brace, incorporating velcro and nylon straps. The operator was responsible for marking a point lateral to the greater trochanter, from which the densitometer began the scan. During the analysis of scans, the operator was responsible for defining the region of interest around the hip joint and the area of the various bone sites, to be analyzed. A "compare" feature, incorporated into the Hologic software allows analysis of duplicate scans by comparison to the original scan made on a subject. This allows for identical placement of regions of interest and bone sites between two scans, making duplicate scans more reproducible.

For the whole body scans, the subjects laid in a supine position, within specific markings upon the scan table, while the densitometer scanned from head to toe. Analysis of the original scan involved the defining of specific subregions of the body, by the operator. Duplicate scans were analyzed by the Hologic software, using the "compare" feature.

All scans were performed and analyzed by the same technician.

Statistics

Reproducibility was expressed as the method error (ME) of duplicate measurements. This is found by dividing the standard deviation of the mean difference of duplicate measurements by the square root of two (25). ME was expressed as a coefficient of variation (CV), as a percentage of the combined means of each pair of measurements.

A one-way between group ANOVA was used, to test if the MEs of the subjects scanned with the same source, on the dual photon densitometer, differed from those scanned with a different source, on the second set of bone measurements.

To test the effect of subject size on DPA reproducibility the MEs of the six largest and six smallest subjects were compared by a two-way ANOVA, with between group factors for size of subject (big or small) and type of Gd-153 source (identical or different) used on the duplicate measurements.

To test the effect of subject size on DPX reproducibility, the MEs of the six largest and six smallest subjects were compared by a one-way between group ANOVA.

Results

Reproducibility of DPA spine BMC and BMD is presented in Table 3. The data for the proximal femur is presented in table 4, which shows that reproducibility of hip BMD and femoral neck BMC was better with DPX, than with DPA measurements.

DPX-measured whole body BMD, BMC, lean mass and fat mass reproducibilities are presented in tables 5,6,7 and 8, with subregional analyses included.

No effect on MEs of DPA or DPX measurements was observed due to subject size. Characteristics of the largest and smallest subjects in each study are given in tables 9 and 10. MEs for large and small subjects measured by DPA are given in tables 11 and 12. MEs for large and small subjects measured by DPX are given in tables 13 to 16. Large subjects tended to have larger BMD MEs when measured by DPX, but the difference was not significant ($p=0.072$).

The changing of the 153-Gd source resulted in higher MEs for spine and hip DPA meausrements (Tables 17 and 18), but the difference was not significant ($p=0.059$).

Discussion

When comparing CVs from previous studies, DPX spine (5-10), hip (5,7,9,11) and whole body (9,12) bone mass and body composition (22) measurements show better reproducibility than DPA measurements (9,10,13-21,24).

Few studies define how their CVs are calculated. Those that used the method error of duplicate measurements, expressed as a CV, show DPA spine BMD and BMC reproducibility of 2.0-3.2% (18,19) and 3.7% (18), respectively. This is in agreement with our DPA spine reproducibility (Table 3). Although DPX lumbar spine BMD was not measured in our study, other studies which make comparisons between DPX and DPA lumbar spine measurements, show better reproducibility when DPX is used (9,10).

Our DPA hip MEs are slightly higher than those found by Shipp et al (19). Their MEs, expressed as CVs were 3.3, 4.0, and 4.9% for left femoral neck, trochanter and Ward's triangle sites, whereas our's were 6.0, 4.7 and 6.2% for the same sites. Our DPX hip measurements showed better reproducibility than DPA measurements (Table 4), in agreement with Stevenson et al (9).

Our DPX whole body bone mineral content and density MEs, expressed as CVs (Tables 5 and 6) are comparable to those of other studies (9,12), although the method of CV calculation is not always mentioned. Stevenson & Lees (1990) show slightly lower CVs of 0.65 and 1.35% for whole body BMD and BMC (23), while Mazess et al. (1989) show a CV of 0.5% for whole body BMD (12). CVs for our

subregion BMDs ranged from 1.1 to 2.9%, which is slightly higher than those reported by Mazess et al. (1989), which range from 1.0 to 1.5% (12). Ormerod et al. (1990) report a reproducibility of 2.0% for DPA-measured total body bone mineral mass and 4.0% for regional bone mineral mass, when differences between two successive measurements are expressed as a percentage of the mean (21). Mazess et al. (1984) report a DPA-measured CV of 2.4% for total body bone mass and CVs ranging from 2.8 to 8.6% for subregion BMDs, but fail to mention the method of CV calculation (20). All subregion BMD CVs found in our study by DPX were better than these.

Significant error in bone reproducibility measurements can result when subjects are large (26,27) or, in the case of DPA, when the Gd-153 radiation source is weak or changed (11,16,18,26-28). MEs of our largest subjects did not differ significantly from MEs of our smallest subjects for DPA and DPX measurements. During the DPA measurements, our Gd-153 radiation source was changed, and a slight, non-significant effect on reproducibility determinations was observed. As the DPA Gd-153 source decays, fewer photons are emitted and detected by the collimator in the scanning arm of the machine, decreasing the precision of measurements. A higher number of photons are emitted by DPX, resulting in greater precision.

Differences in analyses between duplicate DPA scans may have resulted in differences in BMC and BMD readings, increasing the ME. When analyzing duplicate DPX scans, the "compare" feature in the software eliminates errors which may result from a technician's subjective analyses. During the DPA spine scans, the technician

may have repositioned the baselines or bone edges differently on duplicate scans. Defining the individual vertebrae, in the same manner, on duplicate scans can sometimes be difficult. If the vertebrae (L2-4) are shifted from one scan to another, a large error can result (16). With the DPA hip scout scans, an error could result if the operator aligns the cursors, which bisect and run parallel to the femoral neck, differently on duplicate scans. The proper alignment of these cursers is necessary for the femoral neck to be displayed vertically on the resulting scan. During the scan analysis, the computer automatically places two horizontal lines, 1 cm apart over the narrowest region of the femoral neck. The placement of these lines determines the areas of the hip from which bone mass readings are taken. If the neck is not perfectly vertical, the computer may place these lines at points which do not reflect the narrowest region, resulting in differences in analyses of duplicate scans and an increased ME.

The largest errors in DPA or DPX BMC and BMD measurements may result from differences in positioning of subjects on duplicate scans (29), or the movement of subjects during scans. During a few DPA hip scans, the velcro strap holding the feet against the trapezoid block came undone, resulting in slight movement at the hip joint. The leg stabilizing device used in DPX scans is more effective, as this type of movement did not occur. Other movements may have resulted due to subject discomfort during lengthy scans. DPA hip scans take approximately 20 minutes, whereas DPX hip scans take eight minutes, decreasing the amount of discomfort that may be

experienced by a subject.

In summary, DPX measurements are more precise than DPA measurements due to better analyses procedures, greater stabilization of the leg during hip scans and a decreased scanning time. Reproducibility of bone measurements is important when considering the results of longitudinal exercise studies, which typically show changes in BMD of 2-5% (1,2). When a measuring device has a ME, expressed as a CV, of n %, the minimal difference between two scans, to have 95% confidence (1.96 standard deviation units) that real changes in BMD have occurred would be:

(n) x (square root of 2) x (1.96) % (30,31)

Using this equation, the CV of bone measurements would have to be 2% or less to have 95% confidence that changes occurred in longitudinal studies (if these changes are approximately 5%). DPA bone measurements may lack the precision needed for usage in exercise studies. DPX measurements show better precision and are preferred.

Our DPX total lean and fat mass reproducibility values (Tables 7 and 8) were slightly lower than those of Stevenson & Lees (1989) who found CVs of 1.47 and 2.73% for total lean tissue and fat mass (22), but slightly worse than those of Kelly et al. (1991), who gave values of 0.4 and 0.96% (22). Neither study mentioned how CVs were calculated. Our arm and leg subregion lean mass CVs (Table 7) are better than those measured by DPA (24) which show CVs of 7.0

and 2.4% for arm and leg muscle mass, but again the methods of CV calculation were not given.

Exercise studies generally show greater changes in body fat and muscle mass than in bone and do not require methods that are as precise. When measuring body composition changes in elite athletes, who are already well trained, precision becomes more important. Changes with training would be smaller than with untrained individuals.

In conclusion, DPX measurements are more precise than measurements by DPA, and are preferred for use in longitudinal exercise studies.

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Table 1

Physical characteristics of the subjects in study 1

Group	Age (y)	Height (cm)	Mass (kg)
Males (n=10)	22 ± 1	178.4 ± 8.7	77.8 ± 15.4
Females (n=10)	21 ± 0.4	167.2 ± 6.5	56.4 ± 8.1

Values are mean ± SD

Table 2

Physical characteristics of the subjects in study 2

Group	Age (y)	Height (cm)	Mass (kg)
Females (n=21)	20.9 ±1.6	166.5 ±5.6	60.5 ±6.8

Values are mean ± SD

Table 3

Means and reproducibility of spine bone mass and bone mineral density, obtained on two separate days by gama ray dual photon absorptiometry.

	Day 1	Day 2	ME (CV%)
Spine BM (n=20)	58.3±13.0	58.6±13.3	2.4
Spine BMD (n=20)	1.313±0.163	1.308±0.183	3.4

Day 1 and Day 2 values are mean ± SD

ME = Method Error expressed as a percentage of combined mean values (CV= Coefficient of Variation)

BM = Bone mass (g)

BMD = Bone mineral density (g/cm^2)

Table 4

Means and reproducibility of hip BMD and femoral neck BMC, as measured by gamma ray and x-ray based dual photon absorptiometry, on two separate days

	Day 1	Day 2	ME (CV%)
DPA femoral neck BMD (n=20)	1.193±0.231	1.164±0.221	6.0
DPX femoral neck BMD (n=21)	0.968±0.107	0.981±0.110	1.9
DPA trochanter BMD (n=19)	0.974±0.178	0.951±0.172	4.7
DPX trochanter BMD (n=21)	0.821±0.067	0.822±0.070	1.0
DPA Ward's BMD (n=20)	1.126±0.226	1.099±0.241	6.2
DPX Ward's BMD (n=21)	0.855±0.123	0.868±0.121	2.5
DPA femoral neck BMC (n=20)	4.46±1.65	4.74±1.79	8.4
DPX femoral neck BMC (n=21)	4.31±1.69	4.27±1.61	3.7

Day 1 and Day 2 values are mean ± SD

ME= Method Error expressed as a percentage of combined mean values (CV= Coefficient of Variation)

BMD= Bone mineral density (g/cm^2)

BMC= Bone mineral content (g)

DPA= 153 Gd Dual Photon Absorptiometry

DPX= X-ray Dual Photon Absorptiometry

Table 5

Means and reproducibility of Whole body and subregion BMD, as measured by x-ray dual photon absorptiometry, on two separate days

	Day 1	Day 2	ME (CV%)
Whole Body BMD (n=21)	1.112±0.055	1.117±0.061	1.1
Arm BMD (n=21)	0.814±0.043	0.814±0.043	1.8
Leg BMD (n=21)	1.230±0.110	1.235±0.114	2.7
Ribs BMD (n=21)	0.685±0.026	0.680±0.024	2.1
Thoracic Spine BMD (n=21)	0.988±0.110	0.991±0.099	2.9
Lumbar Spine BMD (n=21)	1.106±0.140	1.102±0.140	2.3
Pelvis BMD (n=21)	1.114±0.087	1.123±0.086	1.1

Day 1 and Day 2 values are mean ± SD

ME = Method Error expressed as a percentage of combined mean values (CV=Coefficient of Variation)

BMD = Bone mineral density (g/cm^2)

Table 6

Means and reproducibility of whole body and subregion BMC, as measured by x-ray dual photon absorptiometry, on two separate days

	Day 1	Day 2	ME (CV%)
Whole body BMC (n=21)	2419.8±224.0	2430.9±230.2	1.7
Arm BMC (n=21)	165.5±20.9	169.9±24.6	5.9
Leg BMC (n=21)	472.5±49.4	477.2±57.1	2.6
Ribs BMC (n=21)	112.1±17.9	108.6±15.7	8.6
Thoracic spine BMC (n=21)	111.7±20.2	110.8±21.6	3.0
Lumbar spine BMC (n=21)	74.7±11.0	75.3±11.7	4.6
Pelvis BMC (n=21)	235.7±37.2	232.7±36.4	3.1

Day 1 and Day 2 values are mean ± SD

ME= Method Error expressed as a percentage of combined mean values (CV=Coefficient of Variation)

BMC= Bone Mineral Content (g)

Table 7

Means and reproducibility of whole body and subregion lean tissue mass as measured by x-ray dual photon absorptiometry on two separate days

	Day 1	Day 2	ME (CV%)
Whole body LTM (n=21)	40961.9±4123.9	41315.4±4340.7	1.4
Arms LTM (n=21)	1915.8±253.8	1980.2±241.8	5.7
Legs LTM (n=21)	7321.6±775.7	7352.3±789.2	2.1
Trunk LTM (n=21)	22283.5±2523.8	22473.2±2619.9	2.0

ME = Method Error expressed as a percentage of combined mean values (CV=Coefficient of Variation)

LTM = Lean tissue mass (g)

Table 8

Means and reproducibility of whole body and subregion fat mass as measured by x-ray dual photon absorptiometry on two separate days

	Day 1	Day 2	ME (CV%)
Whole Body FM (n=21)	12820.4±2925.0	12813.6±2828.4	1.8
Arms FM (n=21)	1198.8±345.2	1198.2±360.3	8.4
Legs FM (n=21)	3043.2±724.6	3079.9±766.1	4.6
Trunk FM (n=21)	4316.1±1136.1	4277.5±1060.4	4.4

Day 1 and Day 2 values are mean ± SD

ME = Method Error expressed as a percentage of combined mean values (CV=Coefficient of Variation)

FM = Fat Mass (g)

Table 9

Physical characteristics of largest and smallest subjects in study 1

Group	Height (cm)	Mass (kg)
Large subjects (n=6)	182.8±7.6	87.9±9.5
Small subjects (n=6)	163.6±6.6	52.0±8.4

Values are mean ± SD

Table 10

Physical characteristics of largest and smallest subjects in study 2

Group	Height (cm)	Mass (kg)
Large subjects (n=6)	169.7±4.1	70.0±7.0
Small subjects (n=6)	165.4±5.5	55.1±2.4

Values are mean ± SD

Table 11

Means and reproducibility of bone mineral density and bone mass of large subjects, as measured by gama ray dual photon absorptiometry on two separate days

	Day 1	Day 2	ME(CV%)
Spine BMD (n=6)	1.459±0.113	1.503±0.084	3.5
Spine BM (n=6)	72.84±10.03	74.08±8.80	2.4
Femoral Neck BMD (n=6)	1.398±0.211	1.381±0.168	3.7
Trochanter BMD (n=6)	1.121±0.136	1.112±0.133	3.1
Ward's BMD (n=6)	1.338±0.209	1.347±0.211	3.6

Day 1 and Day 2 values are mean ± SD

ME = Method Error expressed as a percentage of combined mean values (CV=Coefficient of variation)

BMD = Bone Mineral Density (g/cm^2)

BM = Bone Mass (g)

Table 12

Means and reproducibility of bone mineral density and bone mass of small subjects, as measured by gama ray dual photon absorptiometry on two separate days

	Day 1	Day 2	ME(CV%)
Spine BMD (n=6)	1.194±0.063	1.162±0.079	1.7
Spine BM (n=6)	46.97±5.71	47.05±5.85	1.1
Femoral Neck BMD (n=6)	1.033±0.205	0.986±0.093	12.0
Trochanter BMD (n=6)	0.881±0.197	0.821±0.140	7.4
Ward's BMD (n=6)	0.992±0.180	0.915±0.093	10.3

Day 1 and Day 2 measures are mean ± SD

ME = Method Error expressed as a percentage of combined mean values (CV=Coefficient of Variation)

BMD = Bone Mineral Density (g/cm^2)

BM = Bone Mass (g)

Table 13

Means and reproducibilty of bone mineral density of large subjects, as measured by x-ray dual photon absorptiometry on two separate days

	Day 1	Day 2	ME(CV%)
Femoral Neck BMD (n=6)	0.951±0.142	0.937±0.142	2.5
Trochanter BMD (n=6)	0.816±0.087	0.810±0.088	1.3
Ward's BMD (n=6)	0.813±0.137	0.829±0.114	3.5
Arms BMD (n=6)	0.804±0.027	0.813±0.043	2.4
Ribs BMD (n=6)	0.685±0.022	0.675±0.022	1.4
Thoracic Spine BMD (n=6)	1.056±0.100	1.063±0.072	4.7
Lumbar Spine BMD (n=6)	1.058±0.051	1.040±0.071	1.9
Pelvis BMD (n=6)	1.116±0.072	1.124±0.081	1.8
Legs BMD (n=6)	1.231±0.071	1.219±0.070	2.4

Day 1 and Day 2 values are mean ± SD

ME = Method Error, expressed as a percentage of combined mean values (CV=Ccefficient of Variation)

BMD = Bone Mineral Density (g/cm^2)

Table 14

Means and reproducibility of bone mineral density of small subjects, as measured by x-ray dual photon absorptiometry on two separate days

	Day 1	Day 2	ME(CV%)
Femoral Neck BMD (n=6)	0.929±0.093	0.939±0.092	1.2
Trochanter BMD (n=6)	0.785±0.039	0.785±0.043	0.6
Ward's BMD (n=6)	0.821±0.091	0.820±0.104	2.6
Arms BMD (n=6)	0.788±0.029	0.783±0.016	2.0
Ribs BMD (n=6)	0.670±0.016	0.659±0.017	1.9
Thoracic Spine BMD (n=6)	0.903±0.088	0.899±0.085	1.0
Lumbar Spine BMD (n=6)	1.047±0.083	1.024±0.094	1.5
Pelvis BMD (n=6)	1.048±0.067	1.057±0.061	0.9
Legs BMD (n=6)	1.183±0.164	1.214±0.181	2.7

Day 1 and Day 2 values are mean ± SD

ME = Method error, expressed as a percentage of combined mean values (CV=Coefficient of Variation)

BMD = Bone Mineral Density (g/cm^2)

Table 15

Means and reproducibility of bone mineral content of small subjects as measured by x-ray dual photon absorptiometry, on two separate days

	Day 1	Day 2	ME(CV%)
Arms BMC (n=6)	142.3±12.1	148.8±21.0	6.1
Ribs BMC (n=6)	102.2±14.0	93.0±10.5	5.0
Thoracic spine BMC (n=6)	94.4±17.9	89.1±15.2	2.8
Lumbar spine BMC (n=6)	65.1±9.4	65.5±13.1	6.1
Pelvis BMC (n=6)	197.2±19.5	201.1±22.7	3.3
Legs BMC (n=6)	438.2±67.4	458.8±85.6	3.2

Day 1 and Day 2 values are mean ± SD

ME= Method Error expressed as a percentage of combined mean values (CV= Coefficient of Variation)

BMC= Bone Mineral Content (g)

Table 16

Means and reproducibility of bone mineral content of large subjects as measured by x-ray dual photon absorptiometry, on two separate days

	Day 1	Day 2	ME(CV%)
Arms BMC (n=6)	176.4±22.0	181.0±15.7	8.0
Ribs BMC (n=6)	122.0±23.7	120.1±14.3	12.7
Thoracic spine BMC (n=6)	123.6±15.1	126.4±16.1	1.8
Lumbar spine BMC (n=6)	78.2±7.1	80.1±6.3	4.5
Pelvis BMC (n=6)	248.6±25.1	239.0±29.0	3.1
Legs BMC (n=6)	490.8±37.3	492.1±24.4	1.5

Day 1 and Day 2 values are mean ± SD

ME= Method Error expressed as a percentage of combined mean values (CV=Coefficient of Variation)

BMC= Bone Mineral Content (g)

Table 17

Means and reproducibility of bone mineral density and bone mass, measured by gama ray dual photon absorptiometry with the same Gd-153 source on two separate days

	Day 1	Day 2	ME(CV%)
Spine BM (n=11)	55.1±12.6	56.3±13.6	2.3
Spine BMD (n=11)	1.257±0.113	1.278±0.147	2.8
Femoral neck BMD (n=10)	1.138±0.169	1.147±0.190	3.6
Trochanter BMD (n=10)	0.918±0.13	0.914±0.132	2.6
Ward's BMD (n=10)	1.078±0.217	1.066±0.212	3.5

Day 1 and Day 2 values are mean ± SD

ME = Method Error expressed as a percentage of combined mean values (CV=Coefficient of Variation)

BM = Bone Mass (g)

BMD = Bone Mineral Density (g/cm^2)

Table 18

Means and reproducibility of bone mineral density and bone mass, measured by gama ray dual photon absorptiometry with a different Gd-153 source on two separate days

	Day 1	Day 2	ME(CV%)
Spine BM (n=9)	62.1±13.2	61.4±13.1	2.4
Spine BMD (n=9)	1.382±0.194	1.344±0.223	3.4
Femoral neck BMD (n=10)	1.249±0.278	1.181±0.258	7.0
Trochanter BMD (n=9)	1.036±0.209	0.993±0.208	5.8
Ward's BMD (n=10)	1.174±0.235	1.132±0.275	7.9

Day 1 and Day 2 values are mean ± SD

ME = Method Error expressed as a percentage of combined mean values (CV=Coefficient of Variation)

BM = Bone Mass (g)

BMD = Bone Mineral Density (g/cm^2)