# BONE DENSITY AND GYMNASTICS

# THE EFFECT OF GYMNASTIC TRAINING ON BONE DENSITY IN PREPUBESCENT FEMALES

By

KERRY DYSON, B.Sc.(Hons)

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AUTHOR: Kerry Dyson, B.Sc. (Hons) (Crewe and Alsager College H.E.)

SUPERVISOR: Dr. C.J. Blimkie

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## ABSTRACT

The effect of intense gymnastic training on bone mineral density was investigated in 16 elite prepubertal (9.82 ± 0.89 years) (mean ± SD) gymnasts (GYM) and 16 normoactive (9.87 ± 0.75 years) controls (CON). Pubertal status was determined according to the criteria of Tanner. Additionally the mother of each subject was recruited, and was included in all measurements as an estimate of hereditability. GYM were in elite pre-competitive or competitive programs for at least two years, and trained on average 18 hr per week. Areal bone mineral density (BMD) and body composition (%BF) was measured for the whole body, and BMD for the left hip and lumbar spine (L1-L4) by dual energy x-ray absorptiometry (DXA), and volumetric BMD for the distal radius by peripheral QCT (pQCT). There were no significant differences between groups for age, pubertal status or body mass; however GYM were significantly shorter (129.3 ± 5.7 vs. 136.7 ± 4.4 cm; $P<0.01$) and leaner (15.12 ± 1.95 vs. 19.58 ± 4.29 %BF) than CON. In an attempt to account for inter-group height differences, areal DXA measurements were converted to apparent bone mineral density (BMAD). GYM had significantly ($P<0.05$) greater femoral neck (0.698 ± 0.058 vs. 0.648 ± 0.064 $g.cm^{-2}$) and trochanteric (0.616 ± 0.060 vs. 0.530 ± 0.084 $g.cm^{-2}$) BMD. Additionally GYM had significantly greater whole body (0.101 ± 0.009 vs 0.094 ± 0.007 $g.cm^{-3}$) BMAD than CON. GYM also had significantly greater total 367.75 ± 51.61 vs 307.37 ± 27.59 $mg.cm^{-3}$), trabecular (207.93 ± 45.35 vs 163.76 ± 31.41 $mg.cm^{-3}$) and cortical (496.94 ± 67.51 vs 429.80 ± 33.78 $mg.cm^{-3}$) volumetric BMD than CON at the distal radius. There were no significant differences between mothers of GYM or CON for any of the

variables studied. Significant daughter mother correlations existed only for lumbar spine BMD ($r=0.55$). These results suggest that participation in elite gymnastics prior to puberty elicits favourable whole body adaptations in bone mineral density, and also that striking regional differences are observed at the wrist, a site subjected to repetitive high impact loading during training.

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# INTRODUCTION

During recent years there has been a growing interest in the effects of exercise and habitual physical activity on bone density during adolescence and early adulthood. Bone density has been shown to increase in juvenile and young adult individuals involved in high levels of habitual physical activity or in specific exercise training.

The purpose of this review is to identify the nature of adaptation of growing bone to exercise stress. Due to the relative lack of data concerning these adaptations in humans, studies using animals are also discussed. The first part of this review discusses the cellular mechanisms involved in bone metabolism and their control. Theories concerning the manner in which bone responds to exercise stress are then identified and discussed. Studies on growing animals are then examined prior to a review of the existing literature regarding skeletal adaptation in growing humans. The final part of this review examines the skeletal loads induced by gymnastic training.

# BONE METABOLISM

## Introduction

It is believed that up to 90% of the bone surfaces of a growing skeleton are undergoing some form of renewal or replacement (Teitelbaum, 1990). This ensures that the bone grows linearly, and that its macro- and micro-architecture is optimised to fulfil load bearing and muscle attachment functions, while maintaining a relatively low mass. In times of decreased serum or plasma calcium ion concentration, bone also provides calcium ions to maintain homeostasis (Rodan, 1992). In addition to linear growth, in which cartilage cells form bone at the growth plates, two forms of adaptive bone processes have been defined. Modelling is defined as a process in which the size and shape of bone is sculptured, increasing external bone diameter and cortical cross-sectional area. Modelling can only add to total bone mass and occurs only during growth. Remodelling is an ongoing process responsible for bone turnover and replacement in which small basic multicellular units (BMU) operate in response to an activation stimulus. Following activation a resorption phase occurs in which bone is removed, followed by a formation phase in which new bone is added (Frost, 1987). Remodelling of a bone packet by a BMU is thought to take approximately 124 days (Agerbæk et al., 1991).

Cells Responsible for Bone Metabolism

Three main cells are active in bone, namely osteoclasts, osteocytes and osteoblasts. A fourth type, lining cells, is recognised as a specific cell type by some authors, while others maintain that this layer of cells represents inactive, or resting, osteoblasts (Sledge and Rubin, 1989).

*Osteoclasts*

Osteoclasts are thought to be the bone cells responsible for bone resorption. Osteoclasts are large (200-2000$\mu m^2$), motile (up to 100$\mu$m per day), multinucleated cells found in resorption pits or hollows (Sledge and Rubin, 1989). These pits are termed Howship's lacunae in trabecular bone, and cutting zones in cortical tissue (Parfitt, 1993). These two forms of resorption cavities have been suggested to be distinct by Jaworski et al. (1972). Osteoclasts usually contain approximately 20 nuclei, which are surrounded by small amounts of rough endoplasmic reticulum and Golgi complexes (Baron, 1990). The cytoplasm is foamy, due to the presence of many vacuoles and vesicles containing lysosomal enzymes responsible for protein degradation (Sledge and Rubin, 1989). The cell membrane adjacent to the bone surface is characteristically folded, and termed either the brush, or more frequently the ruffled border (Rodan, 1992), increasing the effective surface area of the cell in close proximity to the resorption surface. Surrounding the ruffled border is an area termed the sealing zone, which is responsible for maintaining the enclosed microclimate around the resorption space (Vaess, 1988). Between the ruffled border and the cellular organelles is an area which appears empty, or clear, in cross-

section. This clear zone contains actin filaments thought to aid in motility and in securing the sealing zone to the bone surface (Baron, 1990).

Resorption of bone is essentially biphasic; the first phase involves degradation of the mineral portion, whereas the second involves removing the underlying matrix (Blair et al., 1993). Mineral is removed by acidification of the micro-environment (approximately pH4), this being achieved via the translocation of protons from the osteoclast into the resorption space. Protons are thought to be provided by carbonic anhydrase, which is abundant within osteoclasts, and which facilitates the production of carbonate ions and protons from carbon dioxide (produced by the mitochondria) and water. The protons are actively transported across the cell membrane into the resorption space by a proton pump (Blair et al., 1993). The acidity of the resorption space is responsible for breakdown of the mineral fraction. Bicarbonate ions in the cell are then actively exchanged for chloride ions in the plasma, preventing the alkalisation of the cytosol and preserving the internal acid/base balance in the cell (Blair et al. 1993). Suggestions have been made that lactate and citrate manufactured from glucose in the mitochondria may also participate in mineral degradation due to their calcium chelating properties; however, this effect would be slight, and physiologically non-significant (Vaes, 1988).

Organic matrix is catabolised by the lysosomal enzymes released by the osteoclast into the resorption space, the acidity of which facilitates lysosomal activity (Raisz, 1988). Particles of matter arising from the degradation process are either reabsorbed by the cell,

transported across the cytoplasm and ejected into the extracellular fluid, or escape from the resorption space during lapses in the function of the sealing zone (Baron, 1990).

Osteoclasts arise from multipotential hematopoetic stem cells found in the marrow and other hematopoetic tissue. These cells are termed colony forming unit stem cells (CFU-S), which differentiate to form granulocyte macrophage progenitor cells (GM-CFU). The GM-CFU may either develop into a premonocyte and eventually a tissue macrophage, or become a committed osteoclast progenitor (Vaes, 1988). The osteoclast progenitor then develops some of the organelle characteristics of a mature osteoclast (but does not possess the ruffled border) and resides in the periosteum and endosteum. This storage site is close to the likely site of action, and provides a secondary site for proliferation of osteoclast progenitors (Vaes, 1988). On activation, the progenitors fuse and develop into non-mitotic, mature osteoclasts. The life span of the osteoclast is estimated at seven weeks, during which time it is capable of resorbing 200,000$\mu m^2$ of bone (Sledge and Rubin, 1989).

*Osteoblasts*

Osteoblasts are thought to be the cells responsible for the production of new organic matrix, and its orderly mineralisation. Recently it has also become clear that osteoblasts are important regulators of all bone cell functions and the release of calcium ions into the plasma (Manolagas et al., 1993). Osteoblasts are mononuclear cells which are cuboidal (15-30um thick) when active (Sledge and Rubin, 1989). Organelle distribution is polarised, the rough endoplasmic reticulum being closest to the bone

surface, followed by the Golgi apparatus and the nucleus. Numerous secretory vesicles are also present within the cell (Puzas, 1990). Osteoblasts contain a characteristically high content of alkaline phosphatase, the exact function of which is unclear, but the presence of which is highly correlated with bone formation (Raisz, 1988). Osteoblasts appear in groups of 100-400 per formation site, each producing a 0.5-1.5$\mu$m of osteoid per day (Sledge and Rubin, 1989). Matrix is formed following protocollagen synthesis in the endoplasmic reticulum which transforms to tropocollagen extracellularly. Tropocollagen is laid down in sheets in which the fibres are of parallel orientation, the angle of orientation being altered as each sheet is produced. It has been proposed that a 60° shift is present, serving to increase the tensile strength of the bone tissue (Sledge and Rubin, 1989). This view is challenged by recently published findings (Marotti, 1993) suggesting that the appearance of the organic matrix is due to lamellae being alternately thick and thin, although the putative function of this form of lamellation is undetermined. Matrix remains unmineralised for approximately 10 days, leading to the appearance of an osteoid seam (Puzas, 1990); however the purpose of the time lag between production and mineralisation is not clear. While osteoblasts initiate mineralisation, and mineral crystals have been discovered in the cytosol (Anderson et al., 1986), it appears that the major role is to ensure mineralisation is inhibited and proceeds at a controllable rate. Inhibition is necessary because at the concentrations present around the bone surface, crystallisation could occur rapidly and spontaneously, which would be potentially damaging (Puzas, 1990).

Osteoblasts appear to arise from intra-skeletal mesenchymal cells, which can also become either fat, muscle or cartilage cells. Differentiation into A and A' cells follows, A' cells being committed osteoblast progenitors. Two stages of pre-osteoblast (G1 and G2) follow, G2 cells being mitotic and found in a stratum configuration layered upon active osteoblasts (Puzas, 1990). Osteoblasts appear to survive for approximately eight weeks before returning to the preosteoblast pool, becoming a bone lining cell, becoming an osteocyte, or dying (Menton et al., 1984). It has been demonstrated that osteoblasts appear to play a critical role in the modulation of stimuli responsible for the activity of osteoclasts and osteoclast precursors (Jilka, 1986).

*Osteocytes*

Osteocytes recently have been investigated with respect to their potential as modulators of the skeletal response to mechanical stimuli (Lanyon, 1993). It is believed that osteocytes are formed from active osteoblasts which become entrapped within the new matrix being deposited during bone formation. Osteocytes are found in cavities termed lacunae (Baron, 1990). Osteoblasts lose much of their intra-cellular organelle structure in becoming osteocytes, but retain the ability to communicate with other osteocytes and with bone cells on the surface via cell processes extending through tunnels termed canaliculi (Jee, 1991). It is estimated that lacunae and canaliculi together make up 7-8% of total bone volume, and represent a surface area of 250$m^2$ per litre of bone volume (Sledge and Rubin, 1989). Although Sledge and Rubin (1989) report that the canalicular network is limited in scope due to the existence of few interconnections

between adjacent osteons, this view is not supported by the electron microscopy study of Curtis et al. (1985) which demonstrated numerous such connections. It has been suggested that osteocytic osteolysis takes place, whereby osteocytes take part in the maintenance of plasma calcium concentration by actively resorbing bone in the lacunae and transporting the mineral to the blood via the canalicular system (Sledge and Rubin, 1989). Lanyon (1993) disputes this proposal suggesting no theoretical need for this response, and little evidence of its occurrence. Jee (1991) suggests that the osteocyte may serve as a storage for calcium after a calcium-rich meal, and may facilitate transport of calcium from deep bone to the plasma, in conjunction with cells lining the bone surface. It is also suggested (Lanyon, 1993) that osteocytes may fulfil a significant role in directing cutting zones towards areas of microdamage for renewal of damaged tissue. This is suggested to result either from a direct stimulatory signal from osteocytes in proximity to the damage, or by the failure of osteocytes killed by microdamage to inhibit resorption.

Various authors (Jee, 1991; Lanyon, 1993) have proposed that the osteocyte, or the osteocyte/lining cell complex (Kimmel, 1993) is responsible for detection of some form of mechanical stimulus following increased mechanical usage. There is little research to date examining the role of osteocytes *in vivo* with relation to mechanical loading, although *in vitro* studies have suggested a link between applied peak strain and cellular activity of osteocytes (Skerry et al., 1989). For example, immediately and six hours following loading of the isolated avian ulna there is an increase in the activity of enzymes responsible for RNA production (El Haj et al., 1990). This activity is closely

related to peak strain imposed in the vicinity of the osteocytes. Also, 12 hours following loading in the same avian model the number of osteocytes incorporating previously introduced labelled uridine increased markedly in areas of maximum subsequent bone formation (Pead et al., 1988).

## Control of Bone Metabolism: Systemic Factors

### *Parathyroid Hormone*

Perhaps the most cited function of PTH is that of regulating calcium ion concentration in the plasma. This seems to occur via indirect activation of the osteoclasts, increasing resorption and therefore calcium release from bone, and by stimulation of $1,25(OH)_2D$ release from the kidney.

PTH induced calcium release is associated with rapid changes in morphology and ultrastructure of osteoclasts (Jilka, 1986). The size of the ruffled border and clear zone increase within ten minutes of PTH administration, followed by an increase in overall cell size, and subsequently (12 hours following injection) an increased number of osteoclasts (Holtrop et al., 1979; Miller et al,. 1984). These results were achieved using rats which previously had undergone either removal of both parathyroid and thyroid glands (Holtrop et al., 1979), or an avian model in which the endocrine system is fundamentally different to that of humans (Miller et al., 1984). Similar alterations in osteoclast function were not demonstrated in the intact animal (Miller et al. 1984),

leading to the conclusion that parathyroid hormone may be important, but is not essential to osteoclast function in normal physiological concentrations.

In contrast to the above findings, it has become increasingly clear that PTH also possesses an anabolic effect on bone formation (Gunness and Hock, 1993). On the basis of cell cultures it has been demonstrated that while PTH causes inhibition of osteoblasts in terms of decreased collagen synthesis (Bringhurst and Potts, 1981), reductions in alkaline phosphatase activity (Wong et al., 1977) and osteocalcin synthesis (Beresford et al., 1984), it may have quite different, stimulatory effects on osteoblast precursors (MacDonald et al., 1986). More recent studies have also demonstrated the importance of the mode of administration of PTH on bone cell activity. Canalis et al. (1990), using continuous and cyclical PTH infusion reported that transient PTH treatment had a stimulatory effect on mature osteoblasts, which is in contrast to earlier studies which demonstrated effects only on preosteoblasts (MacDonald et al., 1986). Recently, further studies have shown that slightly elevated systemic PTH levels (as occur in mild secondary hyperparathyroidism) may have a protective effect against involutional loss in cancellous (Parisien et al., 1992) and cortical bone (Ejersted et al., 1992; Oxlund et al., 1992). Thus it seems that while high concentrations of PTH may result in bone loss, transient or slight increases may induce an increase, or at least a maintenance of bone mass.

Therefore, the role of PTH in the healthy human organism is still relatively unclear. It seems that although PTH can indeed increase resorption and increase plasma calcium concentration, this may only occur at high concentrations and represent an

"emergency" or pathological response. The overall role of PTH seems to be to modulate the rate of bone turnover, which initially leads to decreases in bone volume. This is followed by stimulation of bone forming cells which replace bone to an extent probably dependent on other systemic and local factors.

*Vitamin D*

Vitamin D is present in active, and inactive forms. Its active form, 1,25 dihydroxyvitamin $D_3$, is synthesised primarily by the kidney (Sowers, 1993), however ectopic sites suggesting paracrine regulation are now thought to exist in bone (Norman and Hurwitz, 1993). The major role of vitamin $D_3$ is to increase calcium ion concentration in the plasma by facilitating calcium ion transport across the intestine, and by stimulating bone resorption (Norman and Hurwitz, 1993). When calcium ion concentration is normal, vitamin $D_3$ concentration is low, and functions to maintain cell division and calcium transport (Raisz, 1993). When ionic concentration of calcium falls vitamin $D_3$ acts to stimulate bone resorption, inhibit matrix formation, and possibly mineralisation (Sowers, 1993). Vitamin $D_3$ acts on osteoblasts initially, in a similar fashion to PTH, leading to an increase in osteocalcin, which may in turn mediate aspects of osteolytic cell activity (Raisz, 1993). In contrast, Vitamin $D_3$ has also been shown to increase osteoblastic matrix production, and alkaline phosphatase activity (Puzas, 1990). The exact effects of vitamin $D_3$ on bone cell activity are incompletely understood at the present time. It is possible that the actions of Vitamin $D_3$ are similar to those of PTH and

are dependent on the concentration of many local factors produced by and surrounding the bone cells.

*Growth Hormone*

Other than its well known effects on linear growth, the exact nature of the effect of growth hormone (GH) on bone cell activity are not well established (Canalis, 1993). Individuals with GH deficiency possess decreased bone mass (Kaufmann et al. 1992), suggesting that GH is an important factor in the development of bone (Raisz, 1988). Bone cells have few receptors for GH, but it has been suggested that the role of GH may be to modulate bone turnover via a permissive action on other factors (Canalis, 1993).

Control of Bone Metabolism: Local Factors

Numerous locally produced factors and cytokines have been associated with the activity of bone cells, and the control of bone metabolism. Transforming growth factor beta (TGF$\beta$) is produced by bone cells in an inactive form, becoming active in response to changes in pH or increases in concentration of bone resorbing agents (Canalis, 1993). When injected close to the periosteum of young rats TGF$\beta$ resulted in increased bone turnover, and in a relative increase in bone formation, such that bone mass was increased during remodelling (Rodan 1992). It is suggested that TGF$\beta$ inhibits mature osteoclast activity, and acts to inhibit the formation and differentiation of osteoclast precursors (Mundy, 1993). TGF$\beta$ also exerts a powerful stimulatory effect on osteoblasts, increasing proliferation of precursors and activity of mature cells (Mundy, 1993). Due to the

autostimulatory ability of TGF$\beta$ and the fact that TGF$\beta$ is released from bone matrix during resorption, it is suggested that TGF$\beta$ may function as a paracrine or autocrine factor responsible for preventing excessive resorption and stimulating the initiation of formation (Canalis, 1993).

Fibroblast growth factor has also been associated with an increase in the activity of osteoblasts. Insulin-like growth factors (IGF I and IGF II) are formed both in the liver and bone cells, where they act to mediate the effect of systemic growth hormone (Raisz, 1993). IGFs increase the replication of osteoblastic precursors, and matrix production by mature osteoblasts (Canalis, 1993). IGFs are also stimulated by PTH and prostaglandins (Raisz, 1993). Due to their effects on proliferation and activity of osteoblasts, IGFs are believed to be crucial to the control of bone cell activity, and the maintenance of bone mass (Canalis, 1993).

Interleukin-1 (IL-1) is released by active monocytes, and acts to stimulate osteoclasts at all stages of growth and development. Lymphotoxin and tumour necrosis factor (TNF) are produced by activated T-lymphocytes and activated macrophages respectively, and are synergistic to the action of IL-1. It is believed that the effects of IL-1 and TNF on osteoclasts are mediated in some fashion by osteoblasts (Manolagas et al. (1993). A new cytokine, osteoclast poietic factor (OPF), responsible for simulation of differentiation and formation of osteoclasts *in vitro* has been identified (Mundy, 1993).

Prostaglandins of the E series, especially PGE2 are postulated to be involved in the mechanism by which bone responds to increased mechanical usage. Murray and Rushton (1990) and Chow and Chambers (1994) reported a biphasic effect of PGE2,

dependent on the strain placed on cultured cells and on rats *in vivo* utilising indomethacin (which inhibits the production of PGE2) administered at various intervals prior to and subsequent to loading. It has been proposed that PGE2 may function to initiate adaptive remodelling following relatively slight increases in mechanical loading, and may also act to initiate damage repair following loading of greater magnitude, stimulating bone formation (Raisz, 1993).

Therefore, it can be seen that at the cellular level, bone metabolism functions under the effects of various local factors, which make the study of bone adaptation extremely complex. Currently, the manner in which these various local and systemic factors interact to maintain homeostasis is unclear (Raisz, 1993). Perhaps the most interesting development is the finding that bone cells secrete autocrine or paracrine factors into the bone matrix during formation, leading to their release during resorption (Mundy 1993). It is currently felt that this may be crucial to ensuring the close coupling necessary between resorption and formation in the BMU during remodelling (Canalis, 1990).

## BONE ADAPTATION THEORIES

Perhaps the most influential bone adaptation theory since that of Wolff's Law appeared in a textbook published in 1964 (Frost, 1964), in which the term "minimum effective strain" appeared for the first time in relation to bone. Although Frost did not expand on this concept for several years, it eventually became a major influence on how bone mineral research progressed.

Frost (1979) proposed a chondral modelling theory as an explanation for the manner in which connective tissue and hyaline cartilage adapts to mechanical loading. Briefly, Frost (1979) stated that when such tissues are placed in compression, growth rate increases until a certain compressive force is attained, after which greater compression retards growth. Increasing tension of any magnitude was said to increase growth rate. Although not strictly a theory of bone adaptation, the theory included concepts similar to those to be advanced subsequently regarding bone and mechanical loading. Perhaps the most relevant is that of the time averaging property, which is to say that the tissue is "aware" of it's loading history and the average magnitude of loads placed on it on a daily basis.

As mentioned above, the concept of a minimum effective strain (MES) was proposed by Frost (1964) in general terms as the mechanical factor responsible for mediating adaptation of bone mass in response to mechanical stimuli. This idea was

further refined (Frost, 1979), and promoted later as a theoretical concept (Frost, 1983). Briefly this theory proposes that a set-point strain exists for skeletal adaptation, which based on animal research appears to be between 0.0008 and 0.002 unit strain. Strain is the longitudinal distortion of the tissue relative to its original length. This research has demonstrated a remarkable similarity between the typical peak strains encountered across species (Rubin, 1984). Also, during growth, forces placed on the bone increase up to 20-fold, but bone strains remain relatively constant (Indrekvam et al. 1991). In contrast to earlier statements (Frost, 1979) the MES was described in less certain terms as a threshold, but more as a range of values. Furthermore, it was proposed that strains above the MES almost always caused adaptation, whereas those below usually did not.

The MES concept was further developed (Frost, 1987[a,b]), and incorporated into what has become the most widely accepted schema for the effect of mechanical stimuli on bone mass. This theory introduced the term "mechanostat", suggesting that bone mass is controlled by a negative feedback loop, in much the same way as a thermostat controls a domestic heating system. Once strains become greater than the threshold MES the system responsible for adding skeletal mass is "switched on", until strains fall below the MES, when it is "switched off".

Frost (1987[a,b]) distinguished between the processes of skeletal modelling and remodelling. Modelling was proposed to involve large scale addition of bone, which is suggested to occur only prior to skeletal maturity. Remodelling is defined as a BMU-based process, which can only add small (<5%) magnitudes of bone. It was proposed that the processes of modelling and remodelling were activated in response to MES

values of different magnitudes (1500-2500uE and 100-300uE for modelling and remodelling, respectively). This distinction is important, as it suggested that only strains of very small magnitudes would be sufficient to maintain bone mass during adulthood. This in itself is not a large progression from the original MES proposal. However, the most novel inclusion was the attempt to predict how various types and magnitudes of loading would influence the three distinct processes of growth, modelling and remodelling. The theory did not, however, allow for bone loss associated with disuse.

Frost (1990[a,b]) re-examined the modelling/remodelling distinction, and proposed mathematical functions which controlled each process separately in lamellar bone. Modelling (m) (Frost, 1990[a]) was proposed to occur via a three-way rule or function in which adaptation was a function of three operators: an endload operator ($\alpha$), a strain operator ($\beta$), and an operator representing the rate at which modelling would occur (M).

i.e. $$f(m) = \alpha.\beta.M$$

Frost (1990[b]) described a four-way rule governing response of the remodelling system to mechanical loading. Remodelling (r) was proposed to be a function of a mechanical usage operator ($\epsilon$), an activation operator ($\mu$) and operators defining the quantity of bone resorbed ($T_r$) and formed ($T_f$) by each BMU.

i.e. $$f(r) = \epsilon.\mu(T_f - T_r)$$

The definitions of the various operators for modelling and remodelling are fairly (and necessarily) vague, but Frost (1990[a,b]) was the first to attempt to formulate a mathematically based, and testable hypothesis which incorporated these concepts.

Lanyon (1992) stated that while load-related adaptation is controlled by an-error-driven negative feedback system, the relative insensitivity of the adaptive response to the number of loading cycles applied to bone suggested that the error signal is not the time averaged property described by Frost (1979). Lanyon (1992), while supporting the view of a negative feedback system, reflected the uncertainty surrounding the error signal and proposed the far more general term "minimum effective strain related stimulus" as the signal for activation of the adaptive sequence, as opposed to Frost's minimum effective strain.

Frost (1992) expanded the theory to include a second, lower threshold strain. This formed a "mechanical usage window" in combination with the original MES. Strains within the higher and lower thresholds induce little or no adaptation, while those inducing average strains above or below the mechanical usage window induce formation or resorption, respectively. Thus the expansion of the theory allows bone atrophy associated with decreased usage and space travel to be incorporated into the model.

Frost's updated mechanostat theory is very similar to that proposed by Turner (1991). Turner (1991) predicted the actions of various stimuli on bone mass, which are discussed in terms of alterations of bone cell function. These are classified as agents responsible for alterations in cell function (e.g. calcitonin), which are suggested to cause transient alterations in bone mass only, and agents responsible for altering the threshold of the physiological window (e.g. estrogen, parathyroid hormone and growth hormone).

In contrast to the MES concept of Frost, Martin and Burr (1982) suggested that micro-damage was the prime error signal inducing skeletal adaptation. Martin and Burr (1982), however, also suggest that microdamage is associated with changes in the strain magnitude surrounding each damaged osteon. This could be viewed either as support for the MES concept of Frost (1983), or alternatively as a suggestion that more direct regulation of bone remodelling occurs via positive feedback.

Other authors have described very similar negative feedback theories of bone regulation to that of Frost (Carter, 1984; Rubin, 1984 and Turner, 1991). The theory proposed by Carter (1984) differed in that it was limited to cortical bone, and suggested that different mechanisms may govern bone atrophy and hypertrophy. Also, the experimental data collected by Carter and co-workers suggested that fatigue damage occurred in bone after relatively low numbers of load cycles which induced strains of physiological magnitude. Carter (1984) proposed an error signal composed not of time averaged strain, but of a complex function of strain ranges, mean strain magnitude, the number of strain cycles, and also other possible factors such as the maximum strain rate. The relationship between the error signal and adaptation differs compared to that of Frost. The mechanostat theory contends that a near linear relationship exists between applied strain and adaptive response, whereas that of Carter is overtly curvilinear.

Turner et al. (1992) introduced the concepts of homeostatic and epigenetic regulation of the bone mineralisation process. Homeostatic feedback systems rely on a negative feedback loop, incorporating some form of genetically determined error detection mechanism. Epigenetic regulation is not reliant on negative feedback loops or

error detection, but is characterised by positive feedback, in this case from signals arising from mechanical loading of bone. The response of lamellar bone is proposed to follow the accepted mechanism of homeostatic feedback control, i.e. that of the mechanostat theory. The response of woven bone is, however, proposed to be governed by a system under positive feedback control. This suggests that woven bone formation is either active maximally, or inactive, based on the size of the error system. Due to the fact that woven bone is produced quickly, it is suggested that the epigenetic regulation of woven bone serves to provide both a fast adaptation to the increased error system, and also provides a framework for lamellar bone formation. Turner (1992) proposed that an increase in the concentration of certain extracellular factors above a threshold value can cause osteoblasts to switch from lamellar to woven bone formation. When the concentration of these factors falls, typically the newly produced woven bone is replaced by lamellar tissue.

Certain difficulties exist in the bone adaptation theories proposed to date. The nature of the error signal, for example, has yet to be determined. The theories of Frost (1992), Carter (1984) and Turner (1991) all rely on a function including maximum strain. These have been defined as variant (requiring a co-ordinate system for description, e.g. peak longitudinal strain, which requires a directional descriptor) or invariant (requiring no co-ordinate system, e.g. strain energy density which does not require a description of the direction of action) (Rubin, 1990). It has been demonstrated, however, (Lanyon and Bourne, 1979; Rubin, 1984; Rubin and Lanyon, 1984) that the curved architecture of long bones serves to increase strains caused by bending moments during locomotion.

Lanyon (1987) suggests that this may be a result of the bone trying somehow to minimise the more potentially damaging shear strains. It can be seen, therefore, that a theory based on variant or invariant characteristics may be over-simplistic. Also the mechanostat theory and its subsequent adaptations assume that both cortical and trabecular bone types are governed by the same processes, and respond in the same manner. This assumption has recently been challenged (Lanyon, 1992).

Another problem associated with the negative feedback models of bone adaptation is that of the "knowledge" of what an acceptable strain function is which must be present either at a cellular level, or at some other more central site. The existence of a strain memory in bone tissue has been proposed which resides in proteoglycan orientation (Skerry et al. 1988), and which would provide a mechanism for time averaging of strain, and the recognition of a normal strain distribution. Little subsequent research has been published concerning this phenomenon. The problem is further compounded when one considers that the strain environment encountered by various bones and sites within a bone is very different. For example, bones of the skull would be expected to experience few strains of a large magnitude, but bone is maintained such that the skull can perform its vital protective role (Lanyon, 1987). Also, within a bone, strains around the neutral axis are minimal, while those at sites further removed are much greater in magnitude (Rubin, 1984). Rubin (1984), suggested that the optimal strain environment is predetermined genetically for each anatomical site. The requirement for local or regional genetic awareness of the optimal strain environment introduces tremendous complexity into the process governing bone mineral regulation.

Rubin et al. (1990), in contrast to Rubin (1984) and the homeostatic theories described above, propose an epigenetic regulation mechanism for both woven and lamellar bone based on the frequency distribution of strains encountered by bone cells. Rubin et al. (1990) propose that bone cells are epigenetically regulated by the relative amplitude of various bone strain frequencies, and seek to maintain a localised milieu by altering the surrounding matrix. Perhaps the most striking consequence of this proposal is that strain frequencies suggested to be critical to bone regulation are present during quiet standing in the horse (Rubin, 1990). This would lead to the controversial suggestion that dynamic loading is not essential for the maintenance of bone mass, a suggestion which much of Rubin's earlier work argues against. It is possible that the theory of epigenetic regulation of bone mass may become more popular with theorists in future, due to its intrinsic simplicity, as compared to the complex systems involved in genetically controlled negative feedback theories. To date, however, there is little specific experimental evidence supportive of epigenetic regulation of both lamellar and woven bone.

It is evident from the preceding review that both positive and negative feedback systems have been proposed as mechanisms which regulate bone adaptation in response to mechanical loading. It is unclear at present which is the most successful in terms of accounting for and explaining experimental data; however, positive feedback systems relying on epigenetic regulation deserve greater attention and development. It is also apparent that the complex nature of systemic and local factors mediating bone cell

activity serve to ensure that bone adaptation theories remain difficult to test experimentally.

## EFFECTS OF INCREASED MECHANICAL USAGE ON SKELETAL PARAMETERS IN GROWING ANIMALS

### Endurance Exercise

During the last quarter century, numerous studies have addressed the issue of the effects of endurance exercise on the skeleton by using animal models (e.g. Saville and Whyte, 1969; Kiiskinen, 1977; Matsuda et al., 1986 and Yeh et al., 1993). Despite this continued interest, the use of various modes, frequencies, intensities and durations of exercise, coupled with the use of differing species and levels of maturation have led to much controversy in the literature (Salem et al., 1993).

The most common exercise model involves rodents running on a flat or inclined treadmill. Typically, running speeds range from 180m.min$^{-1}$ (Kiiskinen, 1977) to 360m.min$^{-1}$ (Raab et al., 1990), with gradients of between zero (Salem et al., 1993) and 15% (Erich et al., 1985). Studies using this exercise modality have produced conflicting results in terms of morphological skeletal adaptations. Saville and Whyte (1969) and Steinberg et al. (1981) report similar findings of an increase in bone size as a result of exercise. Similarly Yeh et al. (1993) found a 13% increase in tibial mass due to running exercise. Salem et al. (1993) report no changes in morphology of either the femoral neck or the lumbar vertebrae. Li et al. (1991), however, reported a significant (3%) decrease in tibia length and circumference without a decrease in body mass. This suggests that the

relationship between body mass and skeletal hypertrophy suggested by Saville and Whyte (1969) can be perturbed under some circumstances. Raab et al. (1990) reported that while exercise increases femur mass in rats, a similar effect is not seen in the tibia. Thus it can be seen that the issue of the nature of skeletal adaptations to running exercise in young rodents has yet to be resolved.

Gordon et al. (1992) reported that swimming exercise decreases femoral mineralisation in male, but not female mice. These authors also reported a decrease in breaking moment in the femurs of exercised animals. In contrast, Swissa-Sivan et al. (1989, 1990) reported significant increases in BMD, cortical area and compressive breaking strength of rat femora in response to swimming exercise. Swissa-Sivan et al. (1989) also reported a positive effect of swimming on the growth of bone in very young rats. Gordon et al. (1989) reported significant increases in cortical area and breaking strength of mouse femora following both experimentally induced burrowing exercise or daily exposure to hypergravity.

Various authors have also attempted to utilise the running rodent model to examine the effects of exercise on the biomechanical properties of bone. Though Saville and Whyte (1969) reported no effects on the biomechanical properties of rat bone, other investigators have reported both positive (Grundnes and Riekerås, 1992; Kiiskinen, 1977 and Raab et al., 1990) and negative (Hou et al., 1990; Li et al., 1991 and Salem et al., 1993) effects of exercise on the physical properties of growing rodent bone. In almost every case, these studies also reported some aspects of physical properties which remain unchanged. Grundnes and Reikerås (1992) reported relatively large increases in

maximum bending moment and bending rigidity, without significant alterations in energy absorption. Raab et al. (1990) describe large increases in the force at the ultimate and yield points in rat femur, and a significant increase in force at yield in the tibia. These changes are of a greater magnitude than increases in bone mass reported by these authors. Kiiskinen (1977) reported a decrease in femur length and an increase in breaking load as a result of exercise. In contrast, Hou et al. (1990) reported large, but non-significant, decreases in load and energy absorbed at the proportional limit of the femoral neck. Large increases in the stress at the proportional limit and ultimate point occurred in these animals. No changes were observed in structural rigidity. Li et al. (1991) report that the load at the proportional limit and ultimate point decreased significantly by 10% in the tibia of exercised animals. Salem et al. (1993), however, report that though mechanical properties of exercised femurs are unchanged, compressive stress at the proportional limit of the lumbar vertebrae decreased significantly (14%) following exercise.

The effects of exercise on bone density have also been investigated utilising the rodent model. Most studies have reported no effect of running exercise on bone density in the growing rodent (Li et al., 1991; Raab et al., 1990, Salem et al., 1993 and Tuukanen et al., 1992). Steinberg et al, (1981) reported an increase in bone density, but this variable was not measured quantitatively, but rather was estimated by radiography.

Other animal models have been used less extensively to investigate the effect of exercise on the growing skeleton. Martin et al. (1981), for example, reported significant increases in tibial BMC in dogs subjected to running exercise while wearing weighted

jackets. Similarly, Biewener and Bertram (1994) reported significant (10%) increases in bone mass following weighted running in growing chicks. Bourin et al. (1992) reported a significant decrease in the bone mass of monkeys, as studied by biopsy of the iliac crest, following five months of climbing exercise. Puustjarvi et al. (1986) reported decreases in bone density following running exercise in dogs. Matsuda et al. (1986) studied running exercise in growing roosters, and found increases in cortical thickness. Bending stiffness, however, decreased markedly with exercise, as did tensile stress at the yield point. Woo et al., (1981) reported similar results in terms of increased cortical area in young swine following a one year exercise programme. Bone density did not change, though structural properties were improved significantly, suggesting that in this instance, the adaptation to exercise resulted in an increase in the quantity, rather than the quality of bone.

Exercise is usually described in terms of the intensity, duration, frequency and mode of activity. The importance of these descriptors of physical activity on bone adaptation in growing animals has yet to be fully described. Studies using fairly moderate intensity and duration have produced both negative (Bourrin et al., 1992; Hou et al., 1990; Matsuda et al., 1986 and Salem et al., 1993) and positive adaptations (Woo et al., 1981; Yeh et al., 1993) in bone mass, and in physical and structural properties. When intensity is relatively high, but duration and frequency remain moderate, results are also conflicting; Li et al. (1991) reported general decreases in bone structural properties, whereas Raab et al. (1990) found positive adaptations of structural properties with this regime. Grundness and Reikerås (1992), Steinberg et al. (1981) and Saville and Whyte

(1969) all reported positive skeletal effects of exercise of moderate intensity and relatively high volume, whereas Puustjarvi et al. (1991) and Gordon et al. (1992) reported decreases in bone density resulting from such exercise. In perhaps the only study to manipulate training volume (actually reported as intensity) Kiiskinen (1977) demonstrated decreases in femur length in animals undergoing all volumes of running exercise, and a significant decrease in femur mass in those exposed to very high training volume. Although this paper is frequently cited as a demonstration of the effects of exercise intensity on skeletal parameters, the independent variable manipulated was training volume, and criticisms such as large inter-group differences in initial body mass and a high rate of mortality in the high volume group make the results of this paper almost impossible to interpret.

Thus it can be seen that following 25 years of investigation the effect of exercise on the skeleton of immature animals remains unclear.

Artificial Loading

Perhaps the simplest method of artificially loading the skeleton in animal models involves ulnar osteotomy, in which the ulna is resected. The adaptation of the remaining radius is then studied in response to the increased load placed upon it during normal activity. Goodship et al. (1979) reported that ulna osteotomy in pigs initially decreased bone cross-sectional area (CSA) of the lower limb to 60% of that with the ulna present. Bone CSA returned to 90% of its original value after a period of three months. Recorded strains, measured by *in vivo* strain gauges, increased by 100% in the radius immediately

following osteotomy, but were comparable to control values after three months. In comparison, Lanyon et al. (1982) reported an increase in radial strains of only 8 to 20% following ulna osteotomy in sheep. Following six months, strains on the radius of the experimental leg were significantly lower (10 to 19%) than those on the contralateral radius, representing an over-adaptation. Lanyon et al. (1982) suggest that this may be due to a mechanism whereby the adaptation involves an effort to replace the bone area lost via osteotomy, representing an increase in functional characteristics. Little subsequent evidence, however, is available to support this hypothesis. Burr et al. (1989) demonstrated increased strains 3 months following osteotomy in dogs, which decreased in magnitude after 6 months. Prolific woven bone formation was noted in the ulna, leading to a significant increase in CSA, and cortical area.

Another frequently used loading technique involves the resection of the distal and proximal portions of the avian ulna. The remaining ends of the ulna shaft are capped, and pins are drilled through the caps which protrude through the skin on both sides of the wing. These pins can then be manipulated to load the ulna precisely under compression or tension. The bone is also functionally isolated when not being experimentally loaded, reducing artifacts resulting from loading during normal activity. This technique has been used to investigate the response of bone to various loading regimes and has demonstrated both increases in bone formation (Rubin and Lanyon, 1984) and in bone cell activity (Pead et al. 1988). Similar loading techniques have produced comparable results. Hert et al. (1972) reported 100% increases in tibial CSA in the rabbit following 30 days of loading applied via wires introduced into the bone

diaphysis. Bone activity in terms of Haversian system formation and reconstruction was also increased, suggesting that adaptation was not complete at this time. Churches et al. (1979) reported significant increases in CSA of sheep metacarpi loaded dynamically for 28 days via bone pins. Responses to this loading regime were, however, variable. A further study by Churches and Howlett (1988) confirmed these adaptations in response to this experimental model.

Due to criticisms (Turner 1994) of the invasive nature of the above techniques, and possible bone responses to the experimental equipment, a less invasive four point bending protocol has been developed for use with rodents. In this technique, rats are anaesthetised with ether, and the lower hind-leg is placed in an apparatus similar to that used for carrying out four point bending material tests. The tibia is loaded such that the medial surface is placed under tension and the lateral surface under compression. Results include an increase in the quantity of woven and lamellar bone (Turner et al., 1994), the rate of bone formation (as assessed by tetracycline labelling), mineral apposition rate and bone forming surface (Raab-Cullen et al., 1994[a and b]).

## Differential Effects of Loading Parameters

### *Volume of Loading*

Very few studies have addressed the issue of the importance of the volume of loading with respect to bone adaptation. Due to the diverse training regimes utilised in animal studies, volume will be considered in this review as a total for the whole experiment, rather than volume per training session which is associated with the

description of training regimes in humans. A wide variety of volumes (load reversals) have been used, being spread over various lengths of time. Hert et al. (1972) subjected rabbits to 135,000 load reversals over a period of 30 days, whereas Churches and Howlett (1980) utilised a model involving 80,640 cycles over 28 days. In contrast, some authors have used single bouts of 360 (Pead et al., 1988) or 300 load cycles (Pead and Lanyon, 1989). Rubin and Lanyon (1984) addressed the issue of the nature of the relationship between load volume and the degree of adaptation. Utilising the avian ulna preparation, mature roosters were subjected to a range of daily loading cycles between zero and 1800 per day over six weeks. Loads were of identical magnitude and applied at a constant rate. Bone mineral content (BMC) decreased markedly in the group in which no loading occurred. In the group subjected to four cycles per day BMC was essentially maintained. In the other groups, which encountered 36, 360 and 1800 load cycles, BMC increased by comparable amounts for 28 days, followed by a maintenance phase, suggesting that homeostasis had been achieved by this time. Raab-Cullen et al. (1994[b]) compared the responses of rat bone to four point bending induced daily, on alternate days, or for three days per week. Loading increased periosteal bone formation on the medial surface of the tibia and the fibula as a whole in all groups; there was no relationship between volume of loading and the magnitude of response. These two experiments demonstrate the early saturation of the bone adaptation response to increased volumes of loading. A very small volume of loading is apparently sufficient to maintain bone, and any greater volume leads to maximal adaptation (Lanyon, 1992).

*Load Magnitude*

Due to the variations in species used, animal maturity, loading technique and loading regime, it is difficult to compare loads utilised across studies. While load magnitude is sometimes expressed in terms of the strain induced on the experimental bone (Goodship et al., 1979), this is problematic, since it is proposed that the distribution of strains induced is perhaps more relevant in terms of the response induced (Lanyon, 1992). Various studies have sought to address the relationship between load magnitude and the degree of adaptation. Rubin and Lanyon (1985) reported a linear relationship between induced strain and the relative change in CSA of the turkey ulna, following 8 weeks of loading. Strains lower than 1000 microstrain were associated with bone loss, whereas strains above this level induced a strain related increase in CSA ($r=0.83$). Churches and Howlett (1988) artificially loaded sheep metacarpi via an arrangement of bone pins and springs. A significant relationship was reported between applied load and CSA ($r=0.75$), for loads generating a stress above a threshold estimated at $3.7N/mm^2$. Turner et al. (1994) reported a linear relationship between strain magnitude and lamellar bone formation above a threshold strain (1050 microstrain) in rat tibia subjected to 4 point bending.

*Rate of Load Application*

O'Connor and Lanyon (1982) investigated the relationship between loading rate and adaptation via artificial loading of sheep metacarpi. Identical load magnitudes were imposed at various rates. A significant correlation was obtained between maximum

loading rate and both CSA (r=0.91) and the increase in CSA (r=0.89). Also, Lanyon and Rubin (1984) reported that while static loading of the turkey ulna was associated with a similar degree of bone loss as immobilisation, the same load magnitudes applied dynamically were sufficient to induce a 24% increase in CSA after eight weeks. Therefore, evidence exists that the rate of application is a significant variable connected with functional adaptation. In contrast, Meade et al. (1984) reported a positive relationship between applied static load and bone formation in dogs. The results of Meade et al. (1984) may conflict with those of Lanyon and Rubin (1984) due to the fact that while the loads were applied via static springs, animals were able to load the bone dynamically during normal daily activity. This is not the case in the work of Lanyon and Rubin (1984) in which the loaded limb is functionally isolated.

It can be seen that experiments inducing artificial loads on bones in animals have the advantage of allowing experimental control over the loading regimen. It is also apparent that a complex interaction seems to exist between magnitude and rate of loading, which has yet to be addressed. It is also interesting to note that the bone adaptation theory proposed by Rubin (1990) in which the importance of strain frequency distribution is introduced has yet to be tested, and questions the relevance of the animal models (such as the avian ulna preparation) which are constructed such that muscular activity around the bone is minimal.

## FACTORS EFFECTING BONE DENSITY DURING CHILDHOOD

### Developmental Factors

Various authors have studied the change in bone density which occurs during the growth years. Mazess and Cameron (1971) demonstrated a linear increase of bone mineral content at the radius and humerus in children aged 6-14years. Glastre (1990), Ponder et al. (1990), Lloyd et al. (1992) and Southard (1991) reported significant correlations (r=0.87-0.90) between lumbar BMD and variables including body height, total and lean body mass, body mass index, body surface area, percentage body fat and developmental stage. The relationship between age and spinal BMD has been expressed as either a third (Glastre, 1990), or second order exponential (Ponder et al. 1990). The dissimilarity between these predictions is probably explained by the smaller age range of subjects in the study of Ponder et al. (1990), most of whom were yet to complete the growth spurt associated with puberty. Similar results are also presented by Gordon et al. (1991) utilising subjects of a wide variety of ages. Southard et al. (1991) report a linear relationship between lumbar BMD and age, a finding supported at both the spine and femoral neck by Kroger et al. (1992) and Bonjour et al. (1991). Bonjour et al. (1991) report differences in bone density accretion during growth between males and females which coincide with the difference in chronological age at onset of puberty. Miller et al. (1991) reported multiple regression models based on various anthropometric

characteristics which explained between 60 and 90% of the variance in BMD at various measurement sites. Greater prediction strength was associated with measurements of BMC compared to BMD, suggesting perhaps that normalisation of data for anthropometric variables may be more successful when based on BMC, rather than BMD. It seems, therefore that BMC and BMD measured by x-ray or photon absorptiometry relate closely to both age and development in children.

Recently, studies have attempted to address whether the increase in bone density associated with age is merely an artefact of currently used measurement techniques, which express density as a function of bone mineral per unit area of bone scanned. The measure is greatly influenced by the increase in the volume of bone which occurs during growth. DePreister et al. (1991), however, report that following adjustment for the variability associated with height and weight, age was not significantly related to BMC of the wrist. Kroger et al (1992) reported significant correlations between age and BMD at the spine, but not the hip, corrected for size on the basis of allometry. These results are supported by Gilsanz et al. (1988) which displayed no significant increase in BMD of the lumbar vertebrae when measured in terms of a true volumetric measurement by quantitative computerised tomography (QCT). Due to the relatively large patient radiation exposure accompanying QCT scanning, studies using this technique in children are limited in number. Therefore it can be seen that the manner in which true bone density changes during maturation is poorly understood, its measurement being to some extent confounded by the associated increases in body size.

Genetic Influences on Bone Density

Various investigations have attempted to address the importance of genetic influences on bone density. Few studies to date have used young children. Lutz (1986) demonstrated significant mother-daughter correlations for radial BMC ($r=0.4$) and BMD ($r=0.49$) in a study utilising young adult females and their postmenopausal mothers. Seeman (1989) reported that daughters of osteoporotic mothers displayed significantly lower BMD than daughters of women without osteoporosis. Slemenda et al. (1991) reported strong, significant correlations ($0.71 \leq r \leq 0.85$) between bone density at the hip, spine and radius in monozygotic twins. Significant correlations also existed for dizygotic twins, although the strength of the relationship was reduced. These findings are supported by similar results for the radius of adolescent and young adult twins reported by Smith et al. (1973). Matkovic et al. (1990) demonstrated significant correlations ($0.4 \leq r \leq 0.7$) between maternal, paternal and mean parental BMD and BMD of adolescent daughters ($N=31$). It has been demonstrated that by age 14, females had achieved 90% of their mother's height and BMD (Matkovic et al. 1990). In light of the relationship between parental height and that of children, and the significant effect of body size on most bone density measurement techniques, the potential problems inherent in isolating the genetic influences on bone density are apparent. Tylavsky et al. (1989) in a study of BMC and BMD at the wrist attempted to control for similarities in size between mothers and their college-aged daughters by correcting for anthropometric characteristics. Tylavsky et al. (1989) reported significant correlations between mothers and daughters in terms of corrected and non-corrected BMC and BMD. Controlling for anthropometric

characteristics in this manner, however, serves to heighten the influence of anthropometric characteristics. The method of Tylavsky et al. (1989) would be improved by utilising a regression model, or analysis of covariance, in order to estimate the variance in anthropometric variables explained by heredity, and then expressing the variance explained by familial resemblance relative to that explaining anthropometric characteristics. It does seem, however, that genetic inheritance may be a major determinant of bone density in humans.

Calcium Intake

Nutrition is an important requirement of normal growth, and the influence of calcium intake on bone mass has been investigated (Sowers et al., 1993). It has been suggested that low calcium intake during childhood may result in a decreased peak bone mass, and increased risk of osteoporosis (Matkovic, 1992). Calcium intake during childhood is currently a topic of widespread interest, due to recent findings that children are less efficient at absorbing calcium than adults, and the suggestion that the recommended daily intake of calcium may not be sufficient for maximum bone health (Peacock, 1991; Matkovic, 1991). Sentipal et al. (1991) reported that calcium intake as measured by four day dietary diary was significantly associated with vertebral BMD in 49 females aged 8-18 years. Similar results were reported by Chan et al. (1991), where a weak ($r=0.18$), but statistically significant relationship between BMD, adjusted for height and weight, and calcium intake existed in a group of 164 children. Matkovic et al. (1990) reported that while supplementation of calcium over two years increased

calcium retention, it did not increase bone density in adolescent females. Johnston et al. (1992) carried out a prospective study lasting two years, during which time 70 pairs of young monozygotic twins were either administered 1000mg/day of calcium citrate malate or a placebo. In prepubertal twins an increase in forearm BMC was evident following 6 months of supplementation. Following three years of supplementation BMD of the hip and spine were also significantly greater in the group receiving supplementation. These studies suggest, therefore, a strong link between the degree of calcium intake and both calcium retention and bone mass. The interaction between calcium intake and physical activity has also been addressed. In animals subjected to artificial loading, physical activity provided a significant protective effect during calcium deficiency (Lanyon, 1986). Kanders et al. (1988) reported an additive effect of calcium intake and physical activity on bone density in premenopausal females. The interaction between calcium intake and physical activity has not been addressed to date in children (Dalsky 1990).

Habitual Physical Activity

The significance of habitual physical activity with regard to bone density has been investigated in the adult population, with increased levels of activity generally being associated with increases in bone density. Some authors have also addressed this question in the paediatric population. Slemenda et al. (1991) and Katzman (1992) both reported beneficial effects of increased levels of habitual activity on bone density at the hip, but not the spine. Slemenda et al. (1991) studied a sample of 118 children from 5-14 years of age. Physical activity was assessed by questionnaire completed by both child and

mother, and expressed as total time spent per week in weight-bearing activities. Significant correlations were obtained between hours of weight bearing activity and bone density at the hip ($0.37 \leq r \leq 0.41$) and the radius ($r=0.40$). No relationship was observed between spinal BMD and activity. Since both BMD and physical activity increase with age, the authors attempted to investigate the effects of activity alone by use of a linear regression model controlling for the effects of age on BMD. Although the relationship between physical activity and BMD was weakened, it was still statistically significant. The authors predicted that increasing activity one standard deviation above the mean level may result in a 5-10% increase in bone mass.

Turner et al. (1992) classified the physical activity of 138 adolescent females in terms of a 15 point scale on the basis of a questionnaire. The relationship between scores on this index and BMD at the hip and spine were examined. On the basis of regression equations including height, body mass, calcium intake and physical activity, a significant degree of variance in BMD at the femoral neck (4%) and trochanter (5%) was accounted for by physical activity. These results conflict, however, with an earlier study by Katzman et al. (1991) of the effects of habitual activity and other developmental factors on BMD in children. Activity was classified as either high, moderate or low on the basis of a questionnaire. There was no significant difference in BMD between physical activity groups. It is not clear why activity was expressed as a discrete variable in this study, rather than a continuous variable in terms of hours spent engaging in physical activity per week. The frequency distribution of the groups was negatively skewed, with over 50% of the sample classified as high activity individuals. Kroger et al. (1993) reported similar

results using a similar methodology. It seems reasonable to assume that the studies of Katzman et al. (1991) and Kroger et al. (1993) did not express physical activity in a manner sensitive enough to measure the relatively small effects of activity on BMD reported by Slemenda et al. (1991) and Turner et al. (1992). Rice et al. (1992) similarly reported no significant relationship between whole body or spinal BMD and either cardiorespiratory fitness or habitual physical activity.

There have been no studies to date which have investigated the relationship between muscle strength and BMD in young children. In adults, this relationship is equivocal. Snow-Harter and Shelley (1992) reported that 50-60% of the variance in BMD at the hip and spine of adult females could be accounted for by strength of the surrounding musculature. These findings are supported for isokinetic, isometric and isotonic strength measures in young females by Eickhoff et al. (1993). In contrast Peterson et al. (1991) reported that a year long exercise regime in adult women induced increases in muscle strength without increasing BMD. A relationship ($0.4 \leq r \leq 0.52$) between both radial BMC and ulnar bending stiffness and strength of the grip and biceps has been reported to exist (Myburgh et al., 1993). In contrast Sinaki et al. (1974) report no relationship between grip/biceps strength and radial BMC in young men. In a longitudinal study involving postmenopausal women Beverly et al. (1989) reported a significant relationship between grip strength prior to, and following six months of training in which grip strength increased significantly. Therefore it can be seen that the relationship between muscle strength and BMD is at best poorly understood in adults, and remains to be described in children.

Exercise

*Cross-Sectional Studies*

Cross-sectional studies in adults have addressed the issue of participation in regular sport or physical activity and its effects on BMD (e.g. Bailey, 1990). Recently this interest has broadened to include younger individuals (Bailey and Martin, 1994). Faulkener et al. (1993) reported increased BMC and BMD in the dominant arm of children, as compared to the contra-lateral limb. These effects were noted for BMC in children greater than nine years old, BMD of the dominant limb being increased in both genders by age thirteen. Similar effects of arm dominance have been reported in young adults (Calder et al. 1992). Bailey and Martin (1995) reported a study by Watson (1974) which investigated the effects of baseball pitching in little league players; BMC of the humerus was greater in the pitching arm of young baseball players than in the non-pitching arm. It is not clear whether the effects of baseball activity are additive with respect to the effect of arm dominance reported in normal children not involved in regular baseball training (Faulkener et al. 1993).

The effects of increases in specific physical activities have also been investigated in children. When sports or activities which induce relatively low magnitudes of skeletal loading are considered, results are non-conclusive. Rico et al. (1993) reported lower bone mineral content in the legs of adolescent males involved in competitive cycling compared to control subjects of similar age. McCulloch et al. (1992) compared groups of adolescents involved in competitive soccer or swimming, and a group of controls. A trend existed for increased BMD of the os calcis, with soccer players having the highest

average bone density, and swimmers the lowest. These differences seem to be site specific as no trends were present at the distal radius. Slemenda and Johnston (1993) compared the bone density of elite figure skaters aged 10-23 years to control subjects. While 40% of the skaters reported menstrual irregularities (which reportedly induce negative effects on bone), regional increases existed in BMD of the skaters at the trunk, legs and pelvis. Whole body BMD was also greater in the figure skaters. Grimston et al. (1993) compared seventeen adolescents involved in competitive sports which purportedly resulted in large impact forces on the skeleton, to an equal number of size and gender-matched controls. The group participating in impact inducing sports had significantly higher hip, but not spine BMD. When gender effects were examined there were no inter-group differences for females at any site, whereas males had significantly greater BMD at the spine, but not the hip. The large degree of within-group variability makes interpretation of these data difficult. It is unclear why BMD was significantly increased at the hip for the whole group, but absent when the groups were separated by gender. Also, no differences existed for the spine when the data were collapsed across gender, but differences existed between groups for males, but not females when gender was examined. A previously reported study by the same authors (Grimston and Hanley, 1992) reported significant differences between competitive gymnasts and swimmers at both the spine and hip. The addition of other subjects from sports which perhaps place less impact on the skeleton than gymnastics, e.g. running, dancing and tumbling, may have diluted the high impact effect of gymnastics in the earlier study. The studies of Grimston et al.

(1993) and Grimston and Hanley (1992) are limited by the absence of a control group containing normoactive children.

In a similar study, 25 prepubescent gymnasts were compared to 21 swimmers and 10 control subjects (Cassell, 1993). Gymnasts were reported to have greater bone density for the whole body after several data adjustments for weight, lean body mass and peak torque production. The nature of these adjustments is questionable since their precise importance in determining bone density in this population remains to be determined. Robinson et al. (1993) reported higher BMD at the hip for collegiate gymnasts compared to runners and controls, and higher spinal BMD in the gymnasts compared to runners, but not to controls. Nichols et al. (1994) also report significant increases in the BMD of college aged gymnasts in comparison with controls at both the lumbar spine and femoral neck when the covariance of body mass was controlled.

*Longitudinal Studies*

Few data are published regarding the response of the immature skeletal system to increases in activity. Margulies et al. (1986) studied 268 male army recruits aged 18-21 years prior to and following 14 weeks of physically intense basic training. In subjects who successfully completed the training, bone density increased significantly in the right (5.2%) and the left (11.1%) legs. There was no skeletal hypertrophy as measured by radiographs of the tibia, suggesting an increase in the quality, but not the quantity of bone. The loading regime in the study of Margulies et al. (1986) may not have induced optimal skeletal adaptation, as stress fractures occurred in 60% of the subjects. These

subjects were unable to complete the training course. Snow-Harter et al. (1992) studied the effects of eight months of either running or resistance training on females of college age in comparison with control subjects. Both training groups displayed significant increases in BMD of the lumbar spine (of the magnitude of approximately 1.5%) but not at the hip. There was no difference between type of training, and BMD of control subjects did not change significantly. Blimkie et al. (1993) reported that resistance training in adolescent girls increased bone mineral density transiently after 4 months, but that the effect was not present at the completion of the exercise regime after several (4) more months of training. Nichols et al. (1994) reported significant increases in lumbar BMD for college aged gymnasts during the course of 27 weeks of gymnastic training during the college season. There were no significant changes in BMD at the hip.

## FORCES ON THE SKELETON DURING GYMNASTICS

Certain movements in gymnastics are characterised by large peak ground reaction (impact) forces in the vertical plane. McNitt-Gray (1991) simulated landing from gymnastic apparatus of various heights, and reported ranges of impact ground reaction forces between 3.5 and 11 times body weight. Hall (1986) reported maximum impact forces of up to ten times body weight for five regularly used gymnastic movements. Similarly, Panzer et al. (1988) and Miller and Nissenen (1987) both reported ground reaction forces of approximately 14 times body weight during completion of the salto and the running forward somersault, respectively. In contrast, Kinolik et al. (1980) reported ground reaction forces of 3.3 times body weight on take-off for an aerial somersault in female gymnasts. The technique used in this study utilised a one-footed take-off, whereas those in the studies of Panzer et al. (1988) and Miller and Nissenen (1987) involved take-off from both feet. Similarly, Payne and Barker (1976) reported ground reaction forces of between 2 and 3 times body weight during take-off for completion of both back somersaults and flic-flacs in male gymnasts.

Koh et al. (1992) reported impact forces on the hands to be approximately 2.5 times body weight during performance of a back handspring. Hay et al. (1979) and Smith (1981) described forces on the hands of female gymnasts completing movements on the uneven bars to be between 3 and 5 times body weight. During contact with the horse

during vaulting, forces are reportedly of the order of 1.5 times body weight in elite male gymnasts (Takei, 1991). It is worthy of note that these forces are quite considerable, given the usually non-weightbearing nature of the hand and wrist.

The above studies are limited in that ground reaction forces are unable to quantify the forces acting on bones at the joint arising not only from impact, but also from the muscular contraction involved in impact absorption and postural maintenance. In order to estimate these forces, O'Connor (1992) completed a simultaneous force plate, electromyographic (EMG) and kinematic analysis of landing from the back salto. An anatomical model of the knee joint was utilised to estimate moments generated at the knee on the basis of relative amplitude of EMG activity. While vertical ground reaction force was approximately 9 times body weight, estimated tibio-femoral contact forces were approximately 25 times body weight.

The ground reaction forces and those appealed on the hands in the above studies are applied at a relatively fast rate. For example, peak loading in the study of Miller and Nissenen (1987) occurred within 15msec of contact. In other studies, time to peak force is greater in magnitude, being between 200 and 300msec (Kinolik et al. 1980; Takei, 1991).

Therefore, it appears that relatively simple gymnastic movements induce large ground reaction and bone on bone joint forces, which, due to the nature of impact are applied at a high rate. Animal research cited previously is suggestive of maximal skeletal adaptation occurring following loading of high magnitude applied at a rapid rate.

Evidence exists therefore, to support gymnastic training as a suitable exercise model for the study of skeletal adaptation in response to high impact mechanical loading.

## SUMMARY

To summarise, while available literature seems generally supportive of a positive effect of exercise on bone density, few data exist relating to the specific effects of different types and magnitudes of mechanical loading on bone density, especially in children. Studies in adults support the hypothesis that activities which induce high load magnitudes on the skeleton, such as gymnastics, are capable of inducing favourable increases in bone density. These positive effects have also been demonstrated in younger, adolescent individuals engaged in activities associated with skeletal loading of high magnitude. To date no published data exists concerning the effect of such loading on bone density during prepubescence. Also, studies to date have largely ignored the potential for self-selection into such activities by individuals possessing greater genetically determined bone density.

## PURPOSE

This study investigated the effects of high magnitude impact loading resulting from elite standard gymnastic training on bone density in prepubescent females. The effects of diet, habitual physical activity, muscle strength and maternal influence on bone density during prepubescence were also addressed in this investigation.

## HYPOTHESIS

It was hypothesised that gymnasts subjected to high magnitude impact loading during training would have significantly greater bone density than age-matched,-normo-active controls.

# METHODOLOGY

## Subjects

A total of 32 prepubescent females, aged 8.76-11.03 years were recruited and placed into one of two groups on the basis of gymnastic training history. In addition, the biological mother of each subject was recruited and completed the same measurements as the child.

## Recruitment

### *Gymnasts*

Gymnasts were recruited from local clubs via visits and presentations by the research team, following liaison with club executive members and coaching staff. Information letters which included a detailed description of the purpose of the study, the measurements to be made, the likely time commitment and potential risks involved in participation (see Appendix 1), were distributed to children by the club personnel. A response slip was enclosed with the letter, which parents and children could complete and return to the club if they were interested in participating in the study. Interested parents were then contacted by telephone to discuss the project, and to co-ordinate testing appointments.

*Control subjects*

Control subjects were contacted via notices published in the university press, and in local newspapers. Information posters were also placed throughout the university campus. Published information and posters contained contact details for project personnel, and interested parents were requested to telephone the project co-ordinator to discuss the project. In addition to telephone discussions, information letters were also dispatched by mail. Parents were then recontacted to confirm participation and arrange appointments.

## Ethical Approval

Study details were approved by the McMaster Unversity Medical Ethics Committee prior to initiation of the project.

## Informed Consent

Written, informed consent was obtained for each mother-daughter pair on arrival at the university for the initial visit. The written consent was obtained separately for mothers and their daughters (Appendices 2 and 3).

## Inclusion Criteria

*Daughters*

Subjects categorised as gymnasts were involved in either competitive or pre-competitive gymnastic training for at least two years. This was defined as participation

in a regular gymnastic program at a recognised club for a minimum of 15 hours per week. Control subjects were included if they were not regularly competitive in a specific sport or if they participated in regular recreational sports at a sub-elite or non-competitive level only.

*Mothers*

To be included written informed consent was obtained prior to data collection. Subjects had to be premenopausal and the biological mother of a child included in the study. Gynaecological status was based on completion of a medical questionnaire (Appendix 4).

Exclusion Criteria

In addition to non-compliance with any of the inclusion criteria, subjects were excluded from the investigation if they presented with metabolic disorders known to effect bone mineral or participation in physical activity, this information being achieved on the basis of a medical questionnaire (Appendices 4 and 5). Pubertal subjects were also excluded from the study on the basis of self-assessed sexual maturity stages. Maturity status was assessed by both mother and daughter together following a verbal explanation of the procedure and its purpose. The procedure was based on photographic representations (Appendix 6) of the five stages of breast and pubic hair development identified by Tanner (1962). Mothers who were pregnant or lactating were also excluded from participation.

## Independent Variables

### Habitual Physical Activity

#### *Children*

Level and type of habitual physical activity was assessed by questionnaire (Appendices 4 and 5). The method used was adapted from that developed by Bailey et al. (personal communication). Questions related to the frequency and duration of specific activities during the preceding 2 week period. These responses were then totalled to give the number of hours spent in all types of physical activity, and also the number of hours spent in body weight-supported activity. Questionnaires were administered by interview with the investigator, with assistance from the mother. Questions also related to subject's perception of both their physical fitness and physical activity level relative to their peers. In addition, gymnasts were asked questions relating to their gymnastic training history, and their current training schedule.

#### *Mothers*

Questionnaire items relating to current levels of activity were identical to those administered to children. In addition questions relating to life-time physical activity were also completed (Appendix 4). These included items regarding physical activity during school, following school and in the recent past. On the basis of these items a subjective grade on a five point scale was awarded to represent lifetime physical activity. On this scale, a value of five was awarded for lifetime regular activity, four for subjects who

were active for most, but not all of their lifetime, three to subjects who reported some activity for most of their life, two to subjects reporting some activity for some of their life, and one to subjects reporting little lifetime activity.

Medical History

Children and adults alike completed questionnaire items relating to previous and current medical status, previous and current medications, and lifetime incidence of skeletal fracture (Appendices 4 and 5). Mothers also completed items concerned with gynaecological status, incidence of pregnancy and child-birth, and use of the contraceptive pill. Questions administered to the mother included items concerning prior and current smoking habits and alcohol and caffeine consumption.

Lifestyle

Items administered to children addressed involvement in clubs or societies, hobbies and pastimes, and time spent watching television. Mothers were asked identical questions, but in addition were asked questions concerning employment (Appendix 4)

Dietary Intake

A three-day dietary diary was completed by both mother and child (Appendix 7) for two weekdays and one weekend day. Full written instructions were attached, and in addition, the completion of the diary was explained verbally to all subjects. Children were encouraged to assist as much as possible with completion of the diary. Subjects

were requested to complete the diary in as much detail as possible, and to estimate the quantity of each food type consumed as accurately as possible. Subjects were also instructed to maintain normal food consumption during the period of diary completion. Completed diaries were analysed using the Nutrient Analysis Program (Elizabeth Warwick, PEI, Canada, 1991).

Anthropometric Variables

Body height was measured to the nearest 0.1cm for all subjects using a free-standing Harpenden stadiometer. Height was recorded without footwear, and subjects were instructed to stand erect and inhale during measurement.

Body mass was recorded on Harpenden scales to the nearest 0.1kg while subjects wore light clothing, without shoes.

Body composition was measured by dual energy x-ray absorptiometry during bone density measurement. This technique has been shown to be highly correlated ($r=0.86$) with hydrodensitometry (Johansson et al. 1993), and demonstrates good ($CV=1.8$) *in vivo* precision (Chilibeck et al., 1994)

Strength Measurement

Grip strength was assessed for all subjects using a hand held dynamometer. Following an explanation relating to the method of adjusting the width of the dynamometer grip, subjects were advised to adjust the grip width such that the device was held between the distal phalangeal joints and the proximal thumb joint. Subjects were

given the dynamometer for a period of approximately ten minutes for familiarisation purposes, followed by a period of rest during which a bone scan was obtained. Grip strength was then recorded for each hand alternately a total of three times per hand. A period of thirty seconds rest was imposed between each measurement, with one minute therefore elapsing between consecutive measures with the same hand. The greatest grip strength obtained for each hand was used to derive average grip strength.

Bone Density

This was assessed at three sites by dual energy x-ray absorptiometry (DXA), and at a single site by peripheral quantitative computerised tomography (pQCT).

*DXA*

Bone density of the whole body, left hip and lumbar spine was obtained for each subject (daughters and mothers) by DXA (Hologic 1000W, Waltham, Mass.). The Hologic 1000W utilises an x-ray tube as the radiation source, which pulses alternately between 70 and 140 kV. Entrance radiation dosage to the patient ranges between 2-5mRem, and is therefore approximately one tenth that of a standard chest x-ray (manufacturers specifications). Total patient radiation dose for the DXA measures was, therefore, less than 10mRem. *In vivo* precision (i.e. measurement reliability expressed as a coefficient of variation) of DXA has been reported as 1-2%, and *in vitro* accuracy (where accuracy is measured by comparison of DXA density values with those obtained directly from measurements of mass and volume) as 3-5% (Alhava, 1991).

Subjects removed any metal items, and wore light clothing during testing. Following explanation of the procedures and familiarisation with the equipment, scans recording bone mineral content (BMC) and areal bone mineral density (BMD) were completed for the whole body, left hip and first four lumbar vertebrae. Areal BMD is expressed as bone mineral content relative to the projected area of the bone in the region of interest (i.e. $g.cm^{-2}$)

Hip scans were carried out with the left leg inwardly rotated using the positioning aid provided. Hip areal bone density was measured at the femoral neck, at Ward's triangle, and for the trochanteric region. The various regions were defined according to the manufacturers instructions. *In vivo* precision of hip measurement in adults is between 0.9 and 1.0% (Kroger et al., 1992). Lumbar spine areal bone mineral density was measured for vertebrae L1-L4, results being expressed as the average of the L1-L4 region. To minimise natural lordosis lumbar scans were completed with the subject positioned on the back with the legs supported on a box such that the knees and hips were in approximately 45° of flexion. Subjects were scanned alternately, such that children were scanned first for the whole body, followed by whole body scanning for the mother. This allowed completion of the medical, activity and lifestyle questionnaires, and grip-strength assessment during scanning of the other member of the mother-daughter pair. Data were collected by two experienced technicians, were stored on optical disk, and analysed by a single technician.

To attempt to control for the influence of bone size on areal BMD, bone mineral apparent density (BMAD) was calculated for the whole body, femoral neck and L2-L4

region using the equations of Katzman et al. (1991). These formulae are based on the allometric relationship between actual bone volume and projected bone area, BMAD being expressed as $g.cm^{-3}$. Femoral neck and lumbar spine BMAD are a function of bone mineral content and the projected bone area raised to an exponent. Whole body BMAD is a function of bone mineral content, projected bone area, and subject height.

*pQCT*

Bone density of the left distal radius was assessed by pQCT (Stratec XCT 960, Norland Corporation, WI). This device utilises a single energy x-ray beam of 45kV via an x-ray tube. Patient radiation dosage is 6mRem per measurement (manufacturers specifications). *In vivo* precision for adults is reportedly $\pm 5$, $\pm 3$, and $\pm 9 mg.cm^{-3}$ for total, trabecular and cortical density measures respectively (manufacturers specifications). *In vitro* accuracy is reportedly 2%. Precision of this technique in children aged $10.5(\pm 1.7)$ years was assessed in a pilot study (Appendix 8). Results of this study suggested an *in vivo* reproducibility of 7.9%, 5.8, and 14.7% for total, trabecular and cortical density in children. Corresponding values for adults were 3.8%, 2.1%, and 6.0% for total, trabecular and cortical density. The precision of area measurements was also investigated, the measurements being slightly less reliable than those of density (Appendix 8).

Forearm length was measured from the olecron process to the most distal edge of the ulna styloid with the shoulder and elbow flexed in the saggital to 90° and the forearm pronated. The length measurement was obtained with a thirty-centimetre ruler

and expressed to the nearest millimetre. The pQCT scan was positioned at 4% and 6% of forearm length, measured proximally from the distal end of the radius, for mothers and daughters respectively, the higher value being adopted for the children to ensure standardisation proximal to the growth plate. Positioning was made with the assistance of the manufacturer's software, on the basis of a two-dimensional "scout" scan, with the distal end of the radius being defined as the touching point of the articular surfaces of the radius and ulna. The scout scan and CT scan were obtained with the shoulder abducted and elbow flexed at 90°, and with the radio-ulna joint pronated. Results were obtained in terms of total bone density and cross-sectional area for the whole radius, and for cortical and trabecular tissue compartments separately. Definitions of bone surfaces and tissue compartments were obtained via iterative algorithms contained within the computer software. Data were collected by two experienced technicians and were stored on computer diskette, prior to analysis.

Data Analysis

Data were transfered to spreadsheet (Excel version 5.0) and analysed statistically using SPSS version 5.0 software for the personal computer. Independent t-tests for samples with separate or pooled variance were carried where appropriate to examine inter-group differences between gymnasts and controls and their mothers for all variables. Pearson product moment correlations were computed between mother-daughter pairs for the gymnasts and controls separately, and for both groups combined for selected bone density variables. Statistical significance was set at the $p < 0.05$ level.

# RESULTS

## Physical Characteristics

The physical characteristics of the gymnastic and control groups are shown in Table 1. No significant group differences existed for age, body mass or developmental stage. Gymnasts were significantly shorter and had less body fat than controls. Physical characteristics of the mothers included in the study are summarised in Table 2. All mothers reported having regular menses. Eleven gymnast's mothers and nine of the control's mothers reported previous use of oral contraceptives. No mothers had taken oral contraceptives within five years of data collection. Three gymnast's mothers and one control mother reported a family history of bone disease; three gymnast's mothers and five control's mothers reported incidence of skeletal fracture. No significant differences were reported for incidence of skeletal fracture. No significant differences were present in measured characteristics between mothers of gymnasts or control subjects.

## Grip Strength

No significant strength differences were present between gymnasts and controls (Table 1), or between mothers of gymnasts and mothers of controls (Table 2).

Bone Density

*Areal Bone Density*

As can be seen in Figure 1, areal bone density of the gymnasts was significantly greater at the femoral neck (8%) and trochanteric region (16%) in comparison to control subjects. No significant differences in areal bone density were present at the other sites of measurement. As shown in Figure 2, no significant differences in areal bone density were present between gymnast and control mothers.

*Apparent Bone Density*

Gymnasts displayed significantly greater apparent bone density for the whole body (7%), femoral neck (19%) and lumbar spine (8%) (Figure 3). There were no significant group differences in apparent bone density of the mothers (Figure 4).

*Radial Bone Density*

Total bone density at the distal radius was significantly greater (20%) in gymnasts (Figure 5) compared to controls. The density of cortical and trabecular tissue types was also greater in the gymnasts at the distal radius (27% and 15% respectively) (Figure 5). There were no significant group differences for distal radial total, trabecular or cortical; bone density between the mothers of gymnasts and control girls (Figure 6).

Bone Cross-Sectional Area

As can be seen from Figure 7, no significant differences existed between gymnasts and controls for absolute total or trabecular cross-sectional area of the distal radius. Absolute cortical cross-sectional area was, however, significantly higher (15%) in the gymnasts. There were no significant differences in absolute cross-sectional area of the distal radius between the mothers of the gymnasts and the controls (Figure 8). As shown in Figures 9 and 10 respectively, when absolute cortical and trabecular areas were normalised for total distal radial cross-sectional area, there were no differences between gymnasts and control subjects, or between the mothers of gymnasts and control subjects

Table 1. Subject Characteristics - Children

| Group | Number | Age (Years) | Height (cm) | Weight (kg) | Tanner Stage (Breast) | Tanner Stage (Pubic Hair) | Body Fat (%) | Mean Grip Strength (N) |
|---|---|---|---|---|---|---|---|---|
| Gymnast | 16 | 9.82 (±0.89) | 129.3 (±5.7) | 26.6 (±3.6) | 1.1 (±0.2) | 1 (±0) | 15.1 (±2.0) | 15.8 (±2.8) |
| Control | 16 | 9.87 (±0.75) | 136.7* (±4.4) | 28.4 (±4.0) | 1.2 (±0.5) | 1.1 (±0.4) | 19.6* (±4.3) | 17.4 (±3.3) |

*$p<0.05$

Table 2. Subject Characteristics - Mothers

| Group | Number | Age (Years) | Height (cm) | Weight (kg) | Body Fat (%) | Mean Grip Strength (N) |
|---|---|---|---|---|---|---|
| Gymnast | 16 | 38.06 (±2.38) | 159.56 (±5.26) | 60.49 (±10.54) | 26.72 (±5.29) | 33.4 (±6.1) |
| Control | 16 | 38.75 (±5.75) | 162.26 (±4.33) | 61.34 (±7.65) | 26.97 (±4.87) | 36.2 (±6.1) |

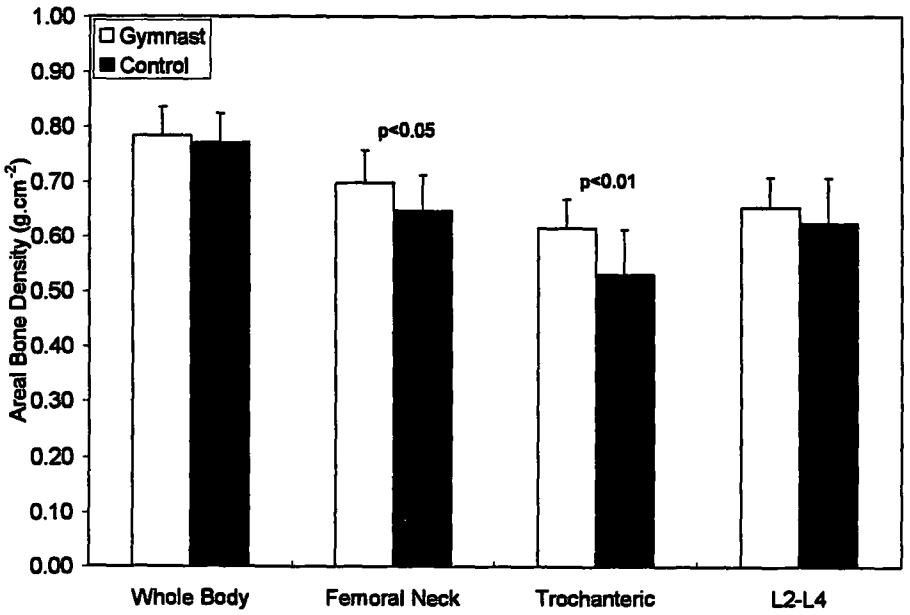


Figure 1. Effect of Gymnastic Training on Areal Bone Density.

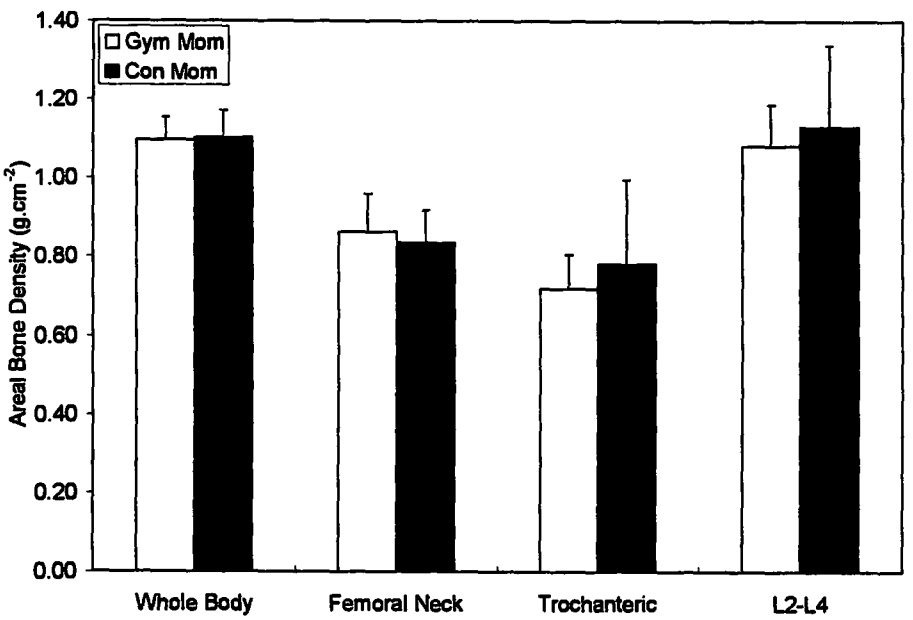


Figure 2. Areal Bone Density of Subject Mothers.

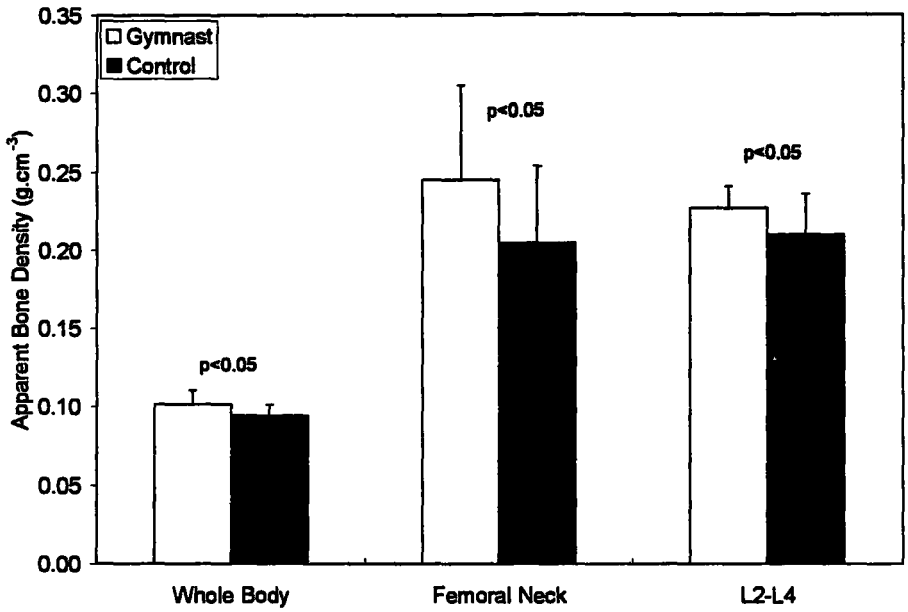


Figure 3. Effect of Gymnastic Training on Apparent Bone Density.

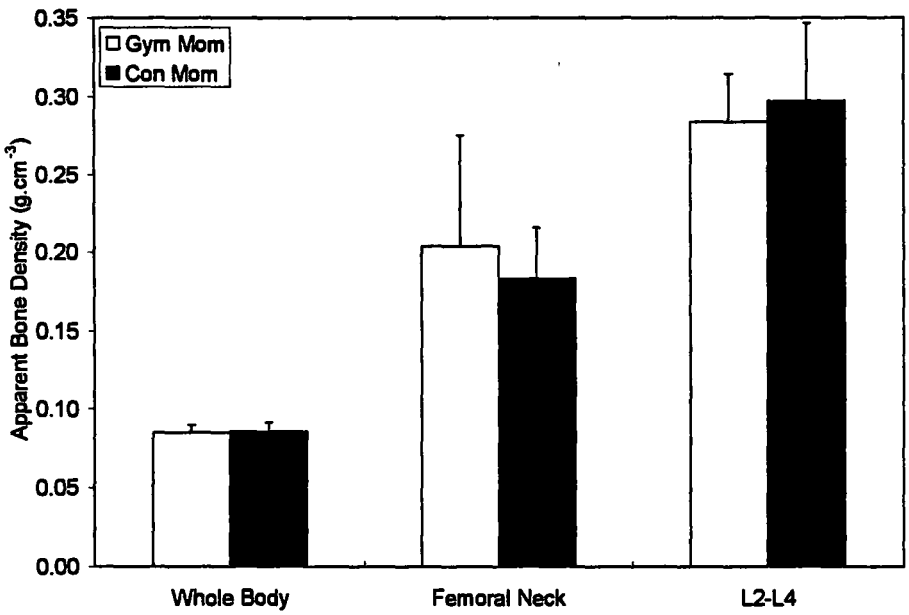


Figure 4. Apparent Bone Density of Subject Mothers.

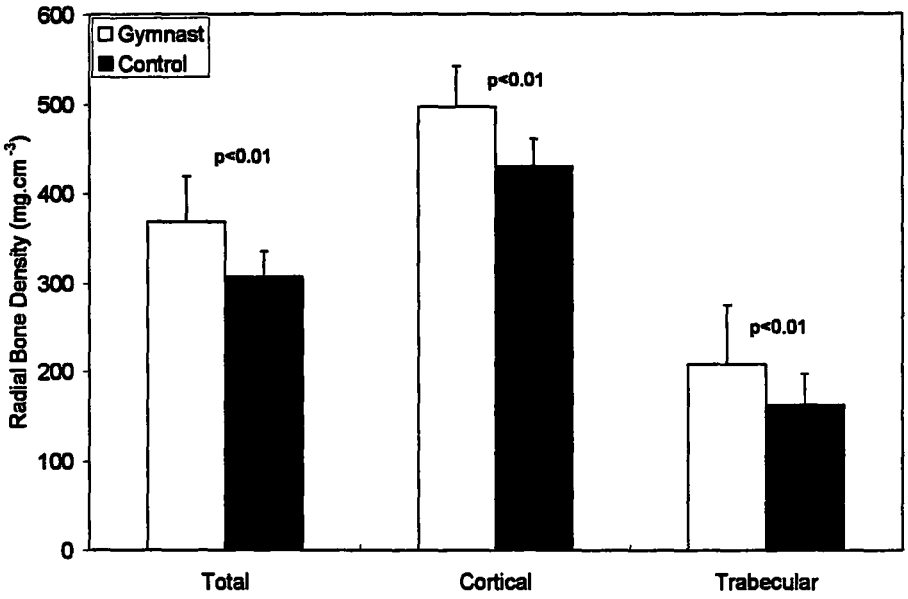


Figure 5. Effect of Gymnastic Training on Bone Density at the Distal Radius.

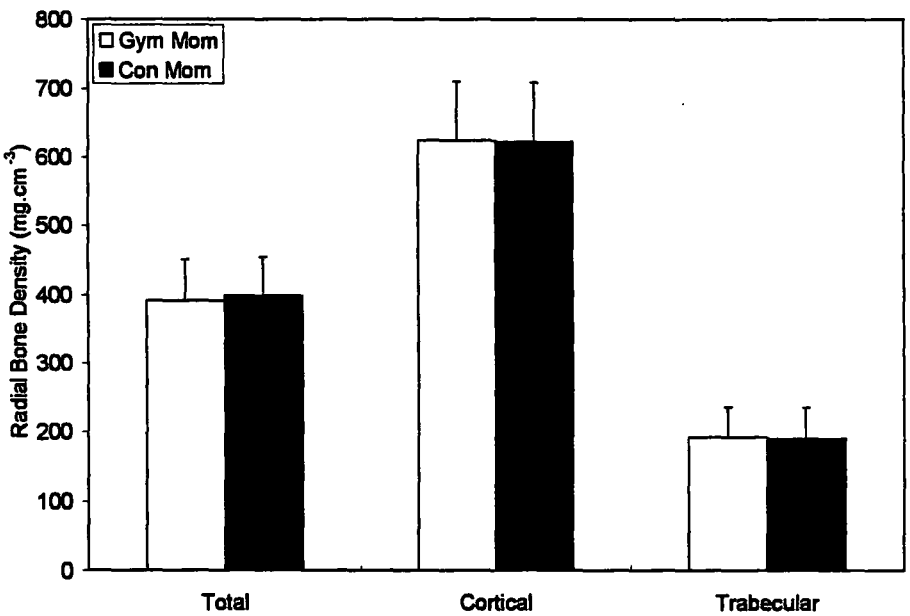


Figure 6. Bone Density of the Distal Radius in Subject Mothers

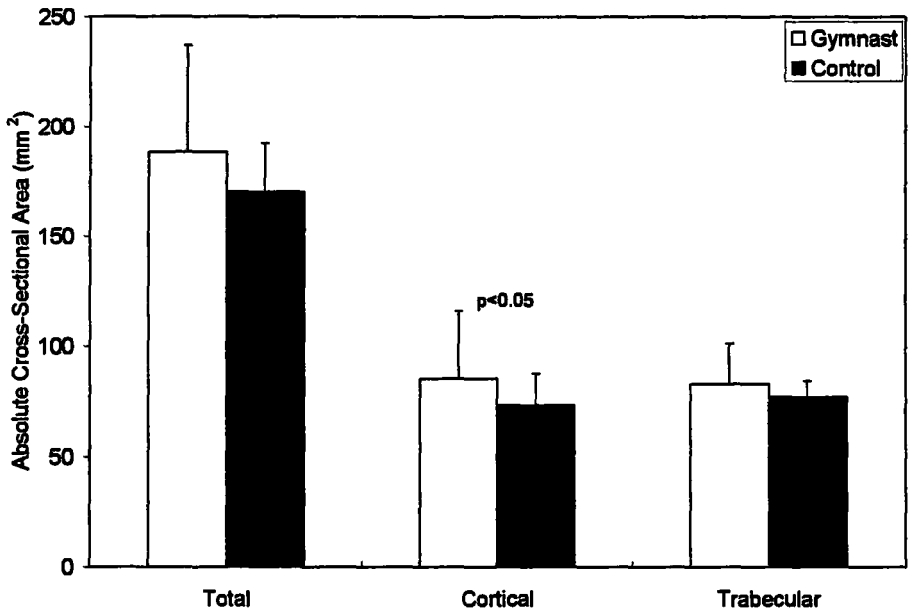


Figure 7. Effect of Gymnastic Training on Absolute Cross-Sectional Area of the Distal Radius.

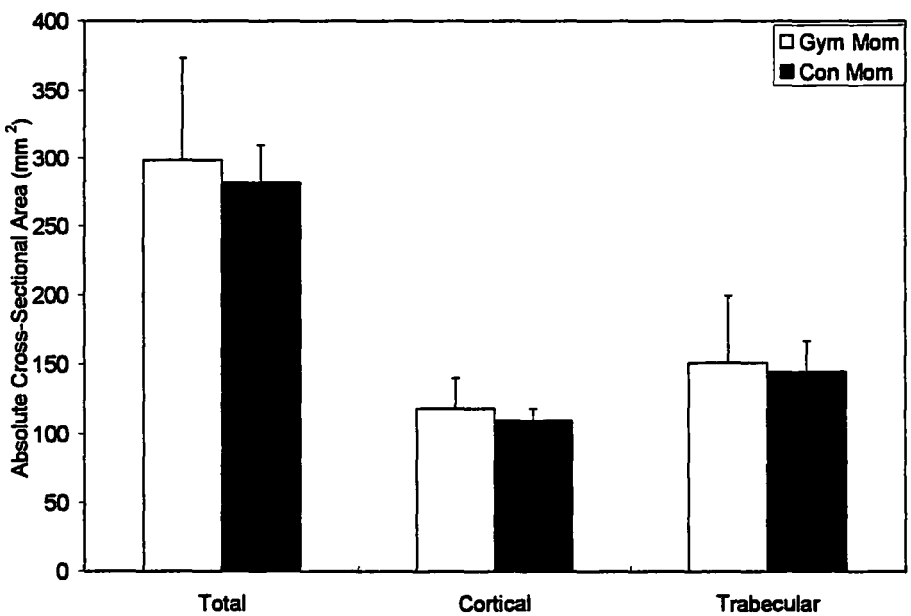


Figure 8. Absolute Cross-Sectional Area of the Distal Radius in Subject Mothers.

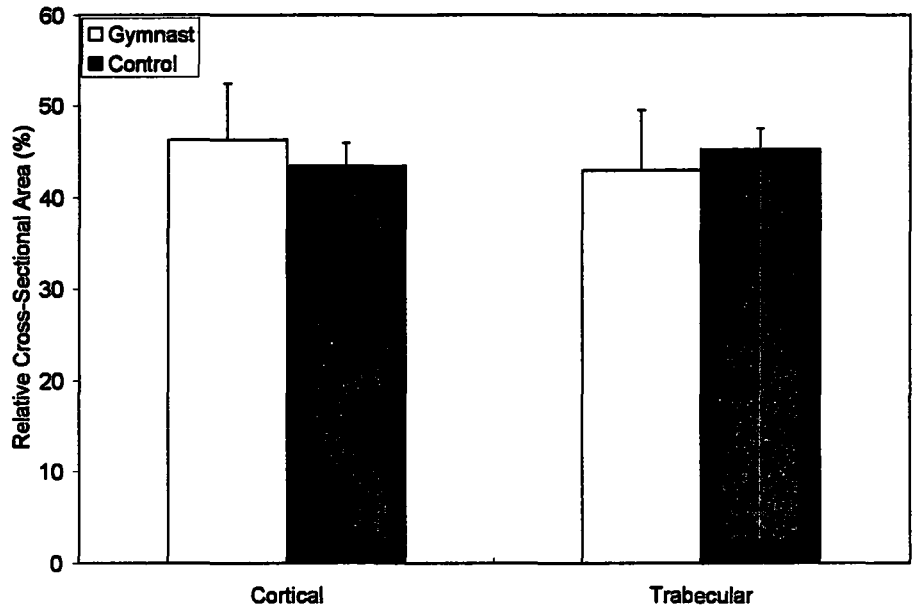


Figure 9.Effect of Gymnastic Training on Relative Area of Cortical and Trabecular Bone at the Distal Radius.

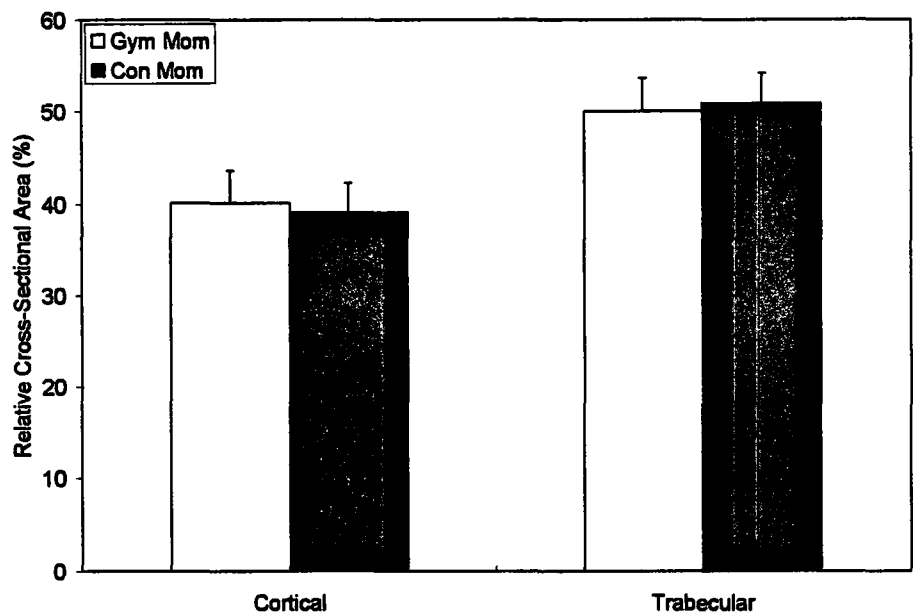


Figure 10.Relative Area of Cortical and Trabecular Bone at the Distal Radius in Subject Mothers

Habitual Physical Activity

Gymnasts spent on average 18 hours per week in intensive training (range = 16 to 23 hours per week), and had been involved in training for gymnastics on average 4.0 years (range = 3.0 to 7.0 years). Excluding gymnastics training time, control girls had significantly greater total and weight bearing hours of weekly physical activity than the gymnasts (Table 3). Gymnasts perceived themselves as being more physically active, and physically more fit than their peers, while control subjects perceived themselves to be average in terms of activity and fitness. Control subjects reported watching significantly more television during the week than gymnasts, but there was no difference between groups in terms of time spent watching television at the weekend. There were no significant differences in habitual physical activity between mothers of gymnasts and controls (Table 4).

Dietary Intake

Dietary information was collected for ten gymnasts and 15 control subjects. There were no significant differences between groups in any of the dietary variables assessed (Table 5). While each group, on average, consumed slightly above the recommended daily intake of calcium, a number of individuals in each group did not. Four individuals in the gymnastic group, and seven in the control group consumed less than the RDI for calcium; the range of calcium intakes relative to the RDI being 54-165% and 40-229% for gymnasts and controls respectively. Similarly, four members of each group reported daily intakes of Vitamin D less than the RDI. The range of intakes relative to the RDI

were 35-309% and 53-399% in the gymnastic and control groups respectively. Average total calorific intake was slightly lower than the RDI (94%) in the gymnasts, and slightly higher (134%) in the controls; gymnasts deriving 14% of total calories from protein, 28% from fat, and 58% from carbohydrates. The corresponding values for the control group were similar, being 13% of total calories from protein, 31% from fat, and 56% from carbohydrates.

There were no significant differences between maternal groups for any of the dietary variables studied (Table 6). Six mothers of gymnasts and seven of controls reported calcium intakes below the RDI, the ranges being 59-163% and 54-194% in gymnast's and control's mothers respectively. Four gymnast mothers, and six control mothers reported daily intakes of Vitamin D below the RDI, the ranges being 52-270% and 6-228% in gymnast's and control's mothers respectively. Total calorific intake was close to the RDI for both groups of mothers; being 101% and 99% for the gymnast's and control's mothers respectively. Total energy intake was derived from approximately 15% protein, 30% fat and 55% carbohydrates in both groups of mothers.

Correlational Analysis of Mother-Daughter Pairs

Significant correlations between mother and daughter were obtained for body weight and percent body fat for gymnasts, but not control subjects (Table 7). There were no significant correlations between mothers and daughters for height in either group.

Significant correlations were present between mothers and daughters of gymnasts and control subjects for areal bone density at the lumbar spine (Table 8). Significant correlations were not present for areal bone density at other measurement sites.

Similarly, for apparent bone density, the lumbar spine was the only site displaying significant daughter-mother correlations (Table 9). There were no significant correlations between daughters and mothers for total, trabecular, or cortical bone density for the distal radius (Table 10).

Significant correlations existed between gymnasts and their mothers, and for all subjects together for total bone cross-sectional area at the distal radius. A significant relationship was also present in absolute trabecular area for all children together, and in absolute cortical area for the gymnasts alone.

Table 3. Physical Activity - Children

| Group | Total (Hrs/wk) | Weight Bearing (Hrs/wk) | Perceived Relative Activity | Perceived Relative Fitness | T.V. Weekday (Hrs/day) | T.V. Weekend (Hrs/day) |
|---|---|---|---|---|---|---|
| Gymnast | 2.0 (±1.3) | 1.7 (±1.3) | 4.9 (±0.4) | 4.9 (±0.4) | 0.7 (±0.6) | 2.0 (±0.95) |
| Control | 5.2* (±4.8) | 4.6** (±3.2) | 3.3 (±0.6) | 3.3 (±0.7) | 2.0** (±0.9) | 2.0 (±1.1) |

Values represent means ± SD.
*$p<0.05$
**$p<0.01$

Table 4. Physical Activity - Mothers

| Group | Total (Hrs/wk) | Weight Bearing (Hrs/wk) | Perceived Relative Activity | Perceived Relative Fitness | T.V. Weekday (Hrs/day) | T.V. Weekend (Hrs/day) |
|---|---|---|---|---|---|---|
| Gymnast | 5.5 (±4.3) | 5.4 (±4.3) | 3.2 (±0.7) | 3.2 (±0.7) | 2.0 (±1.8) | 2.58 (±1.5) |
| Control | 5.1 (±2.5) | 4.5 (±2.8) | 3.4 (±1.0) | 3.5 (±0.7) | 1.7 (±1.1) | 1.75 (±1.5) |

Values represent means ± SD.

Table 5. Dietary Intake - Daughters

| Group | Total Energy Intake (kJ) | Protein (g) | Carbohydrate (g) | Fat (g) | Calcium (mg) | Vitamin D (ug) |
|---|---|---|---|---|---|---|
| Gymnast | 1780 (±1780) | 61 (±6) | 259 (±43) | 57 (±15) | 831 (±248) | 3 (±2) |
| Control | 2044 (±484) | 71 (±18) | 285 (±72) | 72 (±23) | 958 (±295) | 5 (±2) |

Values represent means ± SD.

Table 6. Dietary Intake - Mothers

| Group | Total Energy Intake (kJ) | Protein (g) | Carbohydrate (g) | Fat (g) | Calcium (mg) | Vitamin D (ug) |
|---|---|---|---|---|---|---|
| Gymnast | 1789 (±388) | 67 (±15) | 243 (±69) | 59 (±19) | 704 (±329) | 3 (±2) |
| Control | 1787 (±458) | 64 (±14) | 243 (±74) | 58 (±24) | 746 (±292) | 3 (±2) |

Values represent means ± SD.

Table 7. Daughter-Mother Correlations for Physical Characteristics

| Group | Height (r) | Weight (r) | % Body Fat (r) |
|---|---|---|---|
| All Subjects | 0.20 | 0.18 | 0.20 |
| Gymnast | 0.23 | 0.60* | 0.61* |
| Control | -0.06 | -0.45 | 0.05 |

Values represent Pearson correlation coefficients
*p<0.05

Table 8. Daughter-Mother Correlations Areal Bone Density

| Group | Whole Body (r) | Femoral Neck (r) | Trochanteric (r) | L1-L4 (r) |
|---|---|---|---|---|
| All Subjects | 0.33 | 0.12 | 0.06 | 0.55** |
| Gymnast | 0.33 | -0.01 | -0.07 | 0.52* |
| Control | 0.39 | 0.20 | 0.36 | 0.67** |

Values represent Pearson correlation coefficients
*p<0.05
**p<0.01

Table 9. Daughter-Mother Correlations for Apparent Bone Density

| Group | Whole Body (r) | Femoral Neck (r) | L2-L4 (r) |
|---|---|---|---|
| All Subjects | 0.10 | 0.21 | 0.48** |
| Gymnast | 0.30 | 0.19 | 0.33 |
| Control | -0.03 | 0.13 | 0.73** |

Values represent Pearson correlation coefficients
**p<0.01

Table 10. Daughter-Mother Correlations for Radial Bone Density

| Group | Total Density (r) | Trabecular Density (r) | Cortical Density (r) |
|---|---|---|---|
| All Subjects | 0.04 | -0.19 | 0.04 |
| Gymnast | 0.16 | -0.07 | 0.03 |
| Control | -0.09 | 0.09 | -0.03 |

Values represent Pearson correlation coefficients

Table 11. Daughter-Mother Correlations for Absolute Radial Cross-Sectional Area

| Group | Total Area (r) | Trabecular Area (r) | Cortical Area (r) |
|---|---|---|---|
| All Subjects | 0.58** | 0.41* | 0.73 |
| Gymnast | 0.61* | 0.47 | 0.73** |
| Control | 0.36 | 0.28 | 0.50 |

Values represent Pearson correlation coefficients

*$p<0.05$

**$p<0.01$

# DISCUSSION

There have been a number of recent investigations into the effects of high impact exercise in humans (Cassell et al., 1993; Robinson et al., 1993; Nichols et al., 1994). These studies have utilised adolescent or young adult populations and techniques which measure areal, rather than volumetric, bone density. An additional issue not addressed in previously published studies is that of heredity, and its potential effect on bone density and on-self-selection into the activity under investigation. The present study addressed the effect of gymnastic training (a high impact activity) on bone density in prepubescent individuals, utilised measurement techniques which were sensitive to changes in bone size, and attempted to account for the genetic influence by including measurements on the gymnasts' mothers. The primary finding of this investigation was that bone density is greater in prepubescent female gymnasts compared to age matched controls at all measurement sites following adjustment for bone size variation.

Cassell (1993) reported significantly greater whole body areal BMD in prepubescent gymnasts following data adjustments for anthropometric characteristics and muscle strength. This finding is similar to the increase in whole body BMAD reported in the current study. The use by Cassell (1993) of adjustments for indices such as lean tissue mass and body mass is questionable, firstly because these variables are largely inter-dependent, and secondly since the direct effect of, for example, body mass on

actual bone density has not been addressed in young individuals. Use of the apparent density conversion is, therefore, a more desirable method for minimising the effects of size on areal bone density. Using the apparent density conversion the present study demonstrated increases in BMAD at the hip and lumbar spine, in addition to whole body BMAD.

Studies of BMD in college-aged gymnasts have produced similar results to those of the current investigation. While training volume (hours of training per week) is comparable between these studies, subjects in the current investigation undoubtedly have trained for a shorter period than college-age gymnasts. The current study demonstrated increases in areal BMD at the hip, but not the spine. This finding is identical to that reported by Robinson et al. (1993), for female collegiate gymnasts. Following adjustment for covariance of body mass, Nichols et al. (1994) also reported significantly greater BMD at both the hip and spine in collegiate gymnasts, which is supportive of the finding in the current investigation of increased BMAD of prepubescent gymnasts at these sites.

Gymnasts in the present study were habitually less active than control subjects, when the time spent in gymnastic training is removed from the analysis. This finding is important, because it demonstrates a compensation in normal physical activity associated with intense gymnastic training. Gymnasts had consistently greater bone density, however, despite having significantly reduced habitual physical activity. This observation supports the conclusion that the high intensity gymnastic training was the primary factor associated with the higher bone density in the gymnasts. Also the finding that mothers of the two groups were comparable in terms of physical activity suggests that familial

differences in habitual activity probably were not a major contributing factor to the differences in bone density between the gymnasts and controls in this study.

The measurement of habitual activity in children is difficult to accomplish with great accuracy and precision. In the current study a two week recall was utilised in order to estimate this variable. Due to the relatively slow adaptation of bone to increases in mechanical loading a technique estimating habitual activity over a greater time (e.g. one year) may have been more desirable. The technique used in the current study simply estimated the time spent in weight bearing and non-weight bearing activities per week, and did not attempt an estimation of the intensity of these activities, or their potential for inducing skeletal adaptation. The potential for various activities to induce skeletal adaptation may be graded on a simple scale relating the activity to the magnitude of skeletal loads it is presumed to induce. This may be a more relevant measure of intensity in studies of bone adaptation than, for example, calorific cost of activity. In comparison, however, to other published studies which have simply placed children into one of three activity groups (Katzman et al., 1991; Kröger et al., 1992), the method used in the current study was relatively detailed.

Nutrition is an important determinant of skeletal health (Matkovic, 1991; Johnston et al., 1992). Habitual dietary intake was assessed by three day dietary diary in the present study. Accurate and precise measurement of dietary habits in children is difficult, and the technique utilised in the present study involves limitations relating to the accuracy of food recording by children or their parents, possible influences on dietary choices arising from diary completion, and the relatively small period of data collection. To

address the final point it would be prudent for future studies to include a food frequency based questionnaire in addition to the diary, in order to assess dietary habit over a longer time period. In the current study both gymnasts and controls, and mothers of each exercise group reported similar nutrient intakes.

The dietary information obtained compares favourably with that reported by Grimston et al. (1993), although the magnitude of the measured variables is smaller in the current study. This difference may arise because subjects in the current study were approximately 2 years younger than those in that of Grimston et al. (1993). Chan (1991) reported daily intake values of 364kJ, 65g and 966mg for total energy, protein and calcium respectively in children aged 9-11 years, values which compare favourably with those from the current study. While individual dietary intakes were quite variable, both the gymnast and control group girls as groups appeared to consume at least the Canadian recommended daily intake for each nutrient studied. It appears, therefore, that dietary intake in this study was similar to those previously published for children of comparable ages. The lack of dietary differences between gymnasts and controls suggests that diet was not associated with the observed differences in bone density in the present study.

A significant relationship was observed between mothers and daughters of both groups for lumbar spine areal bone density ($r=0.55$), and for apparent bone density ($r=0.48$) in control subjects only. These values compare favourably with those of Matkovic et al. (1990), who reported similar ($r=0.43$) correlations in lumbar spine areal BMD between mothers and adolescent daughters. The correlation between control mothers and their daughters for areal bone density at the lumbar spine ($r=0.67$) was

considerably higher than that reported by Matkovic et al. (1990). The lower correlation in the study by Matkovic et al. (1990) may be explained in part by the greater growth and development changes in size and sexual maturity in this sample of adolescent girls.

Significant correlations were also obtained between mothers and daughters for total radial cross-sectional area (r=0.58), trabecular area (r=0.41), and cortical area (r=0.73). These results are supported by those of Matkovic et al. (1990), who also reported a significant (r=0.50) correlation between mothers and daughters for cortical area at this site. These findings are difficult to explain in this population with respect to the lack of a significant relationship between mothers and daughters for other anthropometric characteristics, such as height.

A significant amount of variability in the daughter's radial bone density was not explained by bone density of the mother at this site. This finding is in contrast to those reported by Tylvasky et al. (1989) and Lutz (1986) who reported significant heritability at this site for young adult women. Radial bone density in the studies of Tylvasky et al. (1989) and Lutz (1986) was measured by single-photon absorptiometry (which provides only an areal density), which is influenced by bone size. As can be seen in the present study a significant correlation existed between mothers and daughters in radial cross-sectional area, a finding which would explain in part the different results obtained from areal and volumetric techniques. There is some, albeit limited, data to suggest a variable genetic influence on radial bone density, which is dependent on maturity status. In a study by Smith et al. (1973) the genetic influence appeared stronger for bone density at the wrist in post- compared to pre-pubertal children.

It is important to note that an accurate assessment of genetic influence on bone density cannot be obtained solely from mother-daughter relationships. Matkovic et al. (1990), for example, reported similar, significant paternal relationships for bone density at the spine, suggesting that measurement of one parent may be insufficient. Mother-daughter correlations, however, were generally greater than father-daughter correlations. Also, in most cases, combination of maternal and paternal measurements did not markedly increase the amount of explained variance, suggesting that a good estimate of familial similarity was obtained in this study from measurement of the mother only. Environmental factors such as nutrition, health awareness and familial attitudes towards physical activity are also included in the estimate of heredity derived from mother-daughter relationships as assessed in this study. The lack of significant correlations, however, between mother's and daughter's bone density at the sites displaying inter-group differences suggests that the higher bone density of the gymnasts is probably not attributed to familial or genetic influences. While this does not rule out self-selection into the activity by children displaying naturally higher bone density, it can be viewed as evidence supportive of gymnastic training, rather than environmental or genetic factors, being responsible for the observed differences in bone density. Additionally, the similarity of bone density between mothers of gymnasts and controls suggests that there is probably no genetic predisposition towards greater densities in the gymnasts compared to the control girls.

Recently, Theintz et al. (1993) provided persuasive evidence that the smaller stature of gymnasts may not due entirely to heredity or self-selection, but may result

from gymnastic training itself. Theintz et al. (1993) reported that completion of 18 hours/week of gymnastic training during prepubescence and adolescence was capable of permanent growth stunting, which was localised to the lower body. If this report is accurate, subjects in the gymnastic group in the present study would appear to be at risk for stunted growth in stature. Despite this potential for growth stunting, it is also apparent that gymnastic training may also elicit strikingly positive skeletal adaptations, at least in terms of bone mineralisation. The occurrence of these adaptations has been recently confirmed (Robinson et al., 1995; LaRiviere et al., 1995; Padro et al., 1995)

In this study areal and apparent bone density in the gymnastic group were between 7 and 19% greater than the control groups. As reported earlier, precision of the DXA technique is of the order of 1-2%. While pQCT is associated with a lesser degree of precision than DXA, the radial density of gymnasts was increased by 20% when compared to controls. It is, therefore, not reasonable to assume that the observed increases in bone density in gymnasts were due to variability inherent in the measurement technique. Additionally, the greater magnitude of adaptation at the wrist suggests that the relative stresses are greater at this site. To date such forces at the wrist have only been measured as reaction forces.

In addition to the degree of mineralisation, bone strength is also determined by factors including bone size, and the distribution of mineralised tissue within the bone. The current study provided unique information regarding these variables. The lack of significant differences between gymnasts and controls for total radial cross-sectional area suggests that gross hypertrophy of bone is not a primary response to increases in

mechanical loading at the wrist during prepubescence. Gymnasts did, however, possess significantly greater absolute trabecular area, which suggests that some hypertrophy may occur in this bone type. There was no difference in the relative amount of each tissue type between groups, and both cortical and trabecular bone increased in density a similar degree in the gymnasts. This suggests that the primary response to increased mechanical loading at this site is not an increase in bone size, or a change in the proportions of trabecular or cortical bone. Rather, the primary response appears to be a comparable increase in the density of cortical and trabecular bone.

The current study did not investigate the effect of volume of gymnastic training on the skeleton. It is suggested that further research should investigate the relationship between the volume of gymnastic training and bone adaptation, to determine the optimal degree of training for favourable skeletal adaptation in terms of bone density.

Due to the cross-sectional nature of the current study it is unclear whether the observed differences in bone density will persist with continued growth and development and lead to a favourable increase in peak bone mass during late adolescence or early adulthood. Studies of young adult gymnasts (Cassell et al., 1993; Nichols et al, 1994) provide evidence supportive of a positive role for gymnastic training on peak bone mass. Also, it is unclear whether bone density will revert to control values with cessation of training. Recent studies of calcium supplementation in childhood suggest that changes are impermanent, with the withdrawal of treatment (Slemenda, personal communication). Longitudinal and retrospective studies are required to address these areas of uncertainty.

# CONCLUSION

This study demonstrates that elite gymnastic training appears capable of inducing favourable adaptations in bone density in prepubescent individuals. This adaptation appears independent of maternal bone density, dietary intake and general habitual physical activity

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# APPENDIX 1

# Mother/Daughter Physical Activity and Bone Density Study

## Mother's General Information Form

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Dear Parent:

It is believed that physical activity during the growth years may lead to stronger, healthier bones both during childhood and later on in adult life. The amount and type of physical activity, however, which is required to bring about favourable changes in bone during childhood is currently unknown. A group of researchers at McMaster University is interested in this question and have initiated a study involving prepubertal girls of varying activity backgrounds between 8 and 10 years of age. The purpose of this study is to investigate the relationship between physical activity, diet and bone density in young girls and their mothers. We would greatly appreciate your participation in this study.

Participation will require two visits, one to the McMaster University Medical Centre, and another to the Hamilton Osteoporosis Clinic. Bone density and body composition (the amount of bone, muscle and fat in the body) will be measured using special scanning devices. One device measures the bone density of the whole body, spine and hip while the subject lies still for between 12 to 20 minutes, and another measures the density of the hand/wrist region while the subject sits quietly for about 10 minutes. The bone scanning techniques are used extensively with individuals of all ages, and involve safe and very low doses of radiation (about the same as one would acquire in flying return across the Atlantic ocean). Subjects will also be asked to complete extensive questionnaires, with our help, about their past and current physical activity levels and medical histories. In addition, subjects will be required to complete a three day food diary for dietary analysis. Muscle strength of the left and right hands will be determined using a special testing device and height and weight will also be measured. All of the measurements in this study have been approved by a human ethics research committee at the University Medical Centre.

Bone density is in part genetically determined. To account for this influence it is hoped, wherever possible, also to make these measurements on the child's natural mother. Testing will be scheduled to minimize conflicts with school and other activities. Each visit, including measurements for both the mother and daughter, will last approximately 1.5 hours. Subjects will receive individual feedback about their personal results, compared to the group average, at the end of the study. All personal information will be kept strictly confidential, and known only to the researchers. You have the right to withdraw from the study at any time, even after you have agreed to participate.

We hope that you and your daughter will take part in the study, and we think that you will both find it enjoyable and interesting. Please do not hesitate to contact either Dr. Cameron (Joe) Blimkie or Shannon Frazer (study coordinator) at the numbers below if you have any questions. We thank you for your interest in and support of our study, and look forward to meeting you.

Dr. C.J. Blimkie 905-525-9140 ext. 24465
Ms. Shannon Frazer 905-528-6243

Sincerely,

Cameron (Joe) Blimkie, Ph.D.,
Associate Professor,
Department of Kinesiology,
McMaster University,
Hamilton, Ontario.

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**WE NEED YOUR HELP**

Does your daughter have any close friends of the same age who are not involved in Gymnastics (and who are either short for their age or normal height), who you think, along with her mother, might be interested in participating in this study? Yes ☐ No ☐

If yes, could you please provide us with their names and phone numbers so we can contact them regarding their possible involvement in this study.

| Name | Phone Number | City |
|---|---|---|
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |

Do you mind if we use your name as a reference when contacting these individuals? Yes ☐ No ☐

.....
.....

# APPENDIX 2

**Mother/Daughter Physical Activity and Bone Density Study**

**Mother's Consent Form**

I, _____, consent to participate in a study designed to investigate the relationship between physical activity, diet and bone density in mother/daughter pairs. The investigator has explained that I will be invited once to both the McMaster University Medical Centre and the Hamilton Osteoporosis Clinic, as outlined in the information sheet overleaf.

I understand that no known harmful effects will occur as a result of the measurements explained to me in the information sheet. I further understand that there are no direct benefits to myself from taking part in this study. I also understand that I can withdraw at any time from the study, even after I have signed this form. Any information which is collected will be kept confidential, and will not identify me in any way. This will also apply if the results are published.

| _____ | _____ | _____ |
|---|---|---|
| Name (print) | Signature | Date |
| _____ | _____ | _____ |
| Witness (print) | Signature | Date |

I have explained the nature of this study to the subject above, and believe that she fully understands the terms of this agreement.

| _____ | _____ | _____ |
|---|---|---|
| Investigator | Signature | Date |

# APPENDIX 3

**Mother/Daughter Physical Activity and Bone Density Study**

**Child's Consent Form**

I, _____, consent to allow my daughter _____ to participate in a study designed to investigate the relationship between physical activity, diet and bone density in mother/daughter pairs. The investigator has explained that my daughter will be invited once to both the McMaster University Medical Centre and the Hamilton Osteoporosis Clinic, as outlined in the information sheet overleaf.

I understand that no known harmful effects will occur to my daughter as a result of the measurements explained in the information sheet. I further understand that there are no direct benefits to my daughter from taking part in this study. I also understand that my daughter can withdraw at any time from the study, even after she has signed this form. Any information which is collected will be kept confidential, and will not identify my daughter in any way. This will also apply if the results are published.

| | | |
|---|---|---|
| Daughter's Name (print) | Signature | Date |
| Mother's Name (print) | Signature | Date |
| Witness (print) | Signature | Date |

I have explained the nature of this study to the subject above, and believe that she fully understands the terms of this agreement.

| | | |
|---|---|---|
| Investigator | Signature | Date |

# APPENDIX 4

**Mother/Daughter Physical Activity and Bone Density Study**

**Medical/Health Questionnaire**

**MOTHER'S FORM**

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The questions in this survey are directed towards events in childhood, adolescence and adult life which may have some influence on your bone mineral density. Please read the questions carefully and mark the appropriate response with a check mark (✓). Answer questions which are not relevant or to which you are unable to respond with an N/A. All information will be kept strictly confidential.

---

Interviewer:_____ Date:_____

1. **MOTHER'S IDENTIFICATION**

1.1 Surname:_____ Given Name(s):_____

1.2 Address:_____

1.3 City or Town:_____ Postal Code:_____

1.4 Telephone (Home):_____ Other:_____

1.5 Date of Birth: Day_____ Month_____ Year_____

2. **DAUGHTER'S IDENTIFICATION**

2.1 Surname:_____ Given Name(s):_____

2.2 Date of Birth: Day_____ Month_____ Year_____

2.3 Gym (>15 hr/wk) ☐ Gym (>8hr/wk) ☐

Gym (<8hr/wk) ☐ Control ☐

2.3.1 During the current Gymnastic season, how many hours per week on average does your daughter participate in gymnastics training and/or competition? _____ hours per week

2.3.2 How many years has your daughter been training/competing at her current number of hours? _____ years

2.3.3 How many years has your daughter been involved in training/competing in Gymnastics? _____ years

2.3.4 How many hours per week (on average) did your daughter train/compete in gymnastics during the following years?

1989 - 1990 _____ hours/week
1990 - 1991 _____ hours/week
1991 - 1992 _____ hours/week
1992 - 1993 _____ hours/week
1993 - 1994 _____ hours/week

2.3.5 Does your daughter participate in any other organized sport besides gymnastics? Yes ☐ No ☐

2.3.6 If yes, which sports does she participate in regularly, and at what level of competition?

| Sports | Level of Participation Recreational | Competitive | Hours/Week |
|---|---|---|---|
| _____ | ☐ | ☐ | _____ |
| _____ | ☐ | ☐ | _____ |
| _____ | ☐ | ☐ | _____ |
| _____ | ☐ | ☐ | _____ |
| _____ | ☐ | ☐ | _____ |

## 3. MOTHER'S LIFESTYLE INFORMATION - SMOKING, ALCOHOL, AND DIET

3.1 Have you ever smoked? Yes ☐
No ☐ go to question 3.6

3.2 Have you ever smoked for 6 months or more?
Yes ☐ How many years did you smoke? ____
No ☐ go to question 3.6

3.3 Do you still smoke? Yes, daily ☐
Yes, occasionally ☐
No, not at all ☐

3.4 When you are/were smoking, how many cigaretts do/did you usually smoke per day? About _____ per day

3.5 At about what age did you start to smoke daily? _____ yrs.

3.6 At about what age did you stop smoking? _____ yrs of age.

3.7 Do you eat a special diet? Yes ☐ No ☐

3.8 If yes, please specify the type of diet:

Vegetarian ☐
Low sodium ☐
Low cholesterol ☐
Other (please specify) _____

3.9 Do you take a calcium supplement? Yes ☐ No ☐

3.10 If yes, how many times a day do you take it? _____ times/day

3.11 What is the name of the supplement? _____

3.12 How many milligrams of calcium does it contain? _____ mgs.

3.13 Do you take a multivitamin supplement? Yes ☐ No ☐

3.14 If yes, how many times a day do you take it? _____ times/day

3.15 What is the name of the supplement? _____

3.16 How many milligrams of calcium does it contain? _____ mgs.

3.17 Do you take any of the following antacids on a daily basis?

Rolaids, Tums, Yes ☐ No ☐

3.18 If yes, how many times a day do you take it? _____ times/day

3.19 Do you take a bran or fiber supplement? Yes ☐ No ☐

3.20 If yes, how many times a day do you take it? _____ times/day

3.21 What is the name of the supplement? _____

3.22 How many grams of fiber does it contain? _____ gm/serving.

3.23 During **your early childhood** (up to 13 years of age) how often did you eat/drink the following foods?

| Food | Never | Frequency | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1-2 Times Daily | 3 + Times Daily | 1-2 Times Weekly | 3 + Times Weekly | 1-2 Times/ Month | 3+ Times/ Month |
| Alcohol | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Tea/Coffee | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Cola/Pop | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Milk | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Yogurt | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Cheese | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Cottage Cheese | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Pizza w cheese | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Sour Cream | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Ice Cream/Milk | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Beans | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Beets | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Broccoli | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Red Meat | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| White Meat | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Foul eg. Chicken | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Shell Fish | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Fish | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Organ Meat | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

3.24 During **your adolescence** (13 to 1ᑫ years of age) how often did you eat/drink the following foods?

| Food | Never | Frequency | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1-2 Times Daily | 3 + Times Daily | 1-2 Times Weekly | 3 + Times Weekly | 1-2 Times/ Month | 3+ Times/ Month |
| Alcohol | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Tea/Coffee | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Cola/Pop | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Milk | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Yogurt | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Cheese | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Cottage Cheese | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Pizza w Cheese | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Sour Cream | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Ice Cream/Milk | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Beans | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Beets | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Broccoli | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Red Meat | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| White Meat | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Foul eg. Chicken | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Shell Fish | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Fish | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Organ Meat | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

3.25 During **your adulthood** (18 years of age to present) how often did you eat/drink the following foods?

| | | Frequency | | | | | |
|---|---|---|---|---|---|---|---|
| Food | Never | 1-2 Times Daily | 3 + Times Daily | 1-2 Times Weekly | 3 + Times Weekly | 1-2 Times/ Month | 3+ Times/ Month |
| Alcohol | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Tea/Coffee | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Cola/Pop | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Milk | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Yogurt | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Cheese | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Cottage Cheese | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Pizza w Cheese | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Sour Cream | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Ice Cream/Milk | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Beans | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Beets | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Broccoli | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Red Meat | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| White Meat | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Foul eg. Chicken | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Shell Fish | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Fish | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Organ Meat | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

## 4. MOTHER'S LIFESTYLE INFORMATION - PHYSICAL ACTIVITY

4.1 Rate (cirle one) your overall level of physical activity as a **child and youth** (up to 18 years of age).

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| seldom active | sometimes active | active | moderately active | very active |

4.2 How would you describe the games you played most often as a child and youth? (Circle one)

| 1 | 2 | 3 |
|---|---|---|
| games such as board games, drawing, puzzles, etc. | games requiring some running, jumping, climbing, throwing, etc. | mostly running, jumping, climbing, and throwing etc. |

4.3 During which years were you physically active? (Circle more than one if need be)

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| 5-10 yrs | 10-15 yrs | 15-20 yrs | 20-30 yrs | 30-40 yrs |

4.4 During which years were you the **MOST** physically active? (Circle one only)

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| 5-10 yrs | 10-15 yrs | 15-20 yrs | 20-30 yrs | 30-40 yrs |

4.5 Did you participate in organized sport as a child or youth?
Yes ☐ No ☐

4.6 If yes, please list below, the sports you participated in during your **YOUTH (before 18 years of age)**, and the approximate number of years of participation.

| Sport | Age While Participating (yrs) | # of Years of Participation (yrs) |
|---|---|---|
| | | |
| | | |
| | | |
| | | |

4.7 **Since the age of 18 years**, did you participate regularly in sport (e.g. tennis, soccer, basketball)?

Yes ☐ No ☐

4.8 If yes, please list below, the sports in which you participated, your age while participating, and the approximate number of years of participation.

| Sport | Age While Participating (yrs) | # of Years of Participation (yrs) |
|---|---|---|
| | | |
| | | |
| | | |
| | | |

4.9 Were you regularly involved in heavy physical work (e.g. farm chores or heavy lifting) as a child or youth?

Yes ☐ No ☐

4.10 How would you rate your current level of **PHYSICAL ACTIVITY** compared to others your age (Check one only)

very low ☐ low ☐ average ☐ high ☐ very high ☐

4.11 How would you rate your current level of **PHYSICAL FITNESS** compared to others your age (Check one only)

very low ☐ low ☐ average ☐ high ☐ very high ☐

4.12 During the last week, how many times did you do any of the activities listed below, about how much time (average) did you spend doing the various activities on each occasion, and how difficult or strenuous was the activity on average

For difficulty or strenuousness of the activity, use the following guidelines:

Light: slight sweating and slight increase in breathing
Moderate: noticeable sweating and above normal breathing
Heavy: heavy sweating and heavy breathing

| ACTIVITY | # Of Times | MINUTES EACH TIME 1-15 | 16-30 | 31-59 | 60 + | STRENUOUSNESS LIGHT | MOD | HEAVY |
|---|---|---|---|---|---|---|---|---|
| Walking For Exercise | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Calisthenics | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Aerobics | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Weight Lifting | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Stat Cycling/ Bicycling | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Jogging/ Running | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Bowling | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Social Dancing | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Modern/ Jazz Dancing | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Racquet Sports | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Golf | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Swimming | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Gardening/ Yard Work | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| House Work | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Baseball/ Softball | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Basketball | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Volleyball | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Curling | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Skipping | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Skating/ Rollerblading ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Skiing/ Down Hill ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Skiing/ Cross-Country ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Ringette/ Ice Hockey ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Tag ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Others (Please specify) | | | | | | | |
| _____ ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| _____ ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| _____ ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

OR, I DID NOTHING LIKE THIS IN THE LAST WEEK ☐

---

4.13 Approximately how many hours of television do you watch each day?

_____ average hours per day from Monday-Friday

_____ average hours per day on Saturday and Sunday

4.14 Please provide any other comments regarding your lifestyle or physical activity which you think we should know about.

_____

_____

_____

_____

_____

_____

## 5. MOTHER'S REPRODUCTIVE HISTORY AND GYNECOLOGICAL STATUS

5.1 Are you pregnant? ____yes ____ no

5.2 To the nearest half-year, at what age did you have your first menstrual period?

_____ years of age.

5.3 Do you menstruate regularly? Yes ☐ No ☐

5.4 If yes, please indicate the approximate time in days between periods: _____ days

5.5 If no,

a) what is the shortest time you experienced between periods: ____ days

b) what is the longest time you experienced between periods : ____ days

5.6 How many periods do you usually have in a year? (Circle one only)

| 1 | 2 | 3 |
|---|---|---|
| 11-17 | 4-10 | less than 4 |

5.7 When was your last period? ____ day ____ mo. ____ yr.

5.8 Other than when you were pregnant or lactating, have you ever had an absence or loss of periods? Yes ☐ No ☐

5.9 If yes, at what age did these missed periods occur?

1st time: ____ years old

2nd time: ____ years old

5.10 For how long did your periods stop during these occasions?

1st time: _____ mo. ____ yrs.

2nd time: _____ mo. ____ yrs.

5.11 Have you had a hysterectomy? Yes ☐ No ☐

5.12 If yes, were the ovaries removed? Yes ☐ No ☐

5.13 When was this surgery performed? ____ mo. ____ year

5.14 Has your menopause begun (no periods for a year or more after your last period)? Yes ☐ No ☐

5.15 At what age did you begin to experience menopausal symptoms e.g. hot flashes, irregular menstrual cycles?
_____ years old.

5.16 How many times have you been pregnant? ____

5.17 How many children have you given birth to? ____

5.18 How old were you at the birth of your children?

1st child: ____ yrs old 2nd child: ____ yrs old

3rd child: ____ yrs old 4th child: ____ yrs old

5.19 Did you breast feed one or more of your children?

____ no none of them

Yes, I breast fed _____ (number) of them

5.20 List the number of months spent breast feeding each child

1st child: ____ mos. 2nd child: ____ mos.

3rd child: ____ mos. 4th child: ____ mos.

5.21 Do you now or have you ever used oral contraceptives?

Yes ☐ No ☐

5.22 If yes, for how many years? ____ years

5.23 Please indicate the brand name of the contraceptive:

_____

5.24 If you previously used oral contraceptives but are no longer using them, what was the last approximate date of use?

____ mo. ____ yr.

5.25 Do you now or have you ever taken estrogen supplements other than oral contraceptives?

Yes ☐ No ☐

5.26 If yes, what medication did you or are you taking?
_____ (brand name)

5.27 When did you begin taking this medication?
____ mo. ____ yr.

5.28 When did you stop taking this medication?
____ mo. ____ yr.

6. **MOTHER'S DETAILED MEDICAL HISTORY AND STATUS**

6.1 Have you seen a doctor in the last 6 months for a medical concern? Yes ☐ No ☐

6.2 If yes, what was the reason for your visit?

_____

_____

_____

_____

6.3 Has there been any change in your general health during the last 6 months? Yes ☐ No ☐

6.4 If yes, please describe the nature of the change:

_____

_____

6.5 Have you been hospitalized in the last year? ____ yes ____ no

6.6 If yes, please indicate the medical condition(s) which was being treated:

_____

6.7 Have you had any surgery in the past 2 years? Yes ☐ No ☐

6.8 If yes, list the procedure and approximate date of surgery.

| of Surgery | Date | Length of Hospital Stay |
|---|---|---|
| | | |
| | | |
| | | |

6.9 Have you ever been treated for any of the following conditions? [hyper = excess; hypo = deficiency]

| | | | | | |
|---|---|---|---|---|---|
| food allergies | yes ☐ | no ☐ | asthma | yes ☐ | no ☐ |
| other allergies | yes ☐ | no ☐ | kidney disease | yes ☐ | no ☐ |
| back pain | yes ☐ | no ☐ | liver problems | yes ☐ | no ☐ |
| scoliosis | yes ☐ | no ☐ | gastrointestinal disease | yes ☐ | no ☐ |
| epilepsy | yes ☐ | no ☐ | muscular dystrophy | yes ☐ | no ☐ |
| osteoporosis | yes ☐ | no ☐ | osteoarthritis | yes ☐ | no ☐ |
| rheumatoid arthritis | yes ☐ | no ☐ | anemia | yes ☐ | no ☐ |
| diabetes | yes ☐ | no ☐ | malabsorption | yes ☐ | no ☐ |
| excess urinary calcium | yes ☐ | no ☐ | excess blood calcium | yes ☐ | no ☐ |
| hyperparathyroid | yes ☐ | no ☐ | hypoparathyroid | yes ☐ | no ☐ |
| hyperthyroidism | yes ☐ | no ☐ | other (specify): _____ | | |
| hypothyroidism | yes ☐ | no ☐ | | | |

6.10 Have you had a bone scan or a diagnostic X-ray in the last year? Yes ☐ No ☐

6.11 If yes, what body part was X-rayed? _____

6.12 Have you ever had a fractured bone? Yes ☐ No ☐

6.13 If yes, please indicate which bone(s) was/were fractured and when the fractures occurred.

1st fracture: body part_____ ____ mo. ____ yr.
2nd fracture: body part_____ ____ mo. ____ yr.
3rd fracture: body part_____ ____ mo. ____ yr.

6.14 Have you ever been hospitalized or confined to bed for any reason, or had a limb immobilized (e.g. arm in a cast) for 21 days or longer?

Yes ☐ No ☐

6.15 If yes, list the condition, approximate date it occurred, and the length of time you were hospitalized or immobolized.

| | Injury type | Date of Injury | Time Immobolized |
|---|---|---|---|
| e.g. | wrist fracture | July, 1982 | 6 weeks |
| | _____ | _____ | _____ |
| | _____ | _____ | _____ |
| | _____ | _____ | _____ |

6.16 Is there a history of wrist, hip or spine fracture in your immediate family? Yes ☐ No ☐

6.17 If yes, please indicate who was affected.

biological mother ☐
maternal grandmother ☐
maternal grandfather ☐
biological father ☐
paternal grandmother ☐
paternal grandfather ☐

6.18 Is there a history of osteoporosis in your family?
Yes ☐ No ☐

6.19 If yes, indicate who was affected.

biological mother ☐
maternal grandmother ☐
maternal grandfather ☐
biological father ☐
paternal grandmother ☐
paternal grandfather ☐

6.20 Is there a history of any other bone disease in your family?

Yes ☐ No ☐

6.21 If yes, indicate which family member is/was affected and the name of the condition.

| Family Member | Condition |
|---|---|
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |

7. **MOTHER'S MEDICATIONS**

7.1 Are you curently taking any prescription medications?
Yes ☐ No ☐

7.2 If yes, which medications are you taking?

_____

_____

7.3 What are these medications for?

_____

_____

7.4 Have you ever taken any of the following medications? Please indicate at what age you began to use them, and for how long you used them.

| Medication | Currently Using | Age at Start | Duration of Use |
|---|---|---|---|
| e.g. Insulin | _____ | 15 yrs old | 20 years |
| Calcium preparations | _____ | _____ | _____ |
| Antacids | _____ | _____ | _____ |
| Inhaled steroids | _____ | _____ | _____ |
| Anabolic steroids | _____ | _____ | _____ |
| Fluoride | _____ | _____ | _____ |
| Vitamin D compounds | _____ | _____ | _____ |
| Calcitonin | _____ | _____ | _____ |
| Diuretics | _____ | _____ | _____ |
| Heparin | _____ | _____ | _____ |
| Cortisone (oral) | _____ | _____ | _____ |
| Corticosteroids | _____ | _____ | _____ |
| Anti-inflammatories | _____ | _____ | _____ |
| Thyroid preparations | _____ | _____ | _____ |

**8. MOTHER'S MEDICAL DECLARATION**

8.1 Do you have any medical/health condition which might prevent you from participating in this study?
Yes ☐ No ☐

**8.2 I certify that the information provided on this form is correct.**

**Signature:** _____

**Date:** _____

# APPENDIX 5

**Mother/Daughter Physical Activity and Bone Density Study**

**Medical/Health Questionnaire**

**DAUGHTER'S FORM**

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The questions in this survey are directed towards events in childhood which may have some influence on your bone mineral density. Please read the questions carefully and mark the appropriate response with a check mark (✓). Answer questions which are not relevant or to which you are unable to respond with an N/A. All information will be kept strictly confidential.

**INSTRUCTIONS:** It is probably best if the mother assists her daughter in completing this questionnaire.

---

Interviewer:_____ Date:_____

1. **DAUGHTER'S IDENTIFICATION**

1.1 Surname:_____ Given Name(s):_____

1.2 Date of Birth: Day_____ Month_____ Year_____

1.3 Gym (>15 hr/wk) ☐ Gym (>8hr/wk) ☐

Gym (<8hr/wk) ☐ Control ☐

2. **DAUGHTER'S LIFESTYLE INFORMATION - DIET**

2.1 During **your daughter's childhood** (from birth to present) how often did she eat/drink the following foods?

| | | Frequency | | | | | |
|---|---|---|---|---|---|---|---|
| Food | Never | 1-2 Times Daily | 3 + Times Daily | 1-2 Times Weekly | 3 + Times Weekly | 1-2 Times/ Month | 3+ Times/ Month |
| Alcohol | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Tea/Coffee | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Cola/Pop | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Milk | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Yogurt | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Cheese | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Cottage Cheese | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Pizza w Cheese | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Sour Cream | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Ice Cream/Milk | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Beans | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Beets | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Broccoli | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Red Meat | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| White Meat | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Foul eg. Chicken | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Shell Fish | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Fish | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Organ Meat | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

2.2 Does your daughter eat a special diet? Yes ☐ No ☐

2.3 If yes, please specify the type of diet:

Vegetarian ☐
Low sodium ☐
Low cholesterol ☐
Other (please specify) _____

2.4 Does your daughter take a calcium supplement?
Yes ☐ No ☐

2.5 If yes, how many times a day does she take it?
_____ times/day

2.6 What is the name of the supplement? _____

2.7 How many milligrams of calcium does it contain? _____ mgs.

2.8 Does your daughter take a multivitamin supplement?
Yes ☐ No ☐

2.9 If yes, how many times a day does she take it?
_____ times/day

2.10 What is the name of the supplement? _____

2.11 How many milligrams of calcium does it contain? _____ mgs.

2.12 Does your daughter take any of the following antacids on a daily basis?

Rolaids, Tums, Yes ☐ No ☐

2.13 If yes, how many times a day does she take it?
_____ times/day

2.14 Does your daughter take a bran or fiber supplement?
Yes ☐ No ☐

2.15 If yes, how many times a day does she take it?
_____ times/day

2.16 What is the name of the supplement? _____

2.17 How many grams of fiber does it contain? _____ gm/serving.

## 3. DAUGHTER'S LIFESTYLE INFORMATION - PHYSICAL ACTIVITY

3.1 Rate (cirle one) your daughter's overall level of physical activity **compared to her friends.**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| seldom active | sometimes active | active | moderately active | very active |

3.2 How would you describe the types of games your daughter plays **in her free time?** (Circle one)

| 1 | 2 | 3 |
|---|---|---|
| games such as board games, drawing, puzzles, etc. | games requiring some running, jumping, climbing, throwing, etc. | mostly running, jumping, climbing, and throwing etc. |

3.3 Is your daughter regularly involved in heavy physical work (e.g. farm chores or heavy lifting) outside of sports?

Yes ☐ No ☐

3.4 How would you rate your daughter's <u>current</u> level of **PHYSICAL ACTIVITY** compared to others her age (Check one only)

very low ☐ low ☐ average ☐ high ☐ very high ☐

3.5 How would you rate your daughter's <u>current</u> level of **PHYSICAL FITNESS** compared to others her age (Check one only)

very low ☐ low ☐ average ☐ high ☐ very high ☐

3.6 During **the last week**, how many times did your daughter do any of the activities listed below, about how much time (average) did she spend doing the various activities on each occasion, and how difficult or strenuous was the activity on average

For difficulty or strenuousness of the activity, use the following guidelines:

Light: slight sweating and slight increase in breathing

Moderate: noticeable sweating and above normal breathing

Heavy: heavy sweating and heavy breathing

| ACTIVITY | # Of Times | MINUTES EACH TIME 1-15 | 16-30 | 31-59 | 60 + | STRENUOUSNESS LIGHT | MOD | HEAVY |
|---|---|---|---|---|---|---|---|---|
| Walking For Exercise | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Calisthenics | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Aerobics | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Weight Lifting | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Stat Cycling/ Bicycling | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Jogging/ Running | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Bowling | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Social Dancing | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Modern/ Jazz Dancing | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Racquet Sports | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Golf | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Swimming | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Gardening/ Yard Work | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| House Work | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Baseball/ Softball | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Basketball | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Volleyball | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Curling | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Skipping | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Skating/ Rollerblading | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Skiing/ Down Hill | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Skiing/ Cross-Country | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Ringette/ Ice Hockey | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Tag | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Intra-murals | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Others (Please specify) | | | | | | | | |
| _____ | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| _____ | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| _____ | ___ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

OR, I DID NOTHING LIKE THIS IN THE LAST WEEK ☐

---

3.7 Approximately how many hours does your daughter spend watching television or playing video/computer games each day?

_____ average hours <u>per</u> day from Monday-Friday

_____ average hours <u>per</u> day on Saturday and Sunday

3.8 Please provide any other comments regarding your daughter's lifestyle or physical activity which you think we should know about.

_____

_____

3.9 How does your daughter usually get to and from school?

Fall and Spring

Walk ☐ Bike ☐ Car ☐ Bus ☐ Other:_____

Winter

Walk ☐ Bike ☐ Car ☐ Bus ☐ Other:_____

3.12 How far is it from home to your daughter's school?

_____ kilometers.

3.13 Does your daughter usually come home for lunch? Yes ☐ No ☐

3.14 How does your daughter usually get to and from school at lunch and after school?

Fall and Spring

Walk ☐ Bike ☐ Car ☐ Bus ☐ Other:_____

Winter

Walk ☐ Bike ☐ Car ☐ Bus ☐ Other:_____

3.15 After eating lunch, what type of activity, if any, does your daughter do? _____

3.16 How long are your daughter's lunch breaks? _____ minutes.

3.17 Does your daughter take Physical Education at school?

Yes ☐ No ☐

3.18 How many times per week does she have Physical Education classes? _____ times per week.

3.19 How long are Physical Education classes usually? ____ minutes.

## 4. DAUGHTER'S DETAILED MEDICAL HISTORY AND STATUS

4.1 Has your daughter seen a doctor in the last 6 months for a medical concern? Yes ☐ No ☐

4.2 If yes, what was the reason for her visit?

_____

_____

_____

_____

4.3 Has there been any change in your daughter's general health during the last 6 months? Yes ☐ No ☐

4.4 If yes, please describe the nature of the change:

_____

_____

4.5 Has your daughter been hospitalized in the last year? Yes ☐ No ☐

4.6 If yes, please indicate the medical condition(s) which was being treated:

_____

4.7 Has your daughter had any surgery in the past 2 years? Yes ☐ No ☐

4.8 If yes, list the procedure and approximate date of surgery.

| Type of Surgery | Date of Surgery | Length of Hospital Stay |
|---|---|---|
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |

4.9 Has your daughter ever been treated for any of the following conditions? [hyper = excess; hypo = deficiency]

| | | | | | |
|---|---|---|---|---|---|
| food allergies | yes ☐ | no ☐ | asthma | yes ☐ | no ☐ |
| other allergies | yes ☐ | no ☐ | kidney disease | yes ☐ | no ☐ |
| back pain | yes ☐ | no ☐ | liver problems | yes ☐ | no ☐ |
| scoliosis | yes ☐ | no ☐ | gastrointestinal | | |
| epilepsy | yes ☐ | no ☐ | disease | yes ☐ | no ☐ |
| osteoporosis | yes ☐ | no ☐ | muscular | | |
| rheumatoid | | | dystrophy | yes ☐ | no ☐ |
| arthritis | yes ☐ | no ☐ | osteoarthritis | yes ☐ | no ☐ |
| diabetes | yes ☐ | no ☐ | anemia | yes ☐ | no ☐ |
| excess urinary | | | malabsorption | yes ☐ | no ☐ |
| calcium | yes ☐ | no ☐ | excess blood | | |
| hyperparathyroid | yes ☐ | no ☐ | calcium | yes ☐ | no ☐ |
| hyperthyroidism | yes ☐ | no ☐ | hypoparathyroid | yes ☐ | no ☐ |
| hypothyroidism | yes ☐ | no ☐ | other (specify):_____ | | |

4.10 Has your daughter had a bone scan or a diagnostic X-ray in the last year? Yes ☐ No ☐

4.11 If yes, what body part was X-rayed? _____

4.12 Has your daughter ever had a fractured bone? Yes ☐ No ☐

4.13 If yes, please indicate which bone(s) was/were fractured and when the fractures occurred.

1st fracture: body part_____ ____ mo. ____ yr.
2nd fracture: body part_____ ____ mo. ____ yr.
3rd fracture: body part_____ ____ mo. ____ yr.

4.14 Has your daughter ever been hospitalized or confined to bed for any reason, or had a limb immobilized (e.g. arm in a cast) for 21 days or longer?

Yes ☐ No ☐

4.15 If yes, list the condition, approximate date it occurred, and the length of time she was hospitalized or immobolized.

| | Injury type | Date of Injury | Time Immobolized |
|---|---|---|---|
| e.g. | wrist fracture | July, 1982 | 6 weeks |
| | _____ | _____ | _____ |
| | _____ | _____ | _____ |
| | _____ | _____ | _____ |

## 5. DAUGHTER'S MEDICATIONS

5.1 Is your daughter currently taking any prescription medications?
Yes ☐ No ☐

5.2 If yes, which medications is she taking?

_____

_____

5.3 What are these medications for?

_____

_____

5.4 Has your daughter ever taken any of the following medications? Please indicate at what age she began to use them, and for how long she used them.

| Medication | Currently Using | Age at Start | Duration of Use |
|---|---|---|---|
| e.g. Insulin | _____ | 6 yrs old | 2 years |
| Calcium preparations | _____ | _____ | _____ |
| Antacids | _____ | _____ | _____ |
| Inhaled steroids | _____ | _____ | _____ |
| Anabolic steroids | _____ | _____ | _____ |
| Fluoride | _____ | _____ | _____ |
| Vitamin D compounds | _____ | _____ | _____ |
| Calcitonin | _____ | _____ | _____ |
| Diuretics | _____ | _____ | _____ |
| Heparin | _____ | _____ | _____ |
| Cortisone (oral) | _____ | _____ | _____ |
| Corticosteroids | _____ | _____ | _____ |
| Anti-inflammatories | _____ | _____ | _____ |

Thyroid preparations _____ _____ _____

## 6. DAUGHTER'S MEDICAL DECLARATION

6.1 **Does your daughter have any medical/health condition which might prevent her from participating in this study?**
Yes ☐ No ☐

6.2 **I (mother) certify that the information provided on this form is correct.**

**Signature of mother:** _____

**Signature of daughter:** _____

**Date:** _____

# APPENDIX 6

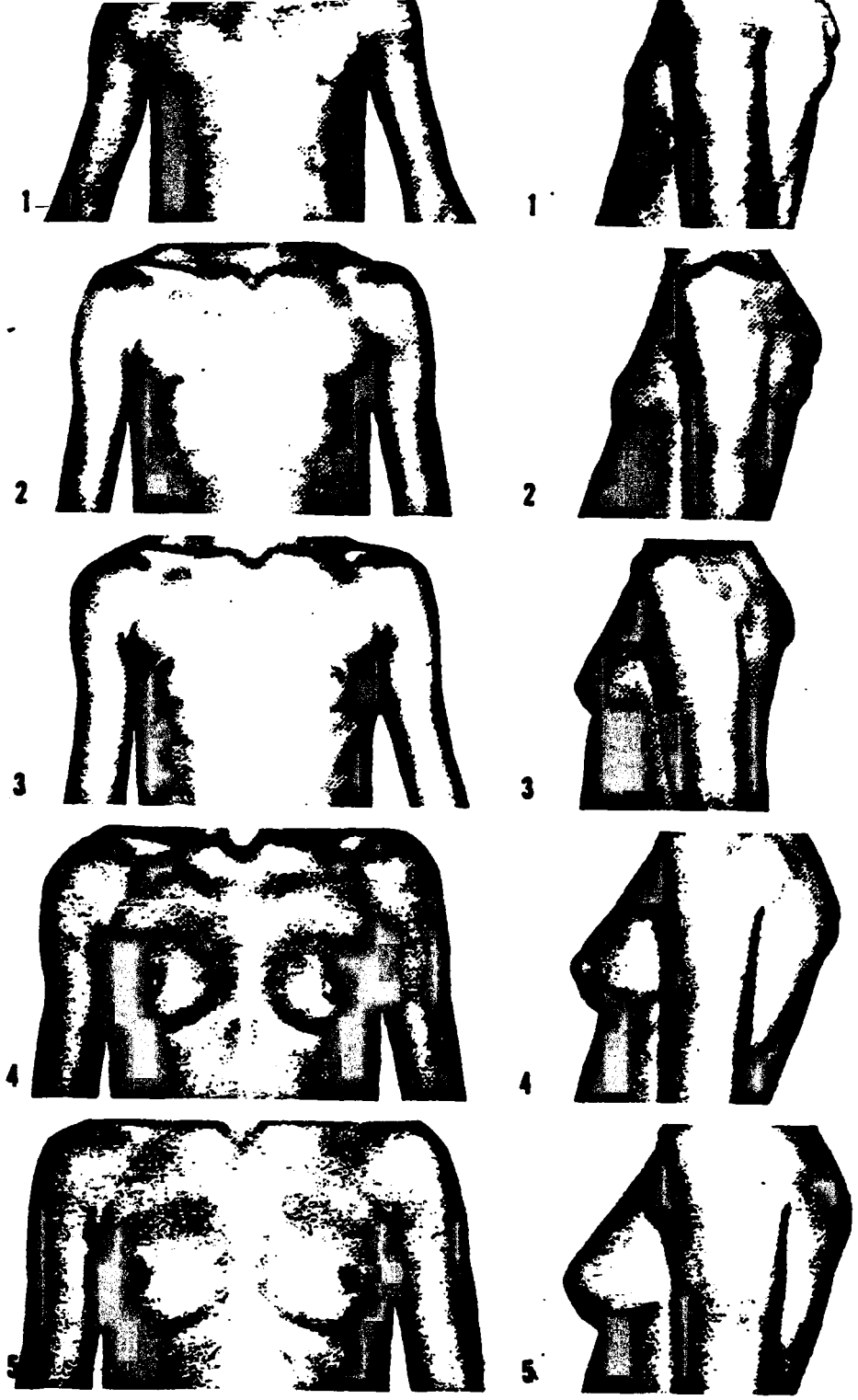
Mother/Daughter Physical Activity and Bone Density Study

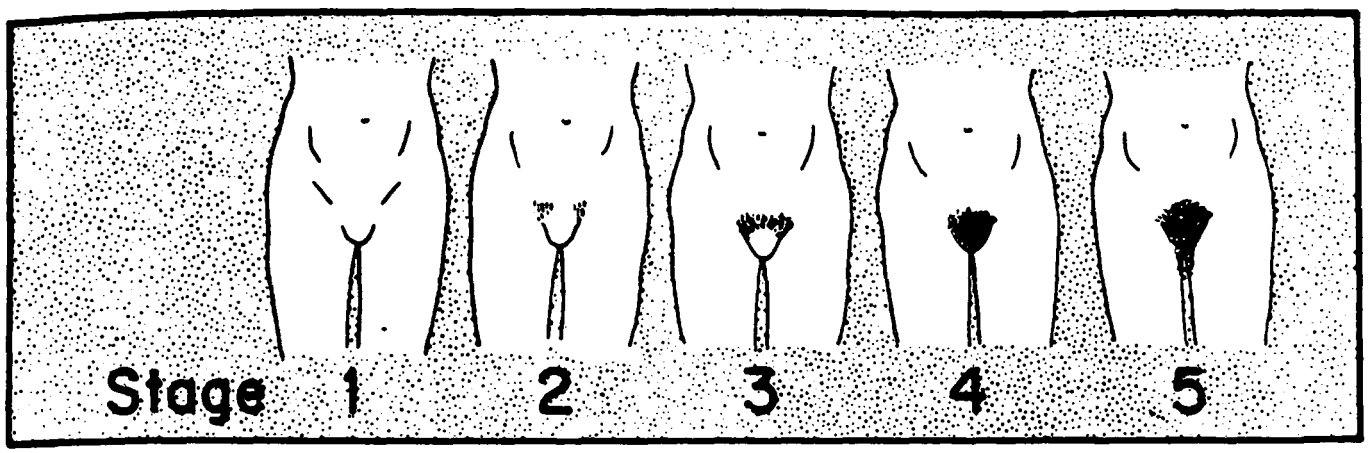
Child's Sexual Maturity Assessment Form

## Mother's Assessment of Child's Maturity Status

Your daughter will experience rather dramatic changes in size and sexual maturity as she advances toward puberty and adulthood. Considerable changes also occur in hormonal status with advancing sexual maturity, and these changes can have a dramatic influence on bone development and mineralization. These hormonal changes are closely correlated with changes in appearance of secondary sexual characteristics such as pubic hair and breast development. It is important that we know the sexual maturity status of your daughter, since this reflects her underlying hormonal status at the time of the bone density measurements. With this information we can compare the bone density measures of girls at the same level of maturity, without having the interpretation of the results complicated by different hormonal levels.

From the attached pictures, please circle the stage of breast and pubic hair development which you think most closely resembles your daughter. **The stages for breast and pubic hair development may be different, so please make independent selections for each characteristic.**


1
1
2
2
3
3
4
4
5
5


Stage 1 2 3 4 5

# APPENDIX 7

Mother/Daughter Physical Activity and Bone Density Study
Dietary Instruction Form

Mother's Form

**DIETARY DIARY**
**INSTRUCTIONS FOR COMPLETION**

**The idea behind the diary is that it enables us to construct an accurate picture of the quantities of many nutrients that you consume each day. For this reason please be as detailed and specific as possible.**

**E.g. If you ate a sandwich for lunch please add more detail than telling us what the filling was. We would like to know what sort of bread was used, whether you had butter or margarine, if you had salt or pepper, whether it contained other dressings such as mayonnaise and any additional information you feel that you would like to include. In short, please tell us exactly what you ate in as much detail as possible.**

**In addition to knowing what you ate, we also want to know how much you ate.**

**E.g. If you drank a glass of milk, please tell us what kind of milk (whole, skimmed, partly skimmed, chocolate etc), and also whether you had a small, medium or large glass.**

**It is important to stress that this is not a "test" of what you and your children eat, and we do not want you to change your eating habits because you are completing the diary.**

**Please also record what you drink during the day (other than water), because this is valuable information as well.**

**E.g. If you drank tea in the morning, please tell us the size of the serving, whether you added whole, partly skimmed or skimmed milk, and how much sugar (or artificial sweetener) you added.**

**IN SUMMARY**

- **Record everything you and your daughter eat.**
- **Record items in as much detail as possible.**
- **Record the quantity of each item that you record.**
- **Try not to let the diary influence your eating habits.**

Please do not hesitate to contact Shannon Frazer at 905-528-6243 if you have any questions regarding the completion of this diary.

# *FOOD DIARY*

**Instructions:**

1) Record all food and beverages you eat or drink over the 3 days and where and when eaten

It's best to write down what you've eaten/drank immediately afterward so that you won't forget any item

2) **List the amount**
- cups (250 mL), ounces or mL
- teaspoons or tablespoons (level or heaping)
- slices or ounces/grams
- dimensions for meat, fish, cheese,... (for example 2"x1"x2")
- scale weight - weigh container then container with food

3) Give **method of preparation**
- broiled, fried, boiled, baked...

4) Give **Brand Names / Restaurant names**
- for example - 2 cups of Kraft dinner (not macaroni and cheese)

5) Note anything added to food or drink
- for example - 1 1/2 cups of coffee with 1 teaspoon of sugar and 1 tablespoon of homogenized milk

6) For combination foods (casseroles, sandwiches, sauces...) record the main ingredients and amounts of each and total amount of recipe along with the portion size that you had

for example:
- egg salad sandwich -2 pieces of whole wheat bread
  -2 teaspoons Becel margarine
  -half of egg salad recipe

egg salad recipe - 3 large eggs, boiled, 3 tablespoons Miracle Whip salad dressing, 1 green onion

7) Include all supplement and vitamin preparations

8) Please record your medications and amounts

***Try to be as descriptive as possible when recording your food diary AND most of all, try not to change what you eat or drink for this study - we're interested in you !***

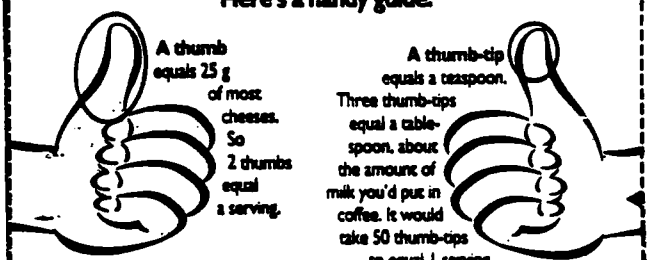
# POCKET SERVING SIZER AND FOOD GUIDE

We all want to enjoy a balanced diet. But what does a serving size actually look like? This Pocket Serving Sizer shows you. Used with Canada's Food Guide to Healthy Eating, it can make sure you're enjoying a variety of foods from the 4 Food Groups. We've included a synopsis of the Food Guide so you can keep track of meals eaten outside the home. (It's on the back of the Serving Sizer.)

## HANDY SERVING SIZER

(With special thanks to the originator, Jane Kirby, R.D. of Glamour Magazine)

**What's a serving size actually look like? Here's a handy guide:**

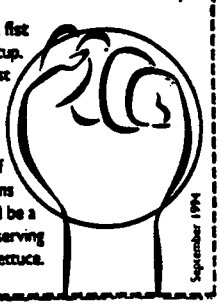


A thumb equals 25 g of most cheeses. So 2 thumbs equal a serving.

A thumb-tip equals a teaspoon. Three thumb-tips equal a tablespoon, about the amount of milk you'd put in coffee. It would take 50 thumb-tips to equal 1 serving of milk.

A palm equals a serving of meat, fish or poultry. That's without fingers and thumb!

A fist equals a cup. A fist would be 1½ servings of yogourt. A fist size of raw leafy greens would be a serving of lettuce.



DAIRY BUREAU OF CANADA
1981 McGill College Avenue, Suite 1330
Montreal, Qc H3A 2X9

September 1994

# DIETARY DIARY SHEET

**Name:** _____ **Date** _____

**Breakfast:-**

**Snacks:-**

**Lunch:-**

**Snacks:-**

**Dinner:-**

**Snacks:-**

**If you need extra space, please continue on another sheet of paper.**

# DIETARY DIARY SHEET

**Name:** _____ **Date** _____

**Breakfast:-**

**Snacks:-**

**Lunch:-**

**Snacks:-**

**Dinner:-**

**Snacks:-**

**If you need extra space, please continue on another sheet of paper.**

# DIETARY DIARY SHEET

**Name:** _____ **Date** _____

**Breakfast:-**

**Snacks:-**

**Lunch:-**

**Snacks:-**

**Dinner:-**

**Snacks:-**

**If you need extra space, please continue on another sheet of paper.**

# APPENDIX 8

# Investigation Into The Short Term Precision Of Bone Density Measurement By Peripheral Quantitative Computerised Tomography.

Several techniques are available for the measurement of bone density. These include radiogrammetry, ultrasonography, single-energy photon absorptiometry (SPA), dual-energy photon absorptiometry (DPA), dual energy x-ray absorptiometry (DXA) and quantitative computerised tomography (QCT) (Tothill, 1989). Various factors must be considered when assessing the value of each of these measurement techniques, including the anatomical site of measurement, speed of measurement, and the radiation dosage to both patient and operator. Perhaps, however, the issues of greatest importance are the precision and accuracy of the technique.

Data are available relating to the accuracy and precision of most of the above methods of measurement. SPA has been reported to have a precision of 1-2%, and an accuracy (based on in vitro measurement) of approximately 5% (Karlalainen, 1973). Similar results have been reported for DPA by both Wahner et al. (1988) and Mazess et al. (1989). The precision of DXA measurements in vivo is approximately 1%, and in vitro accuracy approximately 5% (Wahner et al., 1988; Orwoll et al., 1989). The accuracy of QCT is reported to be slightly lower (5-10%), with an in vivo precision of 1-2% (Alhava, 1991).

While each measurement technique has a unique set of advantages and disadvantages, QCT is the only method which provides a true volumetric density (g/cm3) and has the unique ability to distinguish between cortical and trabecular tissue (Tothill, 1989). The major limitations associated with QCT measurements are its relatively poor accuracy, which can be due in part to overlying layers of body fat (Alhava, 1991) and the fatty composition of bone marrow (Tothill, 1989), and the relatively high radiation dosage received by the patient. The

high radiation dosage is a result of both the high energy x-ray beam required to penetrate the volume of soft tissue at regions such as the spine, and the technique of repeat scanning in order to produce a complete image of the area under scrutiny.

A new QCT technique which minimises problems associated with both fat coverage and radiation exposure via a specific equipment modification to measure at the distal radius has only recently become available. This technology, termed peripheral QCT (pQCT), utilises relatively low levels (6mrem patient dosage) of radiation (the wrist being relatively small in cross-section), making it suitable for the repeated measurements essential in a clinical setting. An iterative contour detection algorithm is also included in the software package which is reported to have the capability of differentiating the soft tissue-bone, and both the trabecular-subcortical and subcortical-cortical boundaries.

The purpose of this study was to investigate the short term in vivo precision of pQCT measurements in children and adults.

## Method

Two groups of subjects were recruited from the Department of Kinesiology at McMaster University, and from subjects already involved in a larger study. Recruitment was verbal and informal in nature. Group A consisted of 9 adults, aged 42.1±3.1 years (mean±SD) with group B composed of 12 children, of mean age 10.5±1.7 years. Subjects were fully informed of the inherent risks and nature of the experiment, and informed consent was obtained in accordance with the McMaster University Medical Centre Ethics Committee.

Short-term precision of pQCT (Stratec XCT 960, location, software version) was measured by repeating measurement s of the distal radius and ulna in both groups during a single visit to the laboratory. Forearm length was measured with a standard ruler from the olecron process to the medial edge of the distal ulna with the elbow flexed at 90° and the radioulna joint pronated.

During measurement, subjects were seated with the right shoulder abducted at 90°, the elbow flexed at 90° and the radio-ulna joint fully pronated. The arm was secured tightly at the elbow and wrist, and subjects were requested to refrain from movement and conversation while the device completed measurement. Following demarcation of the distal radius via a longitudinal (scout) scan, a single (1mm wide) transverse (CT) scan was initiated. This was positioned at a point proximal to the demarcation line equivalent to 4% of forearm length in adult subjects. In children a distance of 6% of forearm length was utilised in order to avoid complications induced by the epiphysial growth plates. Resolution of the scan was set such that voxel size (the smallest area of measurement) represented a cuboid of dimensions 0.59 by 0.59 by 1.00 mm.

On arrival at the laboratory and following explanation of the protocol children were able to nominate either themselves or their mother/father as the first to be scanned. Subjects were scanned twice, with full repositioning between scans. Total measurement time per scan was approximately 7 minutes, with the inter-trial interval being approximately 10 minutes. The software included with the pQCT device allows a comparison with the demarcation points used in previous scans of the same subject, decreasing the likely variability between scans. This technique was not used in this study, as it was felt most appropriate to investigate the reliability

of the technique including all possible sources of error. This allows a true estimation of the errors associated with all processes involved in use of the pQCT device.

The dependent variables measured were total bone density, trabecular bone density and cortical bone density for both the radius and ulna. Also the cross-sectional area of the total bone, and both the cortical and trabecular subfractions was assessed for both radius and ulna.

Results were assessed for each dependent variable in terms of absolute, constant and variable error. Absolute error (AE) represents the average total error present in the repeated scan, regardless of the arithmetic sign of the error.

i.e. $$AE = \text{SQRT}\ \frac{\Sigma(D^2)}{N}$$

(Where D represents the difference between the two measurements)

Constant error (CE) is defined as the average error between measurements and is sensitive to the arithmetic sign of the individual errors.

i.e. $$CE = \frac{\Sigma(D)}{N}$$

Variable error (VE) is represented by the standard deviation of the error scores. The magnitude of the error score in each case was then expressed as a percentage of the first measurement.

Results

Table 1. Error Coefficients for Measurement of Forearm Bone Mineral Density* by pQCT

| | Variable | Density 1 ($mg/mm^3$) | Density 2 ($mg/mm^3$) | AE (%) | CE (%) | VE (%) |
|---|---|---|---|---|---|---|
| **Adults (Radius)** | **Total** | 369.4 ± 54.1 | 370.9 ± 61.8 | 2.51 | 0.07 | 3.8 |
| | **Trabecular** | 208.5 ± 45.3 | 207.9 ± 44.6 | 1.32 | 0.15 | 2.06 |
| | **Cortical** | 600.4 ± 78.9 | 600.2 ± 94.3 | 4.28 | 0.88 | 6.01 |
| | | | | | | |
| **Children (Radius)** | **Total** | 317.6 ± 61.2 | 315.2 ± 59.0 | 5.84 | -4.78 | 7.89 |
| | **Trabecular** | 189.1 ± 33.8 | 190.8 ±31.7 | 4.86 | 1.79 | 5.83 |
| | **Cortical** | 469.1 ± 97.2 | 466.1 ± 103.9 | 10.89 | -6.03 | 14.78 |
| | | | | | | |
| **Adults (Ulna)** | **Total** | 301.2 ± 42.6 | 307.6 ± 45.9 | 3.78 | 0.45 | 4.69 |
| | **Trabecular** | 207.1 ± 62.0 | 211.1 ± 63.8 | 2.77 | 1.78 | 4.55 |
| | **Cortical** | 402.9 ± 38.8 | 408.4 ± 47.2 | 6.69 | 2.23 | 12.56 |
| | | | | | | |
| **Children (Ulna)** | **Total** | 307.7 ± 39.8 | 311.1 ± 41.8 | 3.82 | -0.61 | 4.61 |
| | **Trabecular** | 228.2 ± 31.8 | 211.5 ± 77.4 | 6.06 | -4.20 | 6.31 |
| | **Cortical** | 368.7 ± 70.3 | 365.5 ± 91.8 | 32.4 | 20.89 | 41.69 |

* Densities reported as Mean ± Standard Deviation

Table 2. Error Coefficients for Measurement of Forearm Bone Areas* by pQCT

| | Variable | Area 1 ($mm^2$) | Area 2 ($mm^2$) | AE (%) | CE (%) | VE (%) |
|---|---|---|---|---|---|---|
| **Adults (Radius)** | **Total** | 336.9 ± 113.8 | 339.5 ± 120.6 | 3.47 | 0.31 | 4.79 |
| | **Trabecular** | 184.2 ± 87.0 | 185.3 ± 90.2 | 4.14 | -1.88 | 5.51 |
| | **Cortical** | 121.3 ± 21.3 | 122.5 ± 24.0 | 4.28 | 2.03 | 5.69 |
| | | | | | | |
| **Children (Radius)** | **Total** | 211.8 ± 32.1 | 211.6 ± 31.04 | 8.94 | 0.85 | 12.16 |
| | **Trabecular** | 98.4 ± 21.7 | 103.1 ± 26.4 | 11.07 | -3.26 | 12.34 |
| | **Cortical** | 91.0 ± 16.9 | 85.5 ± 7.5 | 12.96 | -8.51 | 16.51 |
| | | | | | | |
| **Adults (Ulna)** | **Total** | 168.5 ± 43.4 | 167.7 ± 46.1 | 3.57 | -0.74 | 4.77 |
| | **Trabecular** | 73.6 ± 22.49 | 71.7 ± 25.3 | 7.11 | -3.25 | 9.24 |
| | **Cortical** | 75.3 ±18.0 | 76.54 ± 21.7 | 6.70 | 2.23 | 12.57 |
| | | | | | | |
| **Children (Ulna)** | **Total** | 110.9 ± 19.2 | 108.5 ± 16.9 | 8.38 | -3.39 | 11.85 |
| | **Trabecular** | 35.8 ± 13.6 | 27.6 ± 16.8 | 28.77 | 0.27 | 33.87 |
| | **Cortical** | 61.6 ± 14.9 | 69.5 ± 24.7 | 32.32 | 20.81 | 41.51 |

* Areas reported as Mean ± Standard Deviation

## Discussion

It can be seen from Tables 1 and 2 that measurement precision varies by both variable and the age of the subject being studied. While density measurements seem to be fairly robust in terms of achieving a relatively high precision for both adults and children, area measurements, especially in children, appear to be of questionable reliability. It is doubtful if the density of either tissue type or the bone as a whole varies greatly along the relatively small distance potential repositioning error. Changes in the area of tissue types are known to change along the length of the arm, with the distal portion being composed of a greater proportion of trabecular bone than the diaphysis. Therefore it is not surprising that a small error in positioning induces a relatively large change in area measurements, but not in density measurements. Measurements of cortical density, especially at the ulna and in children, were also relatively imprecise, the underlying reason for which is not clear. It is possible that due to the relatively small proportion of cortical tissue that a small change in positioning that, for example, includes a particularly dense area of cortex induces large effects on the data.

In terms of absolute error, which is probably of greatest importance to the clinician, a reproducibility of within 3% is possible for total and trabecular bone densities. This compares favourably with values of 2.8% and 2% reported for the spine (Genenat, 1980) and forearm (Ruegsegger et al., 1976) using traditional QCT techniques.

It is apparent that the pQCT device is less precise when recording area measurements, especially in children. The effect of age may be due to the size difference in cross-section, leading to lower resolution when scanning children. Also it is possible that the iterative

algorithm used to detect tissue type boundaries is effected by the small size, or less differentiated tissue present in children.

The results of this study also show that measurement of density and area at the ulna is relatively imprecise. This may be due to the fact that positioning was made on the basis of the radius, not the ulna itself. Also the positioning of the scan in many subjects was not sufficiently distal for a suitable cross-sectional area of the ulna to be scanned. Also in children the presence of the epiphysial growth plate may have affected the precision and results of ulna scanning.

In summary, the precision of the technique is dependent on factors including the age (or size) of the subject, and the variable being measured. The relatively poor precision of the device for measurement of some variables should not detract from the good precision obtained when measuring important and frequently measured variables of total and trabecular bone density. It is also worthy of note that the sample size utilised in this experiment is probably not sufficient for a detailed description of the performance of the device. Finally it should be noted that repeat scan precision is likely improved by use of the software available for ensuring accurate repositioning.