BONE SPEED OF SOUND IN OVERWEIGHT AND NORMAL-WEIGHT GIRLS AND ADOLESCENTS

Matthew W. Yao

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Supervisor: Bareket Falk, Ph.D.

The Faculty of Applied Health Sciences
Brock University
St. Catharines, ON.

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ABSTRACT

Over the last two decades, the prevalence of obesity in the general population has been steadily increasing. Obesity is a major issue in scientific research because it is associated with many health problems, one of which is bone quality. In adult females, adiposity is associated with increased bone mineral density, suggesting that there is a protective effect of fat on bone. However, the association between adiposity and bone strength during childhood is not clear. Thus, the purpose of this study was to compare bone strength, as reflected by speed of sound (SOS), of overweight and obese girls and adolescents with normal-weight age-matched controls. Data from 75 females included normal-weight girls (G-NW; body fat ≤ 25%; n = 21), overweight and obese girls (G-OW; body fat ≥ 28%; n = 19), normal-weight adolescents (A-NW, body fat ≤ 25%; n = 13) and overweight and obese adolescents (A-OW; body fat ≥ 28%; n = 22). Nutrition was assessed with a 24-hour recall questionnaire and habitual physical activity was measured for one week using accelerometry. Using quantitative ultrasound (QUS; Sunlight Omnisense™), bone SOS was measured at the distal radius and mid-tibia. No differences were found between groups in daily total energy, calcium or vitamin D intake. However, all groups were below the recommended daily calcium intake of 1300 mg (Osteoporosis Canada, 2008). Adolescents were significantly less active than girls (14.7 ± 0.6 vs. 6.3 ± 0.6% active for G and A, respectively). OW accumulated significantly less minutes of moderate-to-very vigorous physical activity per day (MVPA) than NW in both age groups (114 ± 6 vs. 57 ± 5 min/day for NW and OW, respectively). Girls had significantly lower radial SOS (3794 ± 87 vs. 3964 ± 64 m/s for G-NW and A-NW, respectively), and tibial SOS (3678 ± 86 vs. 3878 ± 52 m/s for G-NW
and A-NW, respectively) than adolescents. Radial SOS was similar in the two adiposity groups within each age group. However, tibial SOS was lower in the two overweight groups (3601 ± 75 m/s vs. 3739 ± 134 m/s for G-OW and A-OW, respectively) compared with the age-matched normal-weight controls. Body fat percentage negatively correlated with tibial SOS in the study sample as a whole (r = -0.30). However, when split into groups, percent body fat correlated with tibial SOS only in the A-OW group (r = -0.53). MVPA correlated with tibial SOS (r = 0.40), once age was partialed out. In conclusion, in contrast with the higher bone strength characteristic of obese adult women, overweight and obese girls and adolescents are characterized by low tibial bone strength, as assessed with QUS. The differences between adiposity groups in tibial SOS may be at least partially due to the reduced weight-bearing physical activity levels in the overweight girls and adolescents. However, other factors, such as hormonal influences associated with high body fat may also play a role in reducing bone strength in overweight girls. Further research is required to reveal the mechanisms causing low bone strength in overweight and obese children and adolescents.
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<th>Definition</th>
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<tr>
<td>A-NW</td>
<td>Adolescent normal-weight</td>
</tr>
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<td>A-OW</td>
<td>Adolescent overweight</td>
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<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>BF%</td>
<td>Body fat percentage</td>
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<tr>
<td>BIA</td>
<td>Bioelectrical impedance analysis</td>
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<tr>
<td>BMC</td>
<td>Bone mineral content (g)</td>
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<td>BMD</td>
<td>Bone mineral density (g/cm)</td>
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<td>BMI</td>
<td>Body mass index (kg/m²)</td>
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<tr>
<td>BSI</td>
<td>Bone strength index</td>
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<tr>
<td>DXA</td>
<td>Dual-energy X-ray absorptiometry</td>
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<td>G-NW</td>
<td>Girl normal-weight</td>
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<td>G-OW</td>
<td>Girl overweight</td>
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<td>pQCT</td>
<td>Peripheral quantitative computed tomography</td>
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<td>LBM</td>
<td>Lean body mass (kg)</td>
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<td>METs</td>
<td>Metabolic equivalents</td>
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<td>MVPA</td>
<td>Moderate-to-very vigorous physical activity (min/day)</td>
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<td>NW</td>
<td>Normal-weight</td>
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<td>OW</td>
<td>Overweight</td>
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<td>QCT</td>
<td>Quantitative computed tomography</td>
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<td>QUS</td>
<td>Quantitative ultrasound</td>
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<td>SOS</td>
<td>Speed of sound (m/s)</td>
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CHAPTER 1: INTRODUCTION

1.1: Background

Childhood obesity has been increasing at alarming rates in the Western World. National surveys on Canadian school-aged children revealed that the prevalence of overweight youths increased from 15% in 1981 to approximately 25% in 1996 (Tremblay and Willms, 2000). He and Beynon (2006) concluded that currently 16.6% and 11.8% of Canadian school-aged children are classified as overweight and obese, respectively. Obesity is associated with numerous diseases, including type-2 diabetes and cardiac disease (Sorof & Daniels, 2002; Young et al., 2000). However, in adults, especially in females, obesity may offer a protective effect against osteoporosis (Kirchengast et al., 2002). Yet it is unclear whether this is also the case in childhood and adolescence.

Dual-energy X-ray absorptiometry (DXA) is the most widely used technique to estimate bone mineral density (BMD) and bone strength in the general population. When using DXA to assess bone strength in overweight and obese youths, the results are contradictory. In view of DXA’s two-dimensional measurements of BMD, the conflicting results may be due to differences in the way in which bone and body size of children and adolescents are corrected for.

Quantitative ultrasound (QUS) is a relatively new technique used to assess bone properties in the general population, as well as in children and adolescents. Transaxial QUS measures the speed of sound (SOS) that travels through the bone of interest and so it is reflective of bone mineral density, as well as elasticity and microarchitecture. Thus, QUS may provide a better representation of bone strength than DXA. Another advantage of transaxial QUS over DXA is that its results are not affected by bone size. There are
very few studies that have used QUS to assess bone properties in overweight children and adolescents. The available data suggest that bone strength, especially in the weight-bearing bones, is lower in overweight youths than their normal-weight counterparts (Eliakim et al., 2001; Falk et al., 2008).

There are many factors that can affect bone health. Physical activity is a known determinant of bone mineral accrual during the growing years. Previous studies in overweight and obese youths have tried to evaluate physical activity levels with questionnaires. However, this method is rather crude and is not sensitive to low frequency and intensity of physical activity. Therefore, in the obese subjects, for whom physical activity levels are characteristically low, even small changes in physical activity may have significant effects on bone quality.

The present study compared bone SOS in overweight and obese pre- and late-pubescent girls with normal-weight controls. Physical activity was carefully assessed in the subjects by using accelerometry to help explain differences in bone strength between groups.

1.2: Purpose of the Study

The purpose of the study was to compare bone properties, specifically speed of sound (SOS) as assessed with quantitative ultrasound (QUS), of overweight and obese girls with their normal-weight counterparts, matched for chronological age and sexual maturity, as well as structured physical activity.

1.3: Research Hypotheses

1. The overweight girls will have lower tibial SOS values than normal-weight girls.

   Radial SOS will not differ significantly between the groups.
2. There will be an age-by-adiposity interaction, in which the difference in tibial SOS between the overweight and normal weight groups will be greater in the girls than in the adolescents.

3. All groups will have relatively low physical activity levels, as assessed by accelerometry. However, overweight girls will have lower habitual physical activity levels than normal-weight girls.

4. SOS will increase with age and maturity.

5. SOS will increase with increased physical activity levels.

1.4: Significance of the Study

Childhood obesity is a major problem in today's society as it is linked to many health problems such as poor bone quality. There is still no consensus as to whether the effect of adiposity on bone quality is positive or negative in children and adolescents. Indeed, there is some indication that excess adiposity has a negative impact on bone in children and adolescents (Eliakim et al., 2001; Falk et al., 2008; Goulding et al., 2000a). The present study examined this issue by comparing bone strength of an overweight and obese group with a normal-weight control group.

When assessing adults, BMI is associated with bone strength (Castro et al., 2005). Our study population involves girls as well as adolescents. By studying bone strength in females of different maturity status, this study may bridge the gap between the conflicting results of possible low bone strength in overweight youths and the protective effect of adiposity in obese adults. For example, the findings will clarify whether the protective effect of excess weight appears before adulthood. Moreover, it is in the formative years that most of the peak bone mass in life is accrued (Kroger et al., 1993), after which there
is a steady decline in BMD (Heaney et al., 2000). Thus, it is important to track bone strength in overweight individuals because obesity during childhood may prevent individuals from attaining optimal bone strength, making them susceptible to osteoporosis in later life.

Furthermore, during childhood and adolescence, body weight can independently predict the risk of bone fractures (Goulding et al., 2000a). However, it is unclear whether this increased risk is due to the greater mass straining the bones, or due to a relative weakness of the bones, or both. Potentially, a successful weight-reduction program in obese children may result in normal body weight maintenance, even into adulthood. However, if obese children are characterized by reduced bone strength, those who succeed in weight loss may be exposed to greater risk of osteoporosis-related fractures in adulthood.

Bone development can be enhanced with increased physical activity (MacKelvie et al., 2005). However, overweight children tend to have lower physical activity levels than normal-weight children (Hernandez et al., 1999; Trost et al., 2001). This difference is exacerbated in girls, as their physical activity levels tend to decline more than boys throughout the teenage years (Andersen et al., 1998; French et al., 2000). To our knowledge there is only one other study that has examined the relationship between bone quality, adiposity, and physical activity levels in children (Falk et al., 2008). This study used questionnaires, a crude assessment of physical activity. The present study used accelerometry, an objective method to measure physical activity, thus eliminating recall bias. Thus, the results of this study could highlight the importance of weight-bearing
physical activity and how the latter may serve as a means to improve bone status in obese girls.

Lastly, we used QUS to assess bone quality of our subjects. This method is still in its infancy when assessing children and adolescents. QUS has its advantages over DXA as a superior method to analyze bone quality in children and adolescents because QUS measurements are not affected by bone/body size and it does not involve any ionizing radiation. Thus, our results will validate QUS as a viable method for assessing bone strength in children and adolescents of different obesity status.
CHAPTER 2: LITERATURE REVIEW

2.1: Bone Characteristics

Bones are rigid organs that form the human skeleton. There are 206 bones, each working in a coordinated manner to facilitate a host of functions. The skeleton arrangement enables the body to move by providing places for the muscles to attach, it gives the body structure and it protects the body's vital organs. Its bone marrow continually produces blood cells. Lastly, bone tissue acts as a reservoir for minerals, especially calcium and phosphate (Malina et al., 2004).

The tubular bones of the skeleton are called long bones and they include among others, the tibia, radius, ulna, humerus, femur and fibula. The shaft of a long bone is called a diaphysis and it consists of a bone marrow cavity and cancellous bone surrounded by a dense outer layer called cortical bone. Cortical bone accounts for approximately 80% of the weight of an adult skeleton (Dempster, 2004). The cancellous bone, also referred to as spongy bone because of its low-density and porous structure, contain a network of branching strands of bone called trabeculae and this accounts for approximately 20% of the weight of an adult skeleton. At the ends of the long bones (epiphyses), the entire interior is filled with cancellous bone.

There are three types of bone cells: osteocytes, osteoblasts, and osteoclasts. Osteocytes are embedded within a bone matrix and are interconnected by processes, forming a mechanosensory network to regulate bone remodeling (Klein-Nulend et al., 2003). Osteocytes also serve to regulate nutrient exchange between the bone and blood. The matrix is the hard part of bone and it consists of collagen fibres tightly bound to
minerals of hydroxyapatite by a ground substance. The hydroxyapatite is comprised
mainly of calcium and phosphorus.

The challenge to maintain the shape of bone during growth is governed by the
opposing processes of bone deposition and bone resorption, and the rate at which these
forces act are influenced by maturation and the loads that are applied to the bone. Bone-
forming cells, called osteoblasts, are found on the surfaces of bone and they are
responsible for bone deposition. In this process, the osteoblast becomes an osteocyte
after being entrapped in newly formed collagen and ground substance. To complete the
mineralization process, calcium and phosphorus are added to the collagen. In contrast,
bone resorption is regulated by bone-removing cells, called osteoclasts, by continually
breaking down bone matrix and releasing its minerals into circulation when required
(Malina et al., 2004).

Periosteal apposition occurs when bone cells are added to the outer surface of
bone. Contrastingly, endocortical resorption occurs when bone cells on the inner surface
of bone are degraded. As a long bone grows, its diameter increases because of the
concurrent processes of periosteal apposition and endocortical resorption (Seeman,
2008). When the rate of periosteal apposition exceeds that of endocortical resorption, the
cortex widens and shifts further away from the central long axis of the bone,
strengthening the bone.

In conclusion, bone continually experiences remodeling and undergoes bone
turnover, especially during the formative years as it adapts to changes in body size,
maturation and the physical demands of the body.
2.2: Bone Growth

Bone growth consists of the positive balance between bone deposition and bone resorption. Throughout the formative years, the rate of bone deposition exceeds the rate of bone resorption, resulting in bone mineral accrual. There is a progressive increase in bone mass (measured in grams) during early childhood which accelerates during adolescence (Faulkner et al., 1996). The increase in bone mineral density (BMD; bone mass relative to bone area, usually measured in g/cm²) appears to peak during late adolescence (Kroger et al., 1993) and as much as 90% of one’s peak bone mass can be deposited by this time (Matkovic et al., 1994).

There are several factors that contribute to bone development, including genetics, hormones, nutrition, and physical activity. Genetics can account for up to 70% of the variability in peak BMD (Slemenda et al., 1991). Moreover, hormones such as growth hormone and estrogen affect bone mass during growth and can preserve bone mass, especially in postmenopausal women (Doren, 2000; Ohlsson et al., 1998). Since nutrition and physical activity are readily modifiable in one’s lifestyle, these are attractive factors to target for interventions. Therefore, in order to optimize bone mass, interventions should take place during childhood and adolescence.

During growth and maturation, girls experience an earlier growth spurt than boys, and consequently have slightly greater total bone mineral content (BMC; amount of bone mineral, usually in grams) than boys during early adolescence (Faulkner et al., 1996; Malina et al., 2004). While girls’ BMC plateaus at around 15 to 16 years of age, boys continue to increase their BMC into their 20’s, establishing greater total BMC than girls by late adolescence. A similar pattern exists for the development of sex differences in
BMD (Figure 2.1). The sex differences translate into boys having superior apparent bone strength compared with girls.

Fractures occur when the load applied to the bone exceeds its strength. The relationship between fracture incidence and age is bimodal. Although the elderly are most prone to fractures, children are at high risk for fractures as well, with forearm fractures being most common (Landin, 1983). From longitudinal data, it is estimated that half of all children and adolescents experience a fracture during growth (Jones et al., 2002). This high incidence of fracture is suggested to be the consequence of low BMD (Goulding et al., 1998). It may also be attributed to the six-month lag between the occurrence of peak BMC velocity and peak bone area velocity, suggesting a period of relative bone weakness during childhood (Faulkner et al., 2006). In girls, the peak in BMC gain occurs at approximately 12.7 years of age and the peak in bone area gain occurs at approximately 12.2 years of age. Therefore, the density of the bone may be
lower than optimal because the growth in bone size occurs sooner than its mineralization, leaving the bone susceptible to fracturing. This finding may be explained by the linear growth of bone not having sufficient periosteal apposition. During puberty, boys mainly add bone on the periosteal surface which greatly increases its integrity. Contrastingly, girls mainly add bone to the endocortical surface, which does not have a great contribution to bone strength (Schoenau et al., 2001). Thus, children’s, especially girls’, bones are vulnerable to fractures around puberty.

In early to mid adulthood, the rates of bone deposition and resorption are similar. However, in late adulthood (> 50 years of age), bone resorption exceeds bone deposition and bone mass consequently declines (Heaney et al., 2000). Luckey et al. (1996) showed that the BMD of early menopausal Caucasian women declined 2.4% per year. If severe enough, this progression can result in osteoporosis. Characteristically in osteoporosis, the bones are porous and leave the body's structure in a state of extreme fragility. As a result, bone fractures become a common occurrence, especially in the spine. Fractures in the hip are also of grave importance because of their association with morbidity and mortality (Braithwaite et al., 2003). Osteoporosis is a serious disease that currently affects over 300 million women over 65 years old worldwide (Dennison et al., 2006). Not only do osteoporotic fractures translate to physical (pain, death), social (confined to the home, loss of independence) and psychological (decreased self-confidence) distress (Pasco et al., 2005), they also have financial burdens. It is estimated that osteoporosis directly costs Canada’s healthcare system an estimated 1.3 billion dollars per annum (Lorrain et al., 2003). Although, osteoporotic fractures in the elderly are mainly related to trabecular bone, in children it is at the distal part of long bones, which are cortical and
trabecular bone, that are susceptible to fracture (Goulding et al., 2000a). Moreover, cortical bone can add strength to bones which are comprised of mainly trabecular bone (Halawa et al., 1978).

In summary, bone is a very dynamic organ, especially throughout childhood and adolescence. Its development is dependent on the rate of bone deposition versus the rate of bone resorption, both of which are influenced by biological and lifestyle factors, such as genetics, hormones, nutrition, and physical activity. As the growth curve for BMD, and hence bone strength, typically follows a rise-plateau-decline pattern throughout the course of life, it only makes sense to allocate efforts into optimizing bone mineral accretion during the formative years, the time when bones are increasingly susceptible to fracture.

2.3: Bone Strength

The terms stress and strain, among others, are fundamental to bone strength. When a force is applied to a bone, there is an internal resistance equal in magnitude but in the opposite direction and this counter-force is called stress. Strain refers to changes to the bone’s dimensions that are the consequence of an applied force. Objects under an applied force, whether it is tensile, compressive, torsional or bending, tend to follow a plot of the stress-strain relationship (Einhorn, 1992; Figure 2.2).
First, there is an elastic region where low levels of stress result in transient deformations of the bone. This part of the curve is linear and is known as Young’s modulus or the modulus of elasticity. It is also a measure of the object’s rigidity or stiffness. When the stress is greater than the upper limit of the elastic region (elastic limit), there is a non-linear relationship between stress and strain, called the plastic region. In this region, the bone undergoes permanent deformation. The strength of an object refers to the maximal amount of force that can be applied to the object before it fails. In the case of bone, its true strength is its ultimate strength and this point is at the upper limit of the plastic region (point of failure). At this point, any added stress will result in the bone fracturing (Einhorn, 1992).

The strength of bone is determined by a multitude of material and structural properties. BMD is thought to be a major variable that determines bone strength (Ammann & Rizzoli, 2003). BMC or BMD are often used as surrogates for bone strength because they are correlated and can account for up to 74% of the variability in
bone strength (Ammann & Rizzoli, 2003). The World Health Organization even uses BMD values of the proximal femur and lumbar spine for the diagnosis of osteopenia (T-score ≤ -1.0) and osteoporosis (T-score ≤ -2.5) (Christiansen, 1993). However, BMC or BMD do not always predict bone strength, as evident from a study presented by Turner (1993); despite higher BMD, rats that were fed fluoridated water had a greater (up to 27% greater) age-related bone strength decline than the animals that drank a less concentrated solution. The discrepant results could have been attributed to the weakening of bonds between bone minerals and matrix, or some other mechanical or morphological alterations.

Whatever the mechanism, this contradiction suggests that there are other parameters, independent of BMC or BMD that influence bone strength, namely bone geometry, bone elasticity and micro-architecture including porosity (Ammann & Rizzoli, 2003; Einhorn, 1992; MacDonald et al., 2006). Bone geometry, or bone volume, cross-sectional area and cortical thickness, can account for up to 80% of the variability in bone strength, with a positive relationship between all three parameters and bone strength (Voide et al., 2008). A bone's elasticity is its ability to return to its normal state after it has deformed from an external force. Currey (1999) showed that among various animal species there is a positive relationship between the elasticity of bone and its strength. Moreover, humans who have fractured their femoral necks had higher porosity in those bones than their controls who did not suffer a fracture, suggesting a negative relationship between bone porosity and strength (Crabtree et al., 2001). This relationship is exponential, as small increases in porosity can greatly diminish bone strength (Turner, 2002).
The "gold standard" of bone strength measurement is its ultimate strength, as determined by placing the bone under progressive stress until it breaks. It can be accomplished by bending or compressing the bone until it breaks. However, this can only be performed in an ex-vivo environment. Consequently, this technique is usually reserved for studies using animal models, wherein lies the limitation of generalizing the results to humans. Therefore, researchers need to use surrogate measures for bone strength in humans.

2.3.1. Bone Strength Assessment Techniques

Bone strength assessment throughout life is important so that we are able to identify those at risk of bone diseases, such as osteoporosis. The ideal technique to evaluate bone status would be non-invasive, readily available, cost-effective, accurate and precise, and capable of assessing the whole body and its individual parts. When working with children and adolescents, who are undergoing rapid growth and maturation, the measurements should not be affected by bone size. The three most commonly used methods to evaluate bone properties in the pediatric population are dual-energy X-ray absorptiometry (DXA), quantitative computed tomography (QCT) and quantitative ultrasound (QUS).

DXA is by far the most widely used method of assessing bone strength in the general population by measuring BMC. With the subject lying on this device, an overhead unit scans the body by passing photons through the bones of interest and subsequently converting the attenuation of the signal, through various algorithms, to BMC (in grams). If desired, this value can be corrected for a projected area of the bone to calculate areal BMD (in grams per unit area) (Gilsanz, 1998). It is worth noting that
true volumetric density is in grams per unit volume. Therefore, DXA's measurements are only an estimate of true BMD. Because of its two-dimensional nature, BMD is size-dependent. For example, a short child with small bones will have low BMD relative to a larger child with larger bones, even if the bones' true density was identical in the two boys (Schoenau et al., 2002).

DXA can measure all sites of the body, but it cannot differentiate between cortical and cancellous bone. It generates little radiation exposure of 1-4 μSv, depending on the site of the measurement, as different parts of the body require different radiation doses for the scan. By comparison, a chest X-ray involves approximately 50 μSv of radiation exposure. Lastly, DXA only measures BMC, which can only partially explain the variance in bone strength (Voide et al., 2008). However, with its large reference database, quick speed, high precision and easy operation, it is the preferred method for bone strength assessment, especially in the older population.

Another technique that has been used to assess bone strength, mainly in research, is QCT. This device can estimate true volumetric density by pooling data from multiple cross-sectional images of any desired bone, offering valuable information on bone geometry. Furthermore, QCT can differentiate the BMD between cortical and cancellous bone. BMC of children and adolescents as assessed with QCT has been shown to be highly correlated ($r^2 = 0.94$) with the same measurements using DXA (Wren et al., 2005). The research literature using QCT is limited, as it has relatively high radiation exposure (70-400 μSv) and it is not commonly available. However, its peripheral unit (pQCT), which is a smaller version of QCT, is now gaining more recognition because it has similar capabilities as its parent unit but with vastly reduced total radiation exposure ($< 2$
μSv). The disadvantage of pQCT is its limited availability and its ability to measure the appendicular skeleton only (Specker & Schoenau, 2005).

Transaxial QUS is a relatively new technique used to evaluate bone properties in adults, as well as in children and adolescents. Briefly, the QUS device has a probe with a transmitter that sends ultrasonic waves to measure the speed of sound (SOS) along the length of the bone of interest. The sound waves pass the soft tissue at a critical angle and scatter through the cortical bone and exit at the same critical angle. A receiver in the same probe detects the fastest signal to propagate between the transmitter and receiver, thus determining the velocity of the wave through the bone (Specker & Schoenau, 2005). The SOS will travel faster through media with increased density; cortical bone (approximately 4000 m/s), trabecular bone (approximately 1800 m/s) and soft tissue (approximately 1540 m/s). SOS has been shown to predict the risk of fracture in the elderly independent of BMD (Pluijm et al., 1999). The advantage of QUS over DXA is that it reflects BMD, as well as other bone properties such as elasticity and microstructure, all of which contribute to bone strength. QUS is also relatively inexpensive, portable and lacks ionizing radiation which is ideal for assessing bone strength of children and adolescents. Most importantly, unlike DXA, bone size does not affect its measurements.

There are also drawbacks of using QUS (Gilsanz, 1998; Specker & Schoenau, 2005). Namely, QUS can only measure bone strength at the radius, tibia, phalanges, and calcaneus, so there are limited clinically-relevant sites of measurement. Also, because the outcome of QUS (SOS) reflects several bone properties, there is inability to
distinguish the relative contributions of each parameter (e.g. bone density, elasticity, porosity) to the SOS value.

There is currently no bone strength assessment technique that can evaluate all the qualitative and quantitative factors that govern bone strength in humans. When evaluating the strength or properties of pediatric bone, it must be considered that children and adolescents are experiencing a period of rapid growth. This results in changes to bone, both to its macrostructure and microstructure – all of which will affect the bone parameters of the aforementioned bone assessment techniques. DXA is clearly the most widely used technique. However, in view of DXA's inherent limitations, especially in children, QUS may provide a better alternative, as has recently been recommended by Baroncelli (2008).

2.4: Bone and Physical Activity

Regular physical activity has many health benefits to children and adolescents and can prevent future chronic diseases, including enhancing bone health (Sothern et al., 1999). It is generally accepted that engaging in physical activity during growth enhances bone development (Boot et al., 1997; Janz et al., 2001; Janz et al., 2006; MacKelvie et al., 2003). For example, Bailey et al. (1999) used the PAC-Q to assess physical activity levels in children and found that active children, 8 to 14 years old, had up to 17% greater total BMC than their relatively inactive, maturity- and size-matched peers. Although there is a correlation between increased physical activity levels and increased BMC, the exact mechanism remains to be elucidated. There are two main explanations that may account for the increase in BMC with physical activity: the muscle force theory and the ground reaction force theory.
The muscle force theory is related to the mechanostat hypothesis and it stems from the idea that a bone will provide just enough strength to withstand voluntary physical loads and prevent fractures (Frost, 2000). Gravity exerts a small force on bones. However, the addition of muscle contractions greatly increases the load. Thus, bone strength is most influenced by the largest mechanical loads and strains, which are produced by the muscles (Burr, 1997; Schoenau & Frost, 2002). For example, because muscles have to work against less-than-optimal lever arms and overcome the resistance of body weight, it may require more than two kilograms of force on bone to move a kilogram of body weight (Frost, 1997). The muscle force theory is supported by many studies showing that increased muscle size is associated with increased bone mass in children and adolescents, as well as in adults (Aloia et al., 1995; Morris et al., 1997; Pietrobelli et al., 2002; Schoenau et al., 2002). In 8 to 14 year old children, Rauch et al. (2004) found that the velocity curves for lean body mass (LBM) accrual and BMC accrual followed similar patterns and that the peak for the LBM accrual preceded that of BMC accrual by 0.51 years and 0.36 years for girls and boys, respectively. However, the “muscle-bone unit” of the muscle force theory is still under scrutiny. Blimkie et al. (1996) found a dissociation between muscle strength and bone mass after they put adolescent girls through a 26-week resistance training program and found it to be ineffective in augmenting BMC or BMD despite significant increases in strength. One explanation may be that 26 weeks is an insufficient duration to induce changes in BMC or BMD. However, even when arm strength significantly increased after a 12-month high resistance strength training program, it was still unable to induce BMD or morphological changes to the upper limb bones in young women (Heinonen et al., 1996).
The muscle-force theory is also supported by animal studies. For example, Hamrick et al. (2002) showed that myostatin-deficient mice (greater muscle mass) had increased trabecular BMC in the proximal region of the humerus and increased cortical BMC in the deltoid crest of the humerus, but otherwise similar cortical BMC in the rest of the regions of the humerus as the wild-type mice. Thus, the more muscular mice had high BMC only in certain parts of the bone, especially at sites of muscle-insertion.

Since age-related bone loss appears to precede muscle mass and strength loss, muscle force may not account for all of the changes in bone (Marcus, 1995). Another possible explanation for the association between physical activity and BMC is the premise that weight-bearing, high-impact activities stimulate bone deposition with site-specificity. This theory may be referred to as the ground reaction force theory. Supposedly, the repetitive weight-bearing activity would deform the bone, and in response, more bone will be deposited at the site of loading to maintain bone homeostasis. The theory is defended by Carlson and Patel (2006) who examined BMD of the radii in primates and humans who exhibit different patterns of stress on the forearms. They found that suspensory primates and bipedal humans had less radial BMD than quadrupedal primates, suggesting that compressive ground reaction forces contribute to increasing BMD. There may also be geometrical adaptations to the weight-bearing or high-impact activity. Haapasalo et al. (2000) found that adult male competitive tennis players had greater bone strength, as assessed with pQCT, than their age-, height- and weight-matched controls and that this difference was attributed to increased bone size rather than volumetric BMD.
Weight-bearing physical activities include movements in which gravity exerts forces on the bones, such as running, jumping, and other activities that load the skeleton. It has been reported that ground reaction forces can be two to three times the body weight while running (Fuchs et al., 2001). The ground reaction force theory is supported by many studies on how high-impact weight-bearing sports affect bone quality in children and adolescents. These studies revealed that the BMD of gymnasts, 7 to 16 years old, was up to 16% higher than that of control girls participating in non-weight-bearing sports, such as swimming (Cassell et al., 1996; Courtiex et al., 1998; Lehtonen-Veromaa et al., 2000). Moreover, Courtiex et al. (1998) showed that 10 year old female gymnasts had 15% higher femoral neck BMD and 33% higher distal radial BMD than age-matched swimmers, as gymnasts are characterized by high-impact loading on their legs as well as on their arms. Lastly, it was found that girls, 13 to 18 years of age, who trained at high intensity and volume in running had greater bone strength index (BSI) than inactive girls (Greene et al., 2005). BSI is defined as the product of cortical volumetric BMD and cross-sectional moment of inertia. Since the BSI reflects geometric and material properties of bone, it is considered a superior assessment of bone strength than volumetric BMD alone.

The positive effects of weight-bearing actions on bone are seen not only in youths participating in sports, but also in youths participating in general physical activities. For example, a meta-analysis of bone mineral accrual in children and adolescents revealed that those who participated in physical activity, such as running or jumping, gained nearly 10% more BMC than their controls, or as much as 11% higher BMD at specific sites (Hind & Burrows, 2007). Janz et al. (2001) used accelerometry to assess general
physical activity levels and found that physical activity is significantly correlated to BMC and BMD ($r = 0.15$ to 0.28) in 4 to 6 year old children. Furthermore, the amount of time spent watching television negatively correlated with hip BMD ($r = -0.15$). The same cohort was re-examined after three years and the researchers found that the children who maintained high physical activity levels, as assessed with accelerometry, gained 14% more trochanteric BMC and 5% more whole-body BMC than those who sustained low physical activity levels (Janz et al., 2006). Together, these findings suggest that physical activity intervention programs may help bone development in children. Fuchs et al. (2001) examined the effects of a school-based physical activity intervention program on bones of pre-pubertal children. The students performed daily jumping over seven months and this produced ground reaction forces of up to eight times the body weight. Over a relatively short period, the intervention group accumulated 3.1% to 4.5% more BMC in the lumbar spine and femoral neck, respectively, than the controls who only participated in stretching exercises. MacKelvie et al. (2003) used a long-term school-based jumping program to induce bone accrual in 8 to 11 year old girls. After two years, the intervention group accumulated 3.7% and 4.6% more BMC in the lumbar spine and femoral neck, respectively, than the control group. Some studies suggest that weight-bearing physical activity has the greatest effect on building bone during the pre-pubertal years (Sundberg et al., 2002), especially for girls (Bass et al., 1998; Witzke and Snow, 2000) in a dose-dependent pattern (MacDonald et al., 2007).

Finally, this theory is further supported by studies designed to reduce stress on the bones – namely spaceflight and immobilization studies. Collet et al. (1997) showed that astronauts returning from a six-month space mission had marked bone mass loss, as
assessed with QCT and QUS. The change was seen only in the tibia and calcaneus – two weight bearing sites, but not in the radius. Similar results were seen with laboratory rats in space for several weeks (Vico et al., 1998), and it is suggested that an increase in bone resorption and possibly a decrease in bone deposition contributed to the loss in bone mass. Moreover, Leblanc et al. (1990) showed that 17 weeks of bed rest decreased bone mass in the weight-bearing bones of volunteer men as much as 10% of baseline values. However, the subjects showed a trend to mend the lost bone mass at all sites after six months of reambulation. Other animal studies showed similar results with physical activity attenuating or reversing the negative impacts of immobilization on bone mass (Inman et al., 1999; Uusitalo et al., 2005).

To conclude this section, it is unclear whether dynamic force is countered by the muscles or strain on the bones. Perhaps the muscle force and weight-bearing theories may not be mutually exclusive, but rather work together or produce different effects on different parts of the bone. Whatever the mechanism, increased physical activity can enhance bone strength in children and adolescents. This is very important because this is the time when bone mineral accrual is most sensitive to change.

2.5: Bone and Childhood Obesity

Childhood obesity has arguably established itself as one of the primary concerns in today’s society. Its effects consist of serious health and psychological consequences that may carry into adulthood, such as cardiovascular complications (Freedman et al., 1999; Sorof & Daniels, 2002), type-2 diabetes (Young et al., 2000), and low self-esteem (Strauss, 2000), as well as other metabolic syndromes and orthopedic problems. Not only is childhood obesity clearly visible in the population, but its increasing prevalence is
reaching epidemic proportions, producing a public health crisis (Ebbeling et al., 2002). The incidence of childhood obesity in Canada has nearly tripled over the last two decades (Tremblay & Willms, 2000), with approximately 25% of its school-aged children currently classified as overweight or obese (He & Beynon, 2006).

One health issue that obesity has an effect on is bone strength. In elderly women, excess adiposity appears to have a protective role against osteoporosis, especially after menopause (Kin et al., 1991; Ribot et al., 1988), presumably due to a greater load on bone (Beck et al., 2001) or to hormonal influences (Albala et al., 1996). However, in girls, increased fat mass is associated with an elevated risk of fracture, especially if they have low BMD (Goulding et al., 2000a). High body weight of children and adolescents can independently predict fracture risk (Goulding et al., 1998; Goulding et al., 2000a; Goulding et al., 2005; Skaggs et al., 2001). Furthermore, Rose et al. (2008) found that adolescents in the 50-90th percentile for BMI were most at risk of injury from sports and recreation participation. Thus, weight reduction has been suggested as a prophylactic to prevent fractures (Davidson et al., 2003).

The literature reports conflicting results regarding the bone strength of overweight and obese children and adolescents. Using DXA, Ackerman et al. (2006) found that fat mass was a significant predictor of BMC in child and adolescent girls, with an inverse relationship between the two variables. They concluded that of two children of similar weight, the individual with higher fat mass would have lower bone mass. Similarly, Goulding et al. (2000b; 2002) used DXA to assess bone and found that overweight and obese boys and girls, 3 to 19 years of age, had low bone mass and bone area for their weight, suggesting that their BMC does not increase proportionally with their increase in
weight. However, comparing BMD of an obese child with a normal-weight child of the same weight would reflect differences in bone length rather than bone density, because the obese child would necessarily be shorter.

Using DXA, obese children on a dietary regimen were shown to have similar lumbar spine BMD as a control group (De Schepper et al., 1995). Similarly, Manzioni et al. (1996) found no differences in BMC between obese and normal-weight children after correcting for height, body mass, and lean and fat mass. However, including all of these body composition variables in the multiple regression is not biologically logical because it does not allow for the comparison of obesity status between groups. That is, comparing an obese child with a normal-weight child of similar stature and lean mass means that they must differ in body mass and fat mass. Therefore, although entering height, mass, lean and fat mass into a regression model is statistically possible, it does not make biological sense.

Leonard et al. (2004) stressed that the correction should be made for height and lean mass so that an obese and normal-weight child would then differ only in their fat mass. After these considerations, they used DXA to assess bone and found that obese children and adolescents had higher BMD, bone area and bone mass than their normal-weight counterparts. Similar results have been reported in other studies with large, multiethnic cohorts (Cobayashi et al., 2005; Ellis et al., 2003).

As evident from the above studies, the conflicting results using DXA could be related to the different corrections used for differences in bone size. Transaxial QUS may be a better alternative to compare bone quality between obese or overweight and normal-weight children and adolescents because its results are not affected by bone size.
Few studies have used QUS to investigate the effects of adiposity on bone properties (Eliakim et al., 2001; Falk et al., 2008; Nemet et al., 2006). Eliakim et al. (2001) used QUS to assess bone strength in obese children and adolescents and found that these individuals had lower radial and tibial bone strengths than age-matched norms, as provided by the manufacturer’s reference data. However, bone mineralization and strength is dependent on many factors such as proper nutrition and physical activity (Boot et al., 1997), which were not considered in their study.

Most recently, Falk et al. (2008) used QUS to show that overweight pre-pubertal boys had lower tibial but not radial bone SOS compared with normal-weight controls, while there were no differences between groups in sexual and skeletal maturity, height, calcium intake and structured physical activity. This finding may be explained by differences between groups in their habitual physical activities, such as playing during school recess or walking to a friend’s house, which were not assessed in the questionnaires used in the study. Since both groups were minimally-active (< 2 hr/wk of structured physical activity), even small differences in physical activity may have significant effects on SOS of weight-bearing bones. These subtle, yet important, differences in physical activity are very difficult to assess with questionnaires.

Three months of a combined dietary supplement and physical activity intervention decreased body fat percentage of obese children and adolescents, 6 to 16 years of age, with a concomitant non-significant increase in tibial SOS (Nemet et al., 2006). By contrast, obese age- and gender-matched controls who were not subjected to the intervention significantly increased body fat percentage but decreased tibial SOS over the three-month period. Since both groups had similar baseline nutritional intakes and
subsequently received the same amount of calcium supplementation, it is unlikely that the difference in the change of SOS after the intervention was due to calcium intake. Thus, this finding suggests that either decreasing weight or increasing physical activity or both, can be used to improve bone strength of obese children and adolescents. Further, this improvement occurs within a relatively short period and is measurable by QUS.

The contradictory findings presented above, of either higher or lower bone strength in obese children, likely reflect the fact that the issue is complex and involves several factors. A study of obese Japanese children and adolescents using digital image processing (radiographic absorptiometry) to analyze BMD, found that obese children had higher BMD in the second metacarpal before puberty, but had lower BMD during puberty, compared with the reference data of normal-weight children (Nagasaki et al., 2004). The researchers suggested that some unknown factor during puberty, for example reduced physical activity, may have contributed to the poor development of BMD observed in obese children. Similar maturational effects on the relationship between adiposity and bone have been reported; Clark et al. (2006) found fat mass was positively associated with bone mass before puberty, but the relationship was attenuated after puberty.

Results from several studies examining the association between adiposity and bone strength using animal models have not been any more convincing. Ninety-day old male Sprague-Dawley rats that were fed junk food for one month to induce obesity were found to have similar bone geometry but significantly greater bone mechanical properties, such as increased ultimate load, deflection at the ultimate load, and energy absorption capacity, than normal-weight controls, suggesting that the obese rats had
stronger bones (Brahmabhatt et al., 1998). By contrast, eight-week old female Fischer rats that were fed high-fat-sucrose diets for 10 weeks to induce obesity were found to have altered bone geometry and lower bone mechanical properties than their normal-weight counterparts (Li et al., 1990). Similarly, Zernicke et al. (1995) showed adverse changes to bone properties after feeding four-week old female Fischer 344 rats a high-fat-sucrose diet for two years to induce obesity. These deficiencies in bone properties could be rectified with three months of moderate treadmill exercise (Mathey et al., 2002). The discrepancies between the results of the animal studies may be partly explained by the differences in the type or gender of rat used as the model, or the method of inducing obesity. Nevertheless, these studies do not necessarily support the notion that adiposity has a protective effect on bone, as has been suggested in adult women (Nomeli et al., 2007).

2.6: Conclusion

The relatively high prevalence of fractures during adolescence may reflect that it is a time of relative bone weakness (Faulkner et al., 2006). The vulnerability of children towards fractures may be exacerbated by excess body weight. Although adiposity may provide a protective effect on bone in adults, the results showing the effects of adiposity on bone in children and adolescents are equivocal. Recent findings using QUS to evaluate bone strength suggest that overweight children and adolescents may have weaker bones than normal weight individuals (Eliakim et al., 2001; Falk et al., 2008). Differences in physical activity levels is a viable explanation for the differences in bone quality between the obesity groups, as physical activity is known to positively influence
bone development. However, whether physical activity exerts its effects through muscle forces or mechanical loading remains unclear.

The available literature studying the effects of obesity on bone quality in children and relating it to physical activity levels is limited. Eliakim et al. (2001) and Falk et al. (2008) both found that obese youths had relatively low bone strength. However, their measures of physical activity were not sensitive enough to find differences in habitual physical activity, although their findings suggest a trend towards lower physical activity levels in overweight youths. In overweight youths who have such low physical activity levels, even a slight increase in weight-bearing physical activity may have a great effect on their bone development.
CHAPTER 3: RESEARCH METHODS

3.1: Study Design

This research study was a cross-sectional study that investigated the association of adiposity on bone properties. By using quantitative ultrasound, weight bearing and non-weight bearing bone SOS of overweight girls were compared with those of age- and maturity-matched, normal-weight girls.

3.2: Subject Description

Subjects included females, 8-11 and 14-16 years old, and of Caucasian descent. Obesity status was classified according to the cut-offs used by Ellis et al. (2003). Specifically, groups were categorized by degree of adiposity: Normal-weight controls (NW, body fat ≤ 25%), and in addition, we had a combined overweight and obese group (OW, body fat ≥ 28%).

Subjects were recruited from the Golden Horseshoe area through physician clinics, obesity programs, newspaper ads, flyers and information packages (Appendix A; Appendix B). Potential participants were informed of the purpose, methods, and potential risks of the study. Before testing, an informed consent form was signed by the parent or legal guardian of the participant (Appendix C).

All participants were non-athletic, with minimal involvement in structured physical activities (≤ 2 hr/wk). Those who had prior experiences that could affect bone properties (i.e., use of steroid medication, growth delay, previous and/or current fracture) were excluded from the study. Girls having irregular menstrual cycles or on oral contraceptives were also excluded from the study.
3.3: Research Protocol

All participants were tested on one occasion at either the applied exercise physiology lab at Brock University, a physician’s office in Welland, or at the Children’s Exercise and Nutrition Centre at Chedoke Hospital in Hamilton, between October 2007 and May 2008. Prior to the visit to the lab, the participants were contacted via phone or email and instructed to refrain from exercise, alcohol consumption, and food and fluid consumption at least four hours before testing. Upon arrival at the lab, body composition, anthropometric measurements, nutritional questionnaires, self-assessed pubertal status, and all bone measures were performed. The researchers also instructed the subjects on the use of the accelerometer. The subjects then returned to the lab approximately one week after the initial visit to give back the accelerometers and related data in exchange for a monetary honorarium.

3.4: Methods

Questionnaires: Questionnaires were completed by the subject, with the help of the investigator and possibly parent or guardian, to assess the subject’s medical history (Appendix G), and nutritional intake (Appendix E). For nutritional analysis, a 24-h recall of the most recent day of typical food intake was detailed. This questionnaire is a valid estimate of energy intake and calcium intake in adolescent females (Greger & Etnyer, 1978). Axxya System’s Nutritionist Pro Diet Analysis (Stafford, TX, USA) was used to analyze the 24-hr recall questionnaires to quantify total energy intake, calcium and vitamin D intake. The nutritional questionnaires and analyses were facilitated by the same investigator.
Anthropometry: With the subjects wearing light clothing and no shoes, height, and body mass was measured using an Ellard Instrumentation board length stadiometer (Monroe, WA, USA) and a Zenith digital scale, respectively. The same investigator performed all anthropometric measurements.

With the subjects barefoot and wearing light clothing, percent body fat, lean mass and fat mass were assessed using the Biospace InbodyS20 bioelectrical impedance analysis system (BIA; Beverly Hills, CA, USA). As instructed, subjects had abstained from exercising, consuming alcohol and eating/drinking for at least four hours, prior to arrival. To ensure that the subjects were hydrated, they were given approximately 500 ml of water to drink at least 45 minutes before their assessment with the BIA. The subjects were then asked to void just prior to the BIA body composition assessment. The InbodyS20 BIA device passes different mild electrical currents with multiple frequencies (5 to 500 kHz) through the subject's body via electrodes situated on the palms and feet. The body’s resistance and reactance to the current is related to total body water, which in turn, is highly correlated with fat-free mass. Thus, the measurements can be used with age- and gender-appropriate equations to calculate body composition variables. This BIA system was recently found to be an accurate predictor of body composition in children of a wide range of adiposity (Kriemler et al., 2008). Additionally, BIA was reported to be valid and reliable in estimating body fat in overweight and obese children (Goldfield et al., 2006). There is no discomfort associated with this measurement.

Maturity: Sexual maturity was self-reported using secondary sexual characteristics visual aids (pubic hair), according to Tanner (1962) (Appendix H). Those
who reported Tanner 1, 2 or 3 (and pre-menarcheal) and 4 or 5 (and post-menarcheal) were categorized as girls and adolescents, respectively.

Accelerometry: Accelerometers are small and lightweight devices that measure acceleration of a body in single or multiple planes. An internal cantilever beam emits a charge proportional to the acceleration of the body, which is then digitized. It sums counts over a specified duration or epoch. Cut-off points for the counts/epoch can be defined to determine the time spent in light, moderate, vigorous and very vigorous activity.

The advantage of using accelerometers is that it is an objective measure of physical activity that eliminates recall bias from questionnaires. Moreover, the counts are related to ground reaction forces, which are important when looking at bone development (Janz et al., 2003). Lastly, accelerometers are sensitive to low levels of physical activity. Contrastingly, the disadvantage of an accelerometer is that it is not waterproof and so activities involving water cannot be measured. Moreover, activities in which the torso remains relatively stable, such as cycling, cannot be measured. It has also been reported that there is a low level of compliance with overweight and normal weight children and adolescents, even when strategies to enhance compliance, such as providing written and verbal instructions, constant reminders to wear the accelerometers, and offering rewards for returning the accelerometer, are applied (van Coervering et al., 2005).

Daily physical activity was assessed using Actigraph (formerly known as MTI/CSA) GT1M uniaxial accelerometers (Pensacola, FL, USA). Results of this model have been shown to be correlated ($r = 0.53-0.73$) with children's free play activities as assessed with heart rate monitoring and direct observation (Ott et al., 2000), as well as
with whole-room calorimetry (Puyau et al., 2002). The accelerometers were programmed to record activity counts at 10-second epochs, to measure vertical acceleration from 0.05 to 2.00 G, at a frequency from 0.25 to 2.50 Hz. On the visit to the lab, the participants were instructed on the proper usage of the accelerometers. Using an adjustable elastic belt, the subjects secured the accelerometers snugly on the right side of the hip, against the skin, throughout the waking hours for seven consecutive days (Trost et al., 2000). Because the accelerometer is not waterproof, the subjects were asked to remove the accelerometer while swimming or bathing. At the same time, the subjects were provided with a log sheet (Appendix I), in which they were asked to record the times when the accelerometers were removed and the time and type of structured activities that were performed throughout the day. The accelerometers were programmed to commence data collection the following early morning (5 a.m.). A researcher phoned the participant or guardian during the week to facilitate compliance. Accelerometers and all log sheets were returned to the lab by the participants or picked up by a researcher from the participant’s residence. At this time, a twenty-dollar honorarium was given to the participant.

Output for the accelerometry consisted of counts/10-sec and were used to estimate metabolic equivalents (METs) using the following age-specific formula (Freedson et al., 2002):

\[
METs = 2.757 + (0.0015 \times cnts/min) - (0.08957 \times age (y)) - (0.000038 \times cnts/min \times age (y))
\]

METs between 3 to 5.9, and 6 to 8.9, and 9 and greater, correspond to moderate, vigorous, and very vigorous physical activity, respectively (Trost et al., 2001). Thus, the amount of physical activity at different intensities was quantified. Although seven days
of monitoring was expected of the subjects, a minimum of three weekdays and one weekend day of full data (≥10 hours/d) were required to be included in data analysis (Penpraze et al., 2006). Raw data was entered into a Microsoft Excel macro to calculate a weighted average of the amount of time spent performing moderate, vigorous and very vigorous activities, average counts per 10-sec epoch and the percentage of the day that the subjects were active (Appendix AL).

**Bone Strength:** Each subject was asked which hand she preferred to write with and what leg she preferred to kick with, to determine the dominant limb. Bone SOS of the distal one-third radius and mid-shaft tibia was assessed bilaterally using Sunlight Medical Ltd.'s Sunlight Omnisense™ model 7000P (Tel Aviv, Israel). The area of measurement for the radius was determined by the midpoint between the olecranon process and the tip of the third phalanx. The mid-shaft tibia was determined by the midpoint between the calcaneus and the top of the knee while the subject was seated (knee and ankle at 90°).

To measure radial SOS, wide scans of 140 degrees around the radius were performed. To measure tibial SOS, scans from the tibial crest to the medial end were performed. All measurements consist of at least three consistent cycles. A system quality verification of the QUS was performed with a Perspex phantom before the first test of each day (Appendix F). The intra-operator coefficient of variation of the QUS measurements in 10 children was 0.98 for the radius and 0.94 for the tibia.

### 3.5: Statistical Analyses

The data was first cleaned by checking for outliers (> 2 standard deviations from the mean) and normality (kurtosis and skewness) of all of the dependent variables
A Levene's test for homogeneity of variance of tibial SOS revealed that the high variance of A-OW relative to that of A-NW violated the assumption for ANOVA analyses \[ F(3,67) = 3.54, p = 0.019 \]. The violation affects the \( F \)-statistic of the overall estimate of the error variance. Ultimately, the probability of making a type 1 error is underestimated (the true \( p \)-value is higher than that which was reported). Advanced statistical analyses (such as log transformations) are required, however, for the purposes of this thesis, two-way ANOVAs were used to assess differences in SOS.

Two-way ANOVAs were also used to assess differences in nutrition, physical characteristics and physical activity between the adiposity and age groups. A Chi square analysis was used to compare the pubertal stage distributions and other categorical variables. Pearson Product Moment correlations were used to determine the correlations between SOS and possible influencing factors, such as habitual physical activity. When significant correlations were found between SOS and possible influencing factors, the latter was used as a covariate in an ANCOVA analysis. Additionally, data was split by age and adiposity group and bivariate correlations were assessed separately.

Data was analyzed using SPSS ver. 16.0 and is presented as means ± SD with significance set at \( p < 0.05 \) (2-tailed).
CHAPTER 4: RESULTS

One hundred and five girls volunteered to participate in the study. Nine girls were excluded because they were not between 8 to 11, or 14 to 16 years of age. Ten girls were excluded because their body fat percentages were between 25 and 28%. Two girls were excluded because they were using corticosteroids. Two girls were excluded due to ethnicity. One girl was excluded because she reported having irregular menstrual cycles. Lastly, six girls reported participating in more than two hours of structured physical activities per week and were consequently excluded from the study. Thus, a total of 75 girls were included for data analyses.

The physical characteristics of the subjects are displayed in Table 4.1. Adolescents were older, taller and more sexually mature than girls, with no significant differences in maturity between adiposity groups. There was also no difference in age of menarche between normal-weight (12.0 ± 0.8 years old) and overweight (11.8 ± 1.3 years old) adolescents. There was an age-by-adiposity interaction for height reflecting the fact that among the girls, OW were taller than NW, while among the adolescents, the pattern was reversed. Adolescents were significantly heavier, had greater fat mass, as well as greater lean mass than girls. This was also the case when comparing OW with NW. There was an age-by-adiposity interaction for weight and fat mass, reflecting the fact that the difference between NW and OW groups was much greater in the adolescents compared with the girls.

BMI percentile was similar in the younger and older NW, as well as in the younger and older OW. Body fat percentage was similar in the younger and older NW.
However, among the OW groups, body fat percentage (BF%) was higher in the older compared with the younger group.
Table 4.1: Physical characteristics of the overweight and normal-weight girls and adolescents.

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<td></td>
<td>Normal-weight</td>
<td>Overweight</td>
<td>Normal-weight</td>
</tr>
<tr>
<td></td>
<td>(n = 21)</td>
<td>(n = 19)</td>
<td>(n = 13)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>10.0 ± 1.1 a</td>
<td>10.3 ± 1.1 b</td>
<td>15.3 ± 0.8 a</td>
</tr>
<tr>
<td>Tanner Stage (I,II,III,IV,V)</td>
<td>16,3,2,0,0 a</td>
<td>11,7,1,0,0 b</td>
<td>0,0,0,9,4 a</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>139.8 ± 8.4 a</td>
<td>146.3 ± 10.6 b</td>
<td>166.4 ± 4.6 a</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>33.0 ± 7.2 a,b</td>
<td>51.5 ± 11.8 a,c</td>
<td>54.9 ± 7.0 b,d</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>6.2 ± 1.8 a,b</td>
<td>18.7 ± 6.7 a,c</td>
<td>11.5 ± 3.1 b,d</td>
</tr>
<tr>
<td>Fat-free Mass (kg)</td>
<td>28.0 ± 4.9 a,b</td>
<td>32.8 ± 6.1 a,c</td>
<td>43.4 ± 4.7 b,d</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.6 ± 1.8 a,b</td>
<td>23.8 ± 3.9 a,c</td>
<td>19.9 ± 2.7 b,d</td>
</tr>
<tr>
<td>BMI Percentile</td>
<td>40.7 ± 23.5 a</td>
<td>90.8 ± 6.5 a</td>
<td>45.8 ± 24.4 b</td>
</tr>
<tr>
<td>Body Fat Percentage (%)</td>
<td>18.0 ± 3.1 a</td>
<td>35.4 ± 5.9 a,b</td>
<td>20.6 ± 4.0 d</td>
</tr>
</tbody>
</table>

Values are presented as M ± SD. Similar superscripts indicate pairwise significant differences (p < 0.05). A = Age effect, Ad = Adiposity effect, AxAd = Age and adiposity interaction (p < 0.05).
There were no significant differences between age or adiposity groups in total energy intake or in calcium and vitamin D intake (Table 4.2). All groups had calcium intakes that were only 69% to 80% of the recommended daily intake of 1300 mg (Osteoporosis Canada, 2008). All groups had vitamin D intakes that were 55% to 98% of the recommended daily vitamin D intake of 6 μg (~300 IU), with a tendency of OW to consume less vitamin D than NW, although it was not significant.
<table>
<thead>
<tr>
<th></th>
<th>Girls</th>
<th>Adolescents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal-weight (n = 21)</td>
<td>Overweight (n = 19)</td>
</tr>
<tr>
<td>Total Caloric Intake (kcal)</td>
<td>1651 ± 404</td>
<td>1630 ± 205</td>
</tr>
<tr>
<td>Calcium Intake (mg)</td>
<td>1000 ± 537</td>
<td>914 ± 393</td>
</tr>
<tr>
<td>Vitamin D intake (µg)</td>
<td>5.6 ± 4.6</td>
<td>3.3 ± 2.6</td>
</tr>
</tbody>
</table>

Values are means ± SD; There were no significant differences between groups.
SOS of the dominant and non-dominant radii and tibiae were highly correlated (r = 0.97 in upper and lower limbs) and there were no significant differences between the non-dominant and dominant limbs. Thus, only data for the non-dominant limbs are presented below. Adolescents had significantly higher radial SOS than girls. However, there were no differences in radial SOS between adiposity groups (Figure 4.1).

![Figure 4.1: Non-dominant radial SOS of the overweight and normal-weight girls and adolescents. M ± SD. *p < 0.01](image)

Adolescents had significantly higher tibial SOS than girls (Figure 4.2). In both age groups, NW had significantly higher tibial SOS than OW. No age-by-adiposity interactions were found for any of the SOS variables.
We collected the full seven days of accelerometry data in a majority of the girls for all groups except for A-NW, of whom only one-third of the girls wore the accelerometer for the entire week. Although Trost et al. (2000) recommended that children wear the accelerometers for seven consecutive days to accurately measure physical activity, Janz et al. (1995) suggested that three weekdays and one weekend day are sufficient. This approach has been widely utilized in the literature (Janz et al., 2001; Janz et al., 2002; Nilsson et al., 2002; Penpraze et al., 2006). The compliance for wearing the accelerometers for at least three weekdays and one weekend day was 90%, 89%, 100% and 86% for G-NW, G-OW, A-NW and A-OW, respectively. Physical activity levels, as quantified by average counts and percent active (percentage of waking hours spent performing moderate-to-very vigorous physical activity) were significantly higher in girls compared with adolescents, with no differences between adiposity groups (Table 4.3).

**Figure 4.2:** Non-dominant tibial SOS of the overweight and normal-weight girls and adolescents. M ± SD. *p < 0.01
Table 4.3: Daily physical activity levels of the overweight and normal-weight girls and adolescents.

<table>
<thead>
<tr>
<th></th>
<th>Girls</th>
<th>Adolescents</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal-weight (n = 19)</td>
<td>Overweight (n = 17)</td>
<td></td>
</tr>
<tr>
<td>Activity (counts/10-sec)</td>
<td>83.4 ± 19.1 a</td>
<td>67.0 ± 16.7 b</td>
<td></td>
</tr>
<tr>
<td>Activity (% Active)*</td>
<td>16.0 ± 4.1 a</td>
<td>13.3 ± 4.1 b</td>
<td></td>
</tr>
<tr>
<td>Inactivity (min)</td>
<td>400 ± 48 a</td>
<td>516 ± 72 a</td>
<td></td>
</tr>
<tr>
<td>Sedentary Activity (min)</td>
<td>71 ± 10 a</td>
<td>62 ± 15 a</td>
<td></td>
</tr>
<tr>
<td>Light Activity (min)</td>
<td>178 ± 27 a</td>
<td>151 ± 31 a</td>
<td></td>
</tr>
<tr>
<td>Moderate Activity (min)</td>
<td>101.5 ± 25.8 a</td>
<td>58.8 ± 30.7 ab</td>
<td></td>
</tr>
<tr>
<td>Vigorous Activity (min)</td>
<td>17.8 ± 7.2 ab</td>
<td>11.8 ± 7.5 ac</td>
<td></td>
</tr>
<tr>
<td>Very Vigorous Activity (min)</td>
<td>4.6 ± 3.9 ab</td>
<td>2.0 ± 2.3 ac</td>
<td></td>
</tr>
<tr>
<td>Moderate - Very Vigorous Activity (min)</td>
<td>125.5 ± 33.2 ab</td>
<td>65.9 ± 37.9 ac</td>
<td></td>
</tr>
</tbody>
</table>

|                          | Normal-weight (n = 13) | Overweight (n=19)     |        |
| Activity (counts/10-sec) | 48.7 ± 14.7 a          | 48.3 ± 16.6 b         |        |
| Activity (% Active)*     | 6.0 ± 2.3 a            | 6.0 ± 2.6 b           |        |
| Inactivity (min)         | 409 ± 64 b             | 543 ± 77 b            |        |
| Sedentary Activity (min) | 74 ± 12 b              | 66 ± 11 b             |        |
| Light Activity (min)     | 183 ± 34               | 169 ± 36              |        |
| Moderate Activity (min)  | 87.9 ± 21.3 c          | 47.5 ± 19.1 bc        |        |
| Vigorous Activity (min)  | 3.4 ± 3.6 b            | 1.5 ± 1.0 c           |        |
| Very Vigorous Activity (min) | 0.6 ± 0.9 b           | 0.4 ± 0.8 e           |        |
| Moderate - Very Vigorous Activity (min) | 103.0 ± 27.2 bd | 48.5 ± 20.4 ed |        |

Values are presented as M ± SD. Similar superscripts indicate pairwise significant differences (p < 0.05).

* = Calculated as percentage of waking hours spent performing moderate-to-very vigorous activity, A = Age effect, Ad = Adiposity effect (p < 0.05).
When physical activity was expressed in absolute minutes of moderate, vigorous and very vigorous activities, as well as the total moderate-to-very vigorous physical activity (MVPA), adolescents and OW were significantly less active than girls and NW, respectively, for all of the variables (Table 4.3). NW also accumulated more sedentary and light activity than OW, but with no significant differences between age groups. On the other hand, G-OW was more inactive than G-NW (Table 3).

Correlations between non-dominant SOS and descriptive variables are shown in Appendix K through Appendix AE. As a whole group, age, height and lean mass positively correlated with radial and tibial SOS ($r = 0.33-0.78$). Tibial SOS, but not radial SOS, was negatively correlated with body fat percentage ($r = -0.30$), especially so in the A-OW group ($r = -0.53$) (Figure 4.3).

![Figure 4.3: Relationship between tibial SOS and body fat percentage in the overweight and normal-weight girls and adolescents. *p < 0.05; Solid line represents the correlation in the A-OW group only. The correlation was not significant in the other groups.](image-url)
Physical activity decreased with an increase in age while bone SOS increased with an increase in age. Therefore, in order to examine the correlation between bone SOS and physical activity, age was partialed out. When age was partialed out, tibial SOS was positively correlated with the absolute number of minutes spent at moderate-to-very vigorous activity per day ($r = 0.37-0.40$, $p < 0.05$).

In order to examine whether fat-free mass or MVPA explained the difference in tibial SOS between groups, both were entered as covariates in the analysis. However, when entered separately or together, neither covariate was statistically significant (Appendix AF).

Although there were differences between groups in total light, vigorous, very vigorous, and sedentary activities, as well as inactivity, none were significant when entered as covariates (Appendix AG).
CHAPTER 5: DISCUSSION

The objective of this study was to compare bone properties, as measured with QUS, between normal-weight and overweight girls and adolescents. Our main results showed that overweight girls and adolescents had lower tibial SOS than their normal-weight counterparts. This difference was apparent despite the fact that within each age group, overweight and normal-weight groups did not differ in sexual maturity, menarcheal status, body height, mean physical activity and calcium intake. Lastly, body fat percentage negatively correlated with tibial SOS, especially in the adolescent overweight girls. Physical activity correlated with tibial SOS. However, it did not explain the difference in SOS between adiposity groups.

Traditionally, DXA has been used to assess the effects of adiposity on bone strength in children and adolescents. However, the results of these studies are conflicting. The discrepancy between studies may be attributed to the different methods used for correcting BMC for body size. Using DXA, several studies reported that obese children and adolescents, 3 to 19 years of age, exhibit similar BMD to normal-weight children (De Schepper et al., 1995; Manzioni et al., 1996; Goulding et al., 2000b). However, when BMD was adjusted for body mass, the obese subjects demonstrated lower BMD compared with normal-weight children (Goulding et al., 2000b; Rocher et al., 2006). Leonard et al. (2004) argued that BMC should be corrected for height and lean mass, rather than for whole body mass, in order to reflect differences in fat mass between adiposity groups. With this correction method, they found that obese children and adolescents, 4 to 20 years of age, have higher BMD than normal-weight controls. The present study used transaxial QUS to assess bone quality, thus eliminating the need
for body size correction. Our overweight and normal-weight girls were similar in height in both age groups. However, the overweight girls had significantly more lean mass than the normal-weight girls. Lean mass correlated positively with bone SOS in the whole group but was not a significant covariate when analyzing differences in SOS between groups, suggesting that differences in SOS were not attributed to lean mass.

In comparison to studies using DXA, there is little available literature employing QUS to assess bone quality in overweight and obese children and adolescents. Eliakim et al. (2001) used QUS and found that obese children and adolescents, 6 to 17 years of age, have lower tibial and radial SOS compared with age-matched reference values obtained from the ultrasound manufacturer (Sunlight Omnisence™). Specifically, the overweight/obese pre-pubertal children had radial and tibial SOS values of 3634 and 3539 m/s, respectively. The overweight/obese pubertal adolescents had radial and tibial SOS scores of 3721 and 3651 m/s, respectively. However, these numbers are consistently lower (as much as 214 m/s difference in the radius) than the corresponding values in the present study. The discrepancy may be partially explained by the different populations studied, as Eliakim et al. (2001) grouped males and females together, whereas our SOS measurements represent only females. Thus, our values are slightly skewed higher because females have greater radial and tibial SOS scores than boys at all ages between 8 and 17 years (Zadik et al., 2003). For the normal-weight females in our study, the SOS scores are very consistent with the SOS reference values for girls and adolescents produced by Zadik et al. (2003).

Unlike Eliakim et al. (2001), we compared our overweight group with a control group that was similar in age, sexual maturity, nutritional intake and structured physical
activity and found that, although there were no differences in radial SOS between adiposity groups, the overweight girls had significantly lower tibial SOS than normal-weight girls.

Recently, Falk et al. (2008) used QUS to assess bone SOS in overweight and obese 9 to 12 year-old boys. They found that the overweight and obese subjects had lower tibial SOS, but not radial SOS, than their normal-weight counterparts who were matched for sexual maturity, nutritional intake and structured physical activity. The lower tibial SOS values in the overweight and obese groups in the present study is in line with Falk et al.'s (2008) findings in pre-pubertal males and extends them to females, both girls and adolescents.

Fat tissue may be argued to compromise the accuracy of the SOS measurement. Kotzki et al. (1994) removed soft tissue surrounding cadaver heels and showed that the SOS steadily increased. When the researchers added a 10 mm piece of lard to the cadaver heels, the SOS decreased around 30 m/s. Rico et al. (1999) compared phalangeal SOS to total body BMC of obese women and found that SOS is negatively correlated with total BMC and weight. The authors concluded that obesity compromises the accuracy of the QUS. However, the studies previously described used quantitative ultrasound technology that measures SOS through the bone. Our study used transaxial transmission and thus it measured SOS along the bone. Previous studies using transaxial QUS argued that QUS measures bone quality independent of soft tissue (Eliakim et al., 2001; Falk et al., 2008). Ultrasonic waves enter the bone at a critical angle and travel along the bone at various paths. SOS travels through cortical bone at approximately 4000 m/s and much slower through soft tissue at approximately 1500 m/s. The sound waves
then emerge from the bone at the same critical angle. Using a proprietary algorithm, the ultrasound detects the fastest signal that propagates along the bone. Therefore, the SOS value obtained is that which reflects cortical bone and not soft tissue. Additionally, differences between adiposity groups could be seen in the tibia but not radii, suggesting that soft tissue did not affect the QUS measurements. It should be noted, however, that adequate contact between the ultrasound probe and bone surface is necessary to obtain valid results (Pearce et al., 2000). Indeed, tibial SOS could not be measured in two obese subjects in our study possibly due to the excessive underlying soft tissue. Thus, further validation studies for the use of QUS on overweight and obese individuals may be warranted.

Bone quality in children and adolescents may be affected by a number of factors, namely nutrition, physical activity and hormones. The results of the present study showed that all groups were relatively low in daily calcium intake compared with recommended values. Previous studies assessing nutritional intake in children and adolescents reported similar results: Based on 2 weekday and 1 weekend day dietary recall questionnaires, Fiorito et al. (2006) found that 9 and 11 year old girls reported consuming an average of $1842 \pm 403$ Kcal and $936 \pm 366$ mg of calcium per day. Carter et al. (2001) used up to four 24-hour recall questionnaires over a period of a year to assess nutritional intake in 8 to 17 year old girls and found that they consume $1615 \pm 473$ kcal and $902 \pm 372$ mg of calcium daily. Our results (Table 4.2) are strikingly consistent with these previous studies.

Santos et al. (2008) compared nutritional intake between obese and normal-weight adolescents using 24-hour dietary recall questionnaires over three non-
consecutive days and found that the obese subjects reported similar energy intake (1886 vs. 1905 Kcal/day for obese and normal-weight, respectively) but significantly less calcium intake (585 vs. 692 Kcal/day for obese and normal-weight, respectively) than the normal-weight subjects. The discrepancy between our results and those of Santos et al. (2008) may be partially explained by the fact that the latter included males in their subject pool while the present study examined only females. Additionally, Santos et al. (2008) examined only obese individuals while in the present study, overweight subjects were also included.

No differences in nutritional intake were observed between any of the age or adiposity groups in our study. However, it should be noted that overweight females tend to underreport nutritional intake on a 24-hour nutritional questionnaire (Novotny et al., 2003). If this was the case in the present study, the overweight subjects may have had higher calcium, vitamin D and total energy intake, all of which are associated with enhanced bone quality (Heaney et al., 2000). However, despite the possible higher nutritional intake, the overweight groups still had significantly lower tibial SOS than the normal-weight groups. Thus, it is unlikely that any difference in nutritional intake could explain the differences in tibial SOS.

The results of the overweight subjects having low tibial but not radial SOS suggests that a site-specific factor may be acting on the bone. It is generally accepted that weight-bearing, high-impact physical activity is beneficial to bone development, especially at weight-bearing sites of the skeleton (Boot et al., 1997; Bailey et al., 1999; Janz et al., 2001; Janz et al., 2006). For example, Janz et al. (2006) showed that five year old children who maintained high levels of physical activity, as assessed with
accelerometry, accumulated 14% more trochanteric BMC and 5% more total body BMC than those less active. Overweight individuals are characteristic of being relatively inactive (Trost et al., 2001). Their typically low physical activity may explain, at least partially, the lower tibial SOS found in overweight pre-pubertal boys (Falk et al., 2008). However, in the latter study, reported structured physical activity was not different than in normal-weight boys. The authors speculated that the questionnaires were not sensitive enough to measure small differences in habitual physical activity between groups. Therefore, the present study used accelerometry to assess physical activity levels of the subjects. This method eliminates recall-bias and can accurately quantify the typically intermittent physical activity characteristic of children. While MVPA did correlate with tibial SOS, it was not a significant covariate and therefore, does not explain the difference in tibial SOS between the normal-weight and overweight groups. This suggests that other factors associated with adiposity may play a larger role in explaining the lower SOS.

In the present study, all groups performed an average of 48 to 125 minutes of MVPA daily. Riddoch et al. (2005) found that 9- and 15 year old European girls perform up to 171 and 82 minutes/day of MVPA, respectively. These high activity levels relative to those of our study may be explained by differences in demographics and the selection bias of minimally active subjects in the present study. Our results are more consistent with those of Trost et al. (2002) who showed that American child and adolescent girls perform 50 to 100 minutes of MVPA per day, as assessed with 1-minute epoch accelerometry.

The activity levels of our girls declined (19-35 counts/10-sec or 17-23 minutes/day of MVPA; Table 4.3) from childhood to adolescence in both adiposity
groups, which is a common pattern found in the literature (Kimm et al., 2002; Nader et al., 2008; Trost et al., 2002). In accordance with the present study, a review of the National Health and Nutritional Examinational Survey suggests that American girls six years of age may decrease an average of 182 counts/minute (~30.3 counts/10-sec) by the time they turn 15 years old (Troiano et al., 2008), which is in agreement with our results. Furthermore, Treuth et al. (2005) found that high school children had 20 minutes/day less MVPA than elementary school children. Dowda et al. (2006) suggested that reduced family support, self-efficacy and behavioural control contribute to the decline in physical activity throughout a female’s childhood and adolescence. The cause for the marked decline in physical activity after puberty in our study remains unknown, as we did not measure any social factors associated with physical activity levels.

The increase in childhood obesity is commonly blamed on the decreasing levels of physical activity (Moore et al., 2003; Must & Tybor, 2005). Studies using accelerometers set at 1-min epochs found that obese children, especially girls, are 20 to 36% less active than non-obese controls (Trost et al., 2001; Page et al., 2005). Trost et al. (2001) used accelerometry to assess physical activity in 11 year old children and found that obese children accumulate 70 minutes/day of MVPA whereas non-obese children accumulate 92 minutes/day of MVPA. However, the researchers did not distinguish the children by ethnicity or gender. In Europe, 9 to 11 year old obese girls were shown to perform 50 minutes/day of MVPA whereas the normal-weight age-matched controls perform 70 minutes/day of MVPA (Page et al., 2005). Patrick et al. (2004) found that obese adolescent girls (11 to 15 years of age) had 48 minutes/day of MVPA as compared with the normal-weight adolescents who had 54 minutes/day of MVPA. The
The aforementioned studies used accelerometers set at 1-minute epochs, as opposed to the more sensitive 10-sec epoch used in the present study. This discrepancy in epoch length may explain the higher amount of MVPA observed in the present study, especially in the normal-weight groups (Table 4.3).

To increase the sensitivity of the accelerometers to measure physical activity, Treuth et al. (2007) tested a large cohort of girls 11 to 12 years of age using 30-second epochs and found that overweight girls performed significantly less MVPA per day than normal-weight girls (27 vs. 24 minutes, respectively). The apparent lower amount of MVPA reported by Treuth et al. (2007) compared with the present study is explained by the fact that the latter study used higher cut-off values for moderate physical activity (~4.6 METs) and that six hours per day were required for data analysis.

Like the previous studies, our results revealed that the overweight girls accumulated significantly less MVPA per day than the normal-weight girls. However, this difference was as much as 60 minutes/day, or 52%. The large difference in MVPA between the adiposity groups may be partially explained by the increased sensitivity of a 10-second epoch to accurately measure physical activity.

Since physical activity is a known determinant of bone quality and since overweight subjects are characterized by low physical activity levels, even a slight increase in weight-bearing physical activities may partially affect the tibial SOS. However, none of the studies mentioned above related the physical activity levels of the overweight and normal-weight children or adolescents with bone strength. In the present study, there were negative correlations between the physical activity variables measured and tibial SOS, likely attributed to the decreased physical activity levels in adolescence.
Indeed, when age was partialled out, physical activity was moderately correlated with tibial SOS, supporting the theory that weight-bearing physical activity is beneficial to bone. Nevertheless, physical activity was not a significant covariate in the analysis for tibial SOS differences between adiposity and age groups. Thus, other factors associated with adiposity, such as lean body mass or hormonal status, may have a greater influence on bone during childhood and adolescence.

Obesity is often accompanied by a greater amount of lean body mass. Indeed, the overweight subjects in this study had a higher fat-free mass compared with the normal-weight groups (Table 4.1). Lean body mass is sometimes used as a surrogate for muscle force. It is argued that increased lean mass is associated with increased muscle force, which may enhance bone quality (Burr, 1997). However, in the present study, despite the greater fat-free mass in the overweight groups, their tibial SOS was lower.

Hormonal adaptations to obesity may also affect bone. Villareal et al. (2001) showed that postmenopausal women on hormone replacement therapy attenuated the bone loss exhibited by old age. This finding is along the lines of other/previous studies (Kin et al., 1991; Ribot et al., 1988), suggesting that estrogen has positive effects on bone. Overweight girls are characteristic of advanced sexual maturity as compared with girls of similar chronological age (Wang, 2002). Thus, it may be possible that although self-reported pubertal stage and menarcheal status were similar between the adiposity groups within each age group, the overweight girls in our study may have had higher estrogen levels, which would theoretically contribute to enhanced bone quality. Estrogen levels were not measured in the present study. Nevertheless, despite possible greater estrogen levels, tibial SOS of overweight girls was lower than normal-weight girls.
Sufficient vitamin D intake can increase BMD at multiple skeletal sites throughout the growing years of life (Cashman et al., 2008; Fuleihan et al., 2003). However, adiposity is negatively associated with serum vitamin D levels (Arunabh et al., 2003), possibly due to the sequestering of the vitamin in fat cells (Liel et al., 1988). Thus, vitamin D deficiency (reduced bioavailability) is common in obese individuals (Wartsman et al., 2008). In the present study, there were no differences in vitamin D intake between the overweight and normal-weight groups (Table 4.2). While we did not measure serum vitamin D levels in our subjects, it is possible that the overweight individuals had lower levels of vitamin D in circulation. This hypothesis, along with lower physical activity levels in overweight girls and adolescents may help to explain their lower tibial SOS.

Leptin is a hormone produced by adipocytes that is involved in energy homeostasis (Kershaw & Flier, 2004) and is released in proportion to the amount of adipose tissue present (Liuzzi et al., 1999). Obese adults are often characterized by high leptin levels (Magni et al., 2005; Mahabir et al., 2007). Leptin is suspected to produce antiosteogenic effects because mice that are deficient in leptin or the leptin receptor have higher rates of bone formation and bone mass than wild-type mice (Cock & Auwerx, 2003). Furthermore, the injection of leptin into leptin-deficient mice decreased the bone formation rate and bone mass (Cock & Auwerx, 2003). The effect of leptin on bone formation in humans is unclear. It is possible that leptin contributed to the decreased bone SOS in the overweight girls. However, hormonal influences on bone would likely translate into systemic effects and yet the differences between adiposity groups in bone
SOS were seen only in the tibia. Therefore, perhaps hormones and physical activity had a combined effect on bone SOS. More research is required to assess this speculation.

Obesity is a major problem in today’s society as it is linked to many health problems, one of which is bone quality. It is important to understand the effect of fat on bone health because there is an accumulation of studies indicating that excess adiposity has a negative impact on bone in children and adolescents (Goulding et al., 2000a; Eliakim et al., 2001; Falk et al., 2008). The results of this study further support this claim. Although body fat is thought to be protective of bone in adult women, this study demonstrates that overweight child and adolescent girls had lower tibial bone strength than their normal-weight peers. Eliakim et al. (2001) and Falk et al. (2008) suggested that the children may not have had enough time for the cumulative effect of the added body weight to be manifested in the bone properties. It would be expected that the added body weight effect would be at least partially manifested in the adolescents but this was not the case, as we did not find an age-by-adiposity interaction in tibial SOS. This may be due to the relatively small sample size of the A-NW group or to the fact that this is a cross-sectional, rather than a longitudinal study. It is possible that the physical activity of A-OW was so low that their inactivity had a stronger (and conflicting) effect on bone strength than the added body weight. From the ANCOVA analysis, although physical activity could not explain the reduced tibial SOS in the overweight subjects, there may have been an interaction between low activity or inactivity and hormonal influences on bones. If this was the case, it only stresses the importance of physical activity, especially to overweight girls and adolescents, during the formative years. In view of the positive correlation between physical activity and tibial SOS, the possible effects of physical
activity on bone should be considered. Along those lines, the possible negative effects of inactivity should also be considered.

It is noteworthy that the duration of obesity among the overweight subjects was not assessed in the present study. Theoretically, the longer that the individual is overweight, the more she is exposed to the weight-bearing activity (or lack of it) and/or hormonal influences on bone development. However, this factor was not assessed as it is very difficult to determine the onset of obesity. Most of the overweight subjects were recruited or referred from physicians' practices and so it is likely that their overweight problems were chronic.

Limitations:

There are several limitations inherent in the present study. Originally, distinct normal-weight (BF% < 25) and obese (BF% > 30) groups were desired for comparison. However, in view of the difficulties in subject recruitment, it was decided to have a combined overweight and obese group (BF% > 28), which may have introduced variability into the data. If the adiposity groups were more extreme (having an obese group, rather than an overweight and obese group), it would have increased the probability in finding a statistical significance between the adiposity groups. In spite of our recruitment efforts, the A-NW group was still relatively small (n = 13). Tibial SOS was still found to be significantly lower in the A-OW group. However, the low number of subjects may have been insufficient to demonstrate potential interaction between age and adiposity in some variables (e.g. SOS, physical activity) or potential differences between groups in other variables (e.g. nutrition).
Regarding the 24-hour recall, results are based on only one day of the subject’s nutritional intake. Although we made sure that it was a typical day of dietary habit, calcium and vitamin D intake are susceptible to underreporting because these nutrients are not contained in all food. Moreover, the presence of a parent during the 24-hour recall interview may have influenced the subject’s decision of what foods to include when completing the questionnaire. For example, if the child had snacked on junk food, she may have been inclined to omit the fact, in fear of her mother’s response.

We did not analyze any hormone levels that are associated with adipose tissue and puberty, such as leptin, vitamin D and estrogen that may affect bone development. Bone formation and resorption markers were also not assessed. All of these factors could have provided valuable metabolic insight into the effects of adiposity on bone.

There are several possible limitations relating to the accelerometry. First, it is possible that the mere fact that the subjects were wearing accelerometers may have initially influenced their physical activity patterns. For example, upon receiving an accelerometer, a girl may have purposely increased her physical activity to show that she had good habits. Thus, five weekdays and two weekend days of physical activity monitoring is recommended for a good representation of physical activity levels in children (Trost et al., 2000). However, only a little more than half of the girls in our study wore the accelerometer for the full seven days, as requested by the researchers. This was especially apparent in the adolescent normal-weight girls, of whom only 30% of the group wearing the accelerometer for seven days. However, the approach used in the present study (three weekdays and one weekend day) has been used or suggested by numerous other studies investigating physical activity in children (Janz et al., 2001; Janz
et al., 2002; Nilsson et al., 2002; Penpraze et al., 2006). It is also a concern that physical activity was assessed during the school year. It has been shown that children tend to peak in physical activity levels in the summer months then decline as winter approaches (Kohl and Hobbs, 1998). However, since all subjects were monitored between October to May, it is assumed that any potential seasonal effect was similar in all groups.

Furthermore, the 10-second epochs of the accelerometers may not have been sensitive enough to pick up the differences in some of the physical activity variables between children that have relatively low physical activity levels to begin with (Baquet et al., 2007; Nilsson et al., 2002). The physical activity pattern in children and adolescents is complex. From direct observation, moderate to highly intense physical activity in children appear to be short, intermittent bouts, lasting three to six seconds long, with regular periods of rest (Bailey et al., 1995). Girls are even less active than boys (Andersen et al., 1998) and the inactivity increases with age. Girls’ lower activity levels are even more apparent in overweight children and adolescents, especially at moderate and higher intensities of physical activity (Page et al., 2005). Thus, an epoch length of five seconds or less may have been warranted for our study.

Accelerometry is an objective measure of physical activity, reflecting physical activity habits over a relatively short period of time. Accelerometry cannot measure past physical activity. On the other hand, bone properties reflect past, as well as present, physical activity. Therefore, it is recommended that a longitudinal follow up study is conducted.

Although QUS is recommended for assessment of bone status in children (Baroncelli, 2008), it still has its limitations when assessing bone strength. QUS cannot
measure bone geometry, which is an important factor that governs strength, as the bending strength of a bone is proportional to the fourth power of its radius (Seeman, 2008). Furthermore, the increase in a long bone’s diameter can account for up to 55% of the variation in bone strength (Ammann & Rizzoli, 2003). Cortical thickness can also contribute to bone strength, although to a lesser degree. Thus, it would have been beneficial to use pQCT to evaluate other bone properties such as geometry and cortical thickness.

Lastly, SOS penetrates two to six millimeters deep into the cortical bone (Baroncelli, 2008). The cortical thickness of girls is likely smaller than that of adolescents and thus we may be measuring different sites of the cortex. And of clinical significance or lack thereof, QUS only measures the diaphysis, whereas most fractures during childhood and adolescence occur at the distal ends of long bones (Tiderius et al., 1999).

Conclusion:

Overweight girls and adolescents were characterized by lower tibial, but not radial, SOS compared with normal-weight girls. The overweight girls and adolescents were significantly less active than normal-weight girls and adolescents, and both adiposity groups had physical activity levels that drastically declined after puberty. Decreased physical activity levels in addition to possible influences of excess body fat (possibly hormonal) may have contributed to the reduced tibial SOS of overweight girls and adolescents. Future research should examine levels of hormones and bone markers in relation to bone health in overweight and obese individuals in order to shed light on the reasons for the apparent reduced bone strength in these children.
REFERENCES


Appendix A: Invitation Letter

Bone Properties, Bone Turnover, and Secretory Immunity in Girls

Principal Investigators: Dr. Bareket Falk and Dr. Nota Klentrou, Department of Physical Education and Kinesiology, Brock University

We would like to invite you to participate in the present study, which investigates bone strength, bone turnover and immunity in young girls, using a new, ultrasound technique.

The purpose of this research project is to compare bone strength, as measured with ultrasound, bone chemistry and immune function between over-weight, normal weight and athletic girls of various ages. In other words, we would like to know if body composition and participation in certain sports enhance bone status and immune function in young people.

Tests and measurements will require about 1.5 hours. Briefly, measurements include bone strength (using ultrasound), secretory immunity (using saliva samples) and filling out several questionnaires. The evaluation of bone turnover using blood samples is optional and you may or may not agree to participate.

Participation in this project will allow you to have personal information on your bone strength, as well as other information, such as height, weight and percent body fat.

This research is being performed only by Brock University researchers in the Applied Physiology Laboratory.

If you have any pertinent questions about your rights as a research participant, please contact the Brock University Research Ethics Officer (905 688-5550 ext 3035, reb@brocku.ca)

If you have any questions, please feel free to contact us.

Thank you

Principal Investigators:

Bareket Falk and Nota Klentrou
Department of Physical Education and Kinesiology
Faculty of Applied Health Science
Brock University
Tel: 905-688-5550 ext:4979 or 4538
email: <bareket.falk@brocku.ca> or <nota.klentrou@brocku.ca>

This study has been reviewed and received ethics clearance through Brock University's Research Ethics Board (file # XXX)
PARTICIPATE IN BONE HEALTH AND IMMUNE FUNCTION RESEARCH

We would like to examine the effects of body composition and training on YOUR BONES AND IMMUNITY

Principal Investigators: Drs. Nota Klientrou and Bareket Falk
Physical Education & Kinesiology, (905) 688-5550 ext. 4538 and 4979

This study is funded by the Social Sciences and Humanities Research Council
And has received clearance from the Brock University Ethics Board (file # 06-316)

We are looking for:

❖ PRE-ADOLESCENT GIRLS (8-11y)
❖ ADOLESCENT GIRLS (14-16 y)

During a 1.5 hour visit to the Applied Physiology Laboratory participants will complete questionnaires on medical history, physical activities, nutritional habits, perceived stress, fatigue, body image and pubertal status. Physiological assessment will include physical characteristics, bone ultrasound, saliva and optional blood sampling. Physical activity will be monitored for 7 consecutive days with a CSA/MTI Actigraph accelerometer. Participants will record any cold/flu symptoms on a log that they will mail back after a month.

IF INTERESTED PLEASE CONTACT:
Matt Yao or Izabella Ludwa (Graduate students) - (905) 680 5550 ext 5623
or email: matthew.yao@brocku.ca or izabella.ludwa@brocku.ca
Appendix C: Informed Consent

INFORMATION & CONSENT/ASSENT TO PARTICIPATE IN RESEARCH

Bone Properties, Bone Turnover, and Secretory Immunity in Girls

You are being invited to participate in a research study being conducted by the investigators listed below. Prior to participating in this study please read this form to find out about the purpose and the tests of this study. For the tests you will have to visit the Exercise Physiology Laboratory (WH22, Brock University). This study is part of the Faculty of Applied Health Sciences of Brock University and the Department of Pediatrics of McMaster University.

INVESTIGATOR:
Dr. Nota Klentrou
Dr. Bareket Falk
Lauren Corbett
Izabella Ludwa
Matthew Yao

DEPARTMENT:
PEKN, Brock University
PEKN, Brock University
PEKN, Brock University
PEKN, Brock University
PEKN, Brock University

CONTACT:
(905) 688-5550 ex. 4538
(905) 688-5550 ex. 4979
(905) 688-5550 ex. 5623
(905) 688-5550 ex. 5623
(905) 688-5550 ex. 5623

PURPOSE:

This multi-purpose research study focuses on young females. The main objective of this study is to compare bone properties, bone turnover and immune function of overweight and athletic girls, as compared with normal-weight girls.

DESCRIPTION OF TESTING PROCEDURES:

If you agree to volunteer for this study, you will visit our laboratory for 1 session of testing, lasting approximately 1.5 hours. At the end of the study, you will be given a summary of the findings, upon request.

You will undergo the measurements and procedures listed below; please note that in all questionnaires, you may choose not to answer any question, and that you may choose not to provide a blood sample without penalty:

1. You will complete several questionnaires, outlining your medical history, physical activities and training history, nutritional habits and pubertal status. The questionnaire used to measure pubertal status involves looking at pictures of pubic hair development and deciding which stage of puberty you best match. This will be carried out in a private room to avoid any uneasiness.

2. You will complete several questionnaires, outlining your feelings of stress, tiredness, and body image.
3. We will measure your height, weight, and percent body fat. Percent body fat will be estimated using bioelectrical impedance analysis. The BIA device creates a mild electrical current (50kHz, 800 μA) that passes from electrodes situated on the back of your hand, through the body, to electrodes on the top of your feet. This current is very low and you will not feel it. The measurement requires approximately 5 minutes, and no discomfort is associated with this measurement.

4. We will determine your bone strength and bone age using the Sunlight Omnisense™ ultrasound system and the Sunlight BonAge™ system, respectively. This procedure involves the application of gel to the forearms, the lower legs, and the wrist, and moving an ultrasound probe over these regions. This procedure requires approximately 20 minutes and causes no discomfort.

5. You will provide a saliva sample by holding a cotton swab in your mouth for approximately one minute. You will be asked not to consume any food or drink for at least one hour prior to saliva collection. This procedure causes no discomfort.

6. You will also provide a blood sample which will be drawn by a certified lab technician, and will be completed using a standard venipuncture technique. It offers minimal risks, although in rare instances, participants may experience minimal momentary pain and/or tingling in the area and/or a minor bruise from the needle.

7. Upon leaving the laboratory, your physical activity will be monitored for 7 consecutive days. You will be provided with an accelerometer, used to monitor physical activity. You will be asked to wear the accelerometer (a small box the size of a match box, weighing 40 g) on a belt around your hips for all waking hours, for one week. In addition, we will ask you to complete a log sheet of any non-weight bearing activities (e.g., cycling) that you perform while the accelerometer is worn.

8. Upon leaving the laboratory, you will also receive a Health Log. You are asked to record cold and flu symptoms each day for one month, using a set of codes provided with the log, and to rate each symptom as mild, moderate, or severe.

It is recommended that you come for the measurements in shorts and a short-sleeved shirt.

Parents may be present with their child at all stages of the study.
CONFIDENTIALITY

All data collected during this study will remain confidential and will be stored in offices and on secured computers to which only the principal and co-investigators have access. You should be aware that the results of this study will be made available to scientists, through publication in a scientific journal but your name and any personal data of you will not appear in the compiling or publishing these results. Data will be kept for 5 years after the date of publication, at which time all information will be destroyed. Additionally, you will have access to your own data, as well as the group data when it becomes available and if you are interested. This can be provided to you by simply contacting the principal investigators.

PARTICIPATION & WITHDRAWL

You can choose whether to participate in this study or not. You may remove your data from the study if you wish. You may also refuse to answer any questions posed to you during the study and still remain as a subject in the study. The investigators reserve the right to withdraw you from the study if they believe that it is necessary.

RISKS AND BENEFITS

Participation will allow you to gain personal and general knowledge about the human body. Additionally, if any unusually low or high result is attained for any of the measurements, reflecting a possible health-related problem, you and your parents will be alerted and advised to consult your physician. All results will be provided to you and your parents upon request.

There are no foreseeable risks associated with participation in this project. The venous blood drawing procedure is a routine procedure performed by a certified technician and offers minimal risk to participants. In rare instances, participants may experience slight pain and/or tingling in the area and/or a minor bruise from the needle.

RIGHTS OF RESEARCH PARTICIPANTS

You will receive a signed copy of this ethics form. You may withdraw your consent to participate in this study at any time, and you may also discontinue participation at any time without penalty. In signing this consent form or in participating in this study, you are not waiving any legal claims or remedies. This study has been reviewed and received clearance from the Brock University Ethics Board (file #________). If you have any pertinent questions about your rights as a research participant, please contact the Brock University research Ethics Office (905-688-5550 ext. 3035, reb@brocku.ca)
INFORMATION:

Please contact Dr. Nota Klentrou at 905-688-5550 ex 4538, Dr. Bareket Falk at 905-688-5550 ex 4979, or Lauren Corbett, Izabella Ludwa, or Matthew Yao, all available at 905-688-5550 ext 5623, if you have any questions about the study.

I HAVE READ AND UNDERSTAND THE ABOVE EXPLANATION OF THE PURPOSE AND PROCEDURES OF THE PROJECT. I HAVE ALSO RECEIVED A SIGNED COPY OF THE INFORMATION AND CONSENT FORM. MY QUESTIONS HAVE BEEN ANSWERED TO MY SATISFACTION AND I AGREE TO PARTICIPATE IN THIS STUDY.

SIGNATURE of PARENT/GUARDIAN ___________________________ DATE ____________

PRINTED NAME OF PARTICIPANT ___________________________ DATE ____________

WITNESS ___________________________ DATE ____________

PRINTED NAME OF WITNESS ___________________________

INVESTIGATOR

In my judgment, the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent and participate in this research study.

SIGNATURE OF INVESTIGATOR ___________________________ DATE ____________
Appendix D: Thank You Letter

Dear ____________ (participant's name),

We are very pleased to write this letter of appreciation for your participation in our research study on Bone Properties, Bone Turnover, and Secretory Immunity in Obese, Normal-weight and Athletic Girls. Your participation, which involved a number of questionnaires, assessment of bone and blood properties analyzed, and recording of your physical activity for a week after her lab visit allows you to also record volunteer hours towards your OSSD if required.

Your enthusiastic participation in this research project was appreciated by all the researchers involved.

Sincerely,

Panagiota Klentrou, PhD
Associate Professor and Chair
Department of Physical Education & Kinesiology
Brock University
Appendix E: 24-Hour Recall Questionnaire

24-HOUR NUTRITIONAL INTAKE

Record all food and fluids from time of waking to midnight 24 hours prior. If yesterday was not typical for your diet (ie. birthday, restaurant, etc.) go back by 48 hours.

24 Hour Recall Date: ___________________________

Nutritional Intake:
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
Appendix F: Bone SOS Data Collection Sheet

BONE STRENGTH DATA COLLECTION SHEET

Bone Strength Data:
Probe Quality Measurements:

<table>
<thead>
<tr>
<th>Date</th>
<th>SOS</th>
<th>Norm</th>
<th>Temp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

Sunlight Omniscence™

<table>
<thead>
<tr>
<th>Side (circle)</th>
<th>Site</th>
<th>SOS</th>
<th>%</th>
<th>Z-Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>L or R</td>
<td>Dominant Radius</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L or R</td>
<td>Dominant Tibia</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>L or R</td>
<td>Non-Dominant Radius</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L or R</td>
<td>Non-Dominant Tibia</td>
<td></td>
<td></td>
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</tbody>
</table>
Appendix G: Medical/Screening Questionnaire

SUBJECT SCREENING AND MEDICAL HISTORY QUESTIONNAIRE

APPLIED PHYSIOLOGY LABORATORY
DEPARTMENT OF PHYSICAL EDUCATION AND KINESIOLOGY
BROCK UNIVERSITY

Name: __________________ Date: __________________ ID: ________

Date of Birth: ______________

Dominant Hand _____________ Dominant Leg _____________

Your responses to this questionnaire are confidential. If you answer “YES” to any of the following questions, please give additional details in the space provided and discuss the matter with one of the investigators. You may refuse to answer any of the following questions.

1. Have you ever had any major joint instability or ongoing chronic pain such as in the knee, back or elbow?

   YES  NO

2. Are you currently taking any medication (including aspirin) or have you taken any medication in the last two days?

   YES  NO

3. Have you taken any medication in the past six months?

   YES  NO

4. Is there any medical condition with which you have been diagnosed and are under the care of a physician (e.g. asthma, diabetes, anorexia)?

   YES  NO

5. Do you, or have you in the past, consumed any alcohol on a regular basis?

   YES  NO
6. Do you, or have you in the past, smoked on a regular basis?
   YES       NO

7. Are you, or have you in the past, engaged in any extreme diet?
   YES       NO

8. Do you, or have you in the past, consumed any nutritional supplements (e.g. calcium, multi-vitamin) on a regular basis?
   YES       NO

9. Do you, or have you in the past, engaged in physical activity on a regular basis?
   YES       NO

10. Have you had any fractures?
    YES       NO

11. Have you had your period?
    YES       NO
APPENDIX H: Tanner Stage for Females

SEXUAL MATURATION AUTOEVALUATION QUESTIONNAIRE (GIRLS)

EXERCISE-METABOLISM RESEARCH GROUP
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

Directions: You should choose only one of the stages shown below. One stage for Breast development and one stage for Pubic Hair development.

Study Subject No:

- Please put a tick in the box that looks most like you now....

1. The breasts are flat.

2. The breasts form small mounds.

3. The breasts form larger mounds than in 2.

4. The nipple and the surrounding part (the Araca) make up a mound that sticks up above the breast.

5. Only the nipple sticks out beyond the breast.

- Please put a tick in the box that looks most like you now....

1. No hairs

2. Very little hair

3. Quite a lot of hair

4. The hair has not spread over the thighs

5. The hair has spread over the thighs

Appendix I: Physical Activity Diary

7 Day Physical Activity Diary – (Parent Reporting for Child)

Dear Parents,

You may choose to fill in the information throughout the day or at the end of each day. The rows of the chart are separated into “Morning”, “Afternoon” and “Evening” categories. For each category, please fill in under “Activity” any structured physical activities that your child performed for that particular day. Also, please fill in under “Time” the duration your child was doing the related activity. **Example:** On day 1, your child had soccer practice at 7pm for 90 minutes. You would record this activity in the “Evening” row and write 7-8:30 under “Time” and write Soccer under “Activity”.

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Activity</td>
<td>Time</td>
<td>Activity</td>
</tr>
<tr>
<td>Morning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Afternoon</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Evening</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td></td>
<td>Day 6</td>
</tr>
<tr>
<td>--------</td>
<td>-------</td>
<td>--------</td>
<td>-------</td>
</tr>
<tr>
<td>Time</td>
<td>Activity</td>
<td>Time</td>
<td>Activity</td>
</tr>
<tr>
<td>Morning</td>
<td></td>
<td>Afternoon</td>
<td></td>
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</tbody>
</table>
### Appendix J: Bone SOS ANOVAs

<table>
<thead>
<tr>
<th></th>
<th>Girls Normal-weight (n = 21)</th>
<th>Girls Overweight (n = 19)</th>
<th>Adolescents Normal-weight (n = 13)</th>
<th>Adolescents Overweight (n = 22)</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant Radius (SOS)</td>
<td>3794 ± 89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3776 ± 64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3994 ± 47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3957 ± 75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>A</td>
</tr>
<tr>
<td>Non-dominant Radius (SOS)</td>
<td>3794 ± 87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3769 ± 54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3964 ± 64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3935 ± 67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>A</td>
</tr>
<tr>
<td>Dominant Tibia (SOS)</td>
<td>3684 ± 90&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>3604 ± 75&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>3868 ± 67&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>3766 ± 132&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>A, Ad</td>
</tr>
<tr>
<td>Non-dominant Tibia (SOS)</td>
<td>3678 ± 86&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>3601 ± 75&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>3878 ± 52&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>3739 ± 134&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>A, Ad</td>
</tr>
</tbody>
</table>

Values are presented as M ± SD. Similar superscripts indicate pairwise significant differences (p < 0.05). A = Age effect, Ad = Adiposity effect, AxAd = Age and adiposity interaction (p < 0.05).
### Appendix K: Whole Group – Bivariate Correlations

<table>
<thead>
<tr>
<th></th>
<th>Dominant Radius SOS</th>
<th>Non-dominant Radius SOS</th>
<th>Dominant Tibia SOS</th>
<th>Non-dominant Tibia SOS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bone</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Dominant Radius SOS</td>
<td>0.97**</td>
<td>0.61**</td>
<td>0.59**</td>
<td></td>
</tr>
<tr>
<td>Non-dominant Radius SOS</td>
<td>0.97**</td>
<td>0.63**</td>
<td>0.60**</td>
<td></td>
</tr>
<tr>
<td>Dominant Tibia SOS</td>
<td>0.61**</td>
<td>0.63**</td>
<td>0.97**</td>
<td></td>
</tr>
<tr>
<td>Non-dominant Tibia SOS</td>
<td>0.59**</td>
<td>0.60**</td>
<td>0.97**</td>
<td></td>
</tr>
<tr>
<td><strong>Anthropometry</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.80**</td>
<td>0.78**</td>
<td>0.59**</td>
<td>0.57**</td>
</tr>
<tr>
<td>Height</td>
<td>0.71**</td>
<td>0.66**</td>
<td>0.48**</td>
<td>0.50**</td>
</tr>
<tr>
<td>Weight</td>
<td>0.56**</td>
<td>0.51**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.37**</td>
<td>0.35**</td>
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</tr>
<tr>
<td>BF%</td>
<td></td>
<td></td>
<td>-0.24*</td>
<td>-0.30*</td>
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<tr>
<td>Fat Mass</td>
<td>0.36**</td>
<td>0.33**</td>
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<td></td>
</tr>
<tr>
<td>Lean Mass</td>
<td>0.68**</td>
<td>0.64**</td>
<td>0.41**</td>
<td>0.39**</td>
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</tbody>
</table>

* p<0.05, ** p<0.01
**Appendix L: Whole Group – Daily Physical Activity and BF% – Bivariate Correlations**

<table>
<thead>
<tr>
<th></th>
<th>Body Fat Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Counts</td>
<td>-0.43**</td>
</tr>
<tr>
<td>% Active</td>
<td>-0.41**</td>
</tr>
<tr>
<td>Moderate Physical Activity</td>
<td>-0.72**</td>
</tr>
<tr>
<td>Vigorous Physical Activity</td>
<td>-0.67**</td>
</tr>
<tr>
<td>Very Vigorous Physical Activity</td>
<td>-0.49**</td>
</tr>
<tr>
<td>Moderate – Very Vigorous Activity</td>
<td>-0.73**</td>
</tr>
</tbody>
</table>

** p<0.01
Appendix M: Whole Group – Partial Correlations for Age

<table>
<thead>
<tr>
<th>Anthropometry</th>
<th>Dominant Radius SOS</th>
<th>Non-dominant Radius SOS</th>
<th>Dominant Tibia SOS</th>
<th>Non-dominant Tibia SOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
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* p<0.05, ** p<0.01
**Appendix N: Whole Group – Daily Physical Activity and BF% - Partial Correlations for Age**

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* p<0.05, ** p<0.01

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**Appendix O: Whole Group – Anthropometry and Bone SOS - Partial Correlations for Adiposity (BF%)**

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** p<0.01

94
### Appendix P: Girls – Bivariate Correlations

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### Appendix Q: Girls – Daily Physical Activity and Body Fat Percentage Bivariate Correlations

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**Appendix R: Girls – Partial Correlations for Adiposity**

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* p<0.05, ** p<0.01
## Appendix S: Adolescents – Bivariate Correlations

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* p<0.05, ** p<0.01
### Appendix T: Adolescents – Daily Physical Activity and Body Fat Percentage Bivariate Correlations

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* p<0.05, ** p<0.01
## Appendix U: Normal-weight – Bivariate Correlations

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* p<0.05, ** p<0.01
## Appendix V: Normal-weight – Daily Physical Activity and Body Fat Percentage Bivariate Correlations

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* p<0.05, ** p<0.01
## Appendix W: Normal-weight – Partial Correlations for Age

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* p<0.05, ** p<0.01
**Appendix X: Overweight – Bivariate Correlations**

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* p<0.05, ** p<0.01
### Appendix Y: Overweight – Daily Physical Activity and Body Fat Percentage Bivariate Correlations

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* p<0.05, ** p<0.01
### Appendix Z: Overweight – Partial Correlations for Age

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* p<0.05
### Appendix AA: Normal-weight Girls – Bivariate Correlations

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## Appendix AB: Overweight Girls – Bivariate Correlations

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* p<0.05, ** p<0.01
Appendix AC: Normal-weight Adolescents – Bivariate Correlations

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**Physical Activity**

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* p<0.05, ** p<0.01
### Appendix AD: Overweight Adolescents – Bivariate Correlations

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* p<0.05, ** p<0.01

### Appendix AE: Overweight Adolescents – Daily Physical Activity and Body Fat Percentage Bivariate Correlations

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* p<0.05, ** p<0.01
### Appendix AF: Non-Dominant Tibia SOS ANCOVA – Physical Characteristics and Activity Covariates

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\(a\) Computed using alpha = 0.05; \(b\) R Squared = 0.57 \(\text{Adjusted R Squared} = 0.52\)
## Appendix AG: Non-Dominant Tibia SOS ANCOVA – Physical Activity Covariates

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<sup>a</sup> Computed using alpha = 0.05; <sup>b</sup> R Squared = 0.54 (Adjusted R Squared = 0.46)
## Appendix AH: Descriptives for Normal-weight Girls

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### Appendix AI: Descriptives for Overweight Girls

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# Appendix AJ: Descriptives for Normal-weight Adolescents

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<td>7.86</td>
<td>1.9221</td>
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<td>2.45499</td>
<td>6.027</td>
<td>1.832</td>
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<tr>
<td>MVPA (min/day)</td>
<td>12</td>
<td>87.30</td>
<td>63.10</td>
<td>150.39</td>
<td>102.9633</td>
<td>7.85731</td>
<td>27.21851</td>
<td>740.847</td>
<td>-.259</td>
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<tr>
<td>Statistic</td>
<td>N</td>
<td>Range</td>
<td>Min.</td>
<td>Max.</td>
<td>Mean</td>
<td>Std. Dev.</td>
<td>Variance</td>
<td>Skewness</td>
<td>Kurtosis</td>
</tr>
<tr>
<td>-----------</td>
<td>---</td>
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</tr>
<tr>
<td>Age (years)</td>
<td>22</td>
<td>2.89</td>
<td>14.01</td>
<td>16.90</td>
<td>15.4816</td>
<td>.16295</td>
<td>.76428</td>
<td>.584</td>
<td>-.187</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>22</td>
<td>31.00</td>
<td>148.00</td>
<td>179.00</td>
<td>164.4545</td>
<td>1.39468</td>
<td>6.54164</td>
<td>42.793</td>
<td>-.202</td>
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<tr>
<td>Weight (kg)</td>
<td>21</td>
<td>53.80</td>
<td>59.80</td>
<td>113.60</td>
<td>84.2952</td>
<td>3.38531</td>
<td>15.51343</td>
<td>240.666</td>
<td>.107</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22</td>
<td>19.30</td>
<td>21.80</td>
<td>41.10</td>
<td>30.8727</td>
<td>2.2252</td>
<td>5.73412</td>
<td>32.880</td>
<td>-.147</td>
</tr>
<tr>
<td>BMI BF%</td>
<td>22</td>
<td>20.00</td>
<td>75.00</td>
<td>95.00</td>
<td>91.5909</td>
<td>1.52136</td>
<td>7.13582</td>
<td>50.920</td>
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<tr>
<td>Fat Mass (kg)</td>
<td>22</td>
<td>23.20</td>
<td>29.10</td>
<td>52.30</td>
<td>41.2500</td>
<td>1.53266</td>
<td>7.18880</td>
<td>51.679</td>
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<tr>
<td>Lean Mass (kg)</td>
<td>21</td>
<td>40.80</td>
<td>17.40</td>
<td>58.20</td>
<td>35.9592</td>
<td>2.60088</td>
<td>11.91874</td>
<td>142.056</td>
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<td>Menarche (years)</td>
<td>22</td>
<td>20.30</td>
<td>41.30</td>
<td>61.60</td>
<td>48.2381</td>
<td>1.14171</td>
<td>5.23197</td>
<td>27.373</td>
<td>.919</td>
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<tr>
<td>Tanner</td>
<td>22</td>
<td>5.00</td>
<td>9.00</td>
<td>14.00</td>
<td>11.7727</td>
<td>.27273</td>
<td>1.27920</td>
<td>1.636</td>
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<tr>
<td>Energy Intake (Kcal/day)</td>
<td>20</td>
<td>271.00</td>
<td>3834.00</td>
<td>4105.00</td>
<td>3957.4500</td>
<td>16.68745</td>
<td>74.62854</td>
<td>5569.418</td>
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<tr>
<td>Calcium Intake (mg/day)</td>
<td>20</td>
<td>277.00</td>
<td>3811.00</td>
<td>4088.00</td>
<td>3935.2380</td>
<td>14.65377</td>
<td>67.15200</td>
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<tr>
<td>Vitamin D Intake (mg/day)</td>
<td>20</td>
<td>485.00</td>
<td>3507.00</td>
<td>3992.00</td>
<td>3766.1050</td>
<td>30.28151</td>
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<td>-.269</td>
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<tr>
<td>Activity (counts/day)</td>
<td>20</td>
<td>498.00</td>
<td>3474.00</td>
<td>3972.00</td>
<td>3739.3000</td>
<td>29.92298</td>
<td>133.8196</td>
<td>17907.6</td>
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<td>Inactivity (min/day)</td>
<td>20</td>
<td>1452.34</td>
<td>847.16</td>
<td>2299.50</td>
<td>1647.2980</td>
<td>87.70185</td>
<td>392.2427</td>
<td>15385.3</td>
<td>-.230</td>
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<tr>
<td>Sedentary Activity (min/day)</td>
<td>20</td>
<td>1260.55</td>
<td>245.57</td>
<td>1452.12</td>
<td>891.6098</td>
<td>73.41326</td>
<td>336.4218</td>
<td>11317.9</td>
<td>-.226</td>
</tr>
<tr>
<td>Light Activity (min/day)</td>
<td>20</td>
<td>12.12</td>
<td>.00</td>
<td>12.12</td>
<td>4.5460</td>
<td>.87211</td>
<td>4.09057</td>
<td>16.733</td>
<td>.497</td>
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<tr>
<td>Moderate Activity (min/day)</td>
<td>20</td>
<td>53.2</td>
<td>2.0</td>
<td>10.1</td>
<td>6.009</td>
<td>.6050</td>
<td>2.6369</td>
<td>6.953</td>
<td>.108</td>
</tr>
<tr>
<td>Vigorous Activity (min/day)</td>
<td>20</td>
<td>367.71</td>
<td>407.14</td>
<td>774.86</td>
<td>543.3540</td>
<td>17.71081</td>
<td>77.19962</td>
<td>5595.782</td>
<td>1.260</td>
</tr>
<tr>
<td>Very Vigorous Activity (min/day)</td>
<td>20</td>
<td>44.00</td>
<td>48.43</td>
<td>92.43</td>
<td>63.8847</td>
<td>2.60081</td>
<td>11.33665</td>
<td>128.520</td>
<td>.311</td>
</tr>
<tr>
<td>MVPA (min/day)</td>
<td>20</td>
<td>161.71</td>
<td>94.71</td>
<td>256.43</td>
<td>169.0056</td>
<td>8.16919</td>
<td>35.60868</td>
<td>1267.98</td>
<td>.255</td>
</tr>
</tbody>
</table>

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Appendix AL: Data Reduction Program for 10-second Epoch Accelerometry Analysis

Sub resefim()

Dim flagy(0 To 5) As Boolean
Dim dt(1 To 21) As Date ' days for analysis
Dim limit(1 To 4) As Integer
Dim dimlt(1 To 4) As Integer

Dim resef(0 To 5) As Integer
Dim resmx(0 To 5) As Integer
Dim sachs(0 To 5) As Integer
Dim ct5lev(0 To 5) As Integer
Dim ct10lev(0 To 5) As Integer
Dim ct20lev(0 To 5) As Integer
Dim ct5lev9(0 To 7) As Integer
Dim ct10lev9(0 To 7) As Integer
Dim ct20lev9(0 To 7) As Integer
Dim ahoz(0 To 5) As Integer
Dim gilmets(1 To 14, 1 To 4) As Integer
Dim avrg As Integer
Dim levy As Integer
Dim qgil As Integer
Dim ap As Integer
Dim moz As Integer
Dim efes As Boolean

gilmets(1, 1) = 6 ' table of limits for kids(by age) each has 4 numbers: the first is age, after 3 limits

gilmets(1, 2) = 102 ' first upto is light, second upto is moderate, third upto is hard,
gilmets(1, 3) = 495 ' above third is very hard

gilmets(1, 4) = 888

gilmets(2, 1) = 7

gilmets(2, 2) = 105

gilmets(2, 3) = 511

gilmets(2, 4) = 916

gilmets(3, 1) = 8

gilmets(3, 2) = 134

gilmets(3, 3) = 552

gilmets(3, 4) = 970

gilmets(4, 1) = 9

gilmets(4, 2) = 152

gilmets(4, 3) = 587

gilmets(4, 4) = 1022

gilmets(5, 1) = 10

gilmets(5, 2) = 170

gilmets(5, 3) = 616

gilmets(5, 4) = 1062

gilmets(6, 1) = 11

gilmets(6, 2) = 189

gilmets(6, 3) = 651

gilmets(6, 4) = 1174

gilmets(7, 1) = 12

gilmets(7, 2) = 211

gilmets(7, 3) = 689
gilmets(7, 4) = 1168
gilmets(8, 1) = 13
gilmets(8, 2) = 233
gilmets(8, 3) = 730
gilmets(8, 4) = 1227
gilmets(9, 1) = 14
gilmets(9, 2) = 258
gilmets(9, 3) = 774
gilmets(9, 4) = 1291
gilmets(10, 1) = 15
gilmets(10, 2) = 284
gilmets(10, 3) = 822
gilmets(10, 4) = 1360
gilmets(11, 1) = 16
gilmets(11, 2) = 313
gilmets(11, 3) = 874
gilmets(11, 4) = 1435
gilmets(12, 1) = 17
gilmets(12, 2) = 345
gilmets(12, 3) = 930
gilmets(12, 4) = 1516
gilmets(13, 1) = 18
gilmets(13, 2) = 379
gilmets(13, 3) = 992
gilmets(13, 4) = 1605

tm12 = Format(Cells(12, 1), "hh:mm:ss ampm") 'MsgBox ("time of 12 is " & tm12)
nm = InputBox("Enter subject name ") 'get subject info. name & age
gilin:
gil = InputBox("Enter subject age in years, between 6 and 18 ")
If gil < 6 Or gil > 18 Then ' if the age is out of range 6-18 then enter age again
    MsgBox ("the age given is out range for this program")
    vb = InputBox("would you like to chose new age ?")
    If vb = "y" Or vb = "yes" Then
        GoTo gilin
    Else
        GoTo sofy
    End If
Else
    For indy = 1 To 13 ' enter the right limits for the age of the subject
        qgil = gilmets(indy, 1)
        If qgil = gil Then
            limit(1) = 15 ' sedentary limit for all ages
            For h = 2 To 4
                limit(h) = gilmets(indy, h)
                Next h
            End If
        Next indy
    End If

hatchala: ' you can chose start & end of-day times for all days the same
    ' or let the computer find them for each day
stm = InputBox(" Do you want to SET TIME Start & End yes/no? ")
If stm = "yes" Or stm = "y" Then
    tm1 = InputBox(" Enter start time (24hr format eg 0700) ")
tm2 = InputBox(" Enter end time (24hr format eg 2200) ")
t1 = Int(tm1 / 100) 'hours
t2 = Int(tm2 / 100)
td1 = tm1 / 100 - t1 'minutes
td2 = tm2 / 100 - t2
tdl = tdl * 100
td2 = td2 * 100
stcell = t1 * 360 + 12 + tdl * 6 'the start line no. from time given
encell = t2 * 360 + 12 + td2 * 6 'the end line no. from time given
End If

k = 1
t = 1
'write the limits in the results sheet- sheet2
Worksheets("sheet2").Cells(3 + k, 1) = "sedetary <"
Worksheets("sheet2").Cells(4 + k, 1) = "light <"
Worksheets("sheet2").Cells(5 + k, 1) = "moderate <"
Worksheets("sheet2").Cells(6 + k, 1) = "hard <"
Worksheets("sheet2").Cells(7 + k, 1) = "very hard >"
Worksheets("sheet2").Cells(3 + k, 2) = limit(1)
Worksheets("sheet2").Cells(4 + k, 2) = limit(2)
Worksheets("sheet2").Cells(5 + k, 2) = limit(3)
Worksheets("sheet2").Cells(6 + k, 2) = limit(4)
d = 1 'first day
startingday: 'each day's calculations starts here
If d > 8 Then GoTo sofy , if more then 8 days go to end

toprec = 0
sumy = 0

dt(d) = Worksheets("sheet1").Cells(12, 2 * d - 1) 'get the date for this day
If dt(d) = tm12 Then GoTo Nextday 'if the date is 12:00 it means no data
If stm = "yes" Or stm = "y" Then 'do the set times
"SET TIMES"
Else 'find out start & end of day
stcell = findstartcell(d)
encell = findendcell(d)
fcell = encell
cv = Worksheets("sheet1").Cells(encell, 2 * d).Value
If cv < 100 Or cv > 3000 Then
encell = encell - 1
cv1 = Worksheets("sheet1").Cells(encell, 2 * d).Value
If cv1 < 100 Or cv1 > 3000 Then encell = encell - 1
End If
efesc = 0
End If

If Not (0 < encell < 8650) Then encell = 8649 'end of day in 10s lines

For i = stcell To encell '11 to 8650 all day
'write level for each 10s
x = Worksheets("sheet1").Cells(i, 2 * d).Value
If x = tm12 Then 'if the value is 12:00 then write 0 & goto next day
wlevel = 0
Exit For

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GoTo today
End If
sumy = sumy + x
If x > toprec Then toprec = x

If x <= 0 Then 'figure out the level of action for each line
wlevel = 0
ElseIf x < limit(1) Then
wlevel = 1
ElseIf x < limit(2) Then
wlevel = 2
ElseIf x < limit(3) Then
wlevel = 3
ElseIf x < limit(4) Then
wlevel = 4
ElseIf x > limit(4) Then
wlevel = 5
End If

Worksheets("sheet3").Cells(i, d) = wlevel 'write on sheet3 the level of action for each line
Next i 'finish writing level for each count

st = stcell
en = encell
moz = 0
pael = 0

For g = 0 To 5 'write zero = initialize all variables
sach(g) = 0
resef(g) = 0
resmx(g) = 1
flagy(g) = False
tc5lev(g) = 0
tc10lev(g) = 0
tc20lev(g) = 0
tc5lev9(g) = 0
tc10lev9(g) = 0
tc20lev9(g) = 0
Next g

p = st
chkresmx: 'find max resef in each level

w1 = Worksheets("sheet3").Cells(p, d) 'take one line's value

'check the current value (0 to 5), add 1 to the right counter =sach() 'if the last one was the same & the resef is 0 it is 2 now 'if the last one was the same & the resef was not 0 just add 1 'if the current resef is bigger then the maximal then change 'set the flag on this value true & the rest false 'set all other resefim to 0

If w1 = 0 Then 'if it is zero
sach(0) = sach(0) + 1 'add to zero count
If flagy(0) = True And resef(0) = 0 Then resef(0) = 2 'if the one before was zero count 2 for resef
If flagy(0) = True And Not (resef(0) = 0) Then resef(0) = resef(0) + 1 ' if the 1 before was 0 & the
some more before were 0 just add 1
If resef(0) > resmx(0) Then resmx(0) = resef(0) ' if the current resef is bigger the max change
flagy(0) = True ' the last one was 0
For m = 1 To 5 ' write 0 to all ther counts & flags
resef(m) = 0
flagy(m) = False
Next m
End If
If w1 = 1 Then
sach(1) = sach(1) + 1
If flagy(1) = True And resef(1) = 0 Then resef(1) = 2
If flagy(1) = True And Not (resef(1) = 0) Then resef(1) = resef(1) + 1
If resef(1) > resmx(1) Then resmx(1) = resef(1)
For i = 2 To 5
resef(i) = 0
flagy(i) = False
Next i
flagy(1) = True
End If
If w1 = 2 Then
sach(2) = sach(2) + 1
If flagy(2) = True And resef(2) = 0 Then resef(2) = 2
If flagy(2) = True And Not (resef(2) = 0) Then resef(2) = resef(2) + 1
If resef(2) > resmx(2) Then resmx(2) = resef(2)
flagy(2) = True
For g = 3 To 5
flagy(g) = False
resef(g) = 0
Next g
flagy(1) = True
End If
If w1 = 3 Then
sach(3) = sach(3) + 1
If flagy(3) = True And resef(3) = 0 Then resef(3) = 2
If flagy(3) = True And Not (resef(3) = 0) Then resef(3) = resef(3) + 1
If resef(3) > resmx(3) Then resmx(3) = resef(3)
For n = 4 To 5
flagy(n) = False
resef(n) = 0
Next n
flagy(3) = True
End If
If w1 = 4 Then
sach(4) = sach(4) + 1
If flagy(4) = True And resef(4) = 0 Then resef(4) = 2
If flagy(4) = True And Not (resef(4) = 0) Then resef(4) = resef(4) + 1
If resef(4) > resmx(4) Then resmx(4) = resef(4)
flagy(4) = True
flagy(5) = False
End If
If w1 = 5 Then
sach(5) = sach(5) + 1
If flagy(5) = True And resef(5) = 0 Then resef(5) = 2
If flagy(5) = True And Not (resef(5) = 0) Then resef(5) = resef(5) + 1
If resef(5) > resmx(5) Then resmx(5) = resef(5)
For n = 0 To 4

If flagy(n) = True And resef(n) = 0 Then resef(n) = 2
If flagy(n) = True And Not (resef(n) = 0) Then resef(n) = resef(n) + 1
If resef(n) > resmx(n) Then resmx(n) = resef(n)
Next n
flagy(5) = True
End If 'end of check value for max resef

p = p + 1 'add one to the position
If p < en Then GoTo chkresmx 'go through the day till the end = en

'100% first time to look for resef in 20min,10min,5min

en20 = en - 119 'last place to look for 20min (120 lines)
en10 = en - 59 'the same for 10min (60 lines)
en5 = en - 29 'the same for 5min (30 lines)

stch20: 'strt check 20min
s = st 'start of day
limity = 15
ch20:

levy = is20min(d, s) 'is it a 20min resef in any level 5 to 1?
If Not (levy = 0) Then 'found 20min same level
    avrg = memoza(d, s, 20) 'do average of the actual action counts
Else
    GoTo chl0
End If
e = 4 'go down
wh20: If avrg > limity Then 'go through the day till the end = en
    f = e + 1
    ct20lev(f) = ct20lev(f) + 1
    s = s + 120 'move down 20min=120lines
    GoTo ch20
End If
e = e - 1
If e > 0 Then
    GoTo wh20
Else
    If avrg < limity And avrg > 0 Then 'change to limit (1,t)
        ct20lev(1) = ct20lev(1) + 1
        s = s + 120
        GoTo ch20
    End If
End If

End If' end of check value for max resef

ch10:
levy = is10min(d, s) 'is it a 20min resef in any level 5 to 1?
If Not (levy = 0) Then 'found 20min same level
    avrg = memoza(d, s, 10)
Else
    GoTo ch5
End If
e = 4
wh10: If avrg > limity(e) Then
    f = e + 1
    ct10lev(f) = ct10lev(f) + 1
s = s + 60  'move down 10min=60lines
GoTo ch10
End If

e = e - 1
If e > 0 Then
    GoTo wh10
Else
    If avrg < limity And avrg > 0 Then
        ct10lev(1) = ct10lev(1) + 1
        s = s + 60
        GoTo ch10
    End If
End If
End If

ch5:
levvy = is5min(d, s)  'is it a 5min resef in any level 5 to 1?
If Not (levvy = 0) Then    'found 5min same level
    e = 4
    avrg = memoza(d, s, 5)
Else
    GoTo endy
End If

wh5: If avrg > limite Then
    f = e + 1
    ct5lev(f) = ct5lev(f) + 1
    s = s + 30  'move down
    GoTo ch5
End If

e = e - 1
If e > 0 Then
    GoTo wh5
Else
    If avrg < limity And avrg > 0 Then
        ct5lev(1) = ct5lev(1) + 1  'sedentary
        s = s + 30
        GoTo ch5
    End If
End If
End If

endy: s = s + 1
If s < en20 Then GoTo ch20
chlast: If s < en10 Then
    GoTo ch10
ElseIf s < e5 Then
    GoTo ch5
End If

'second time - look for 90% of resefim 20min,10min,5min
e9n20 = en - 119    'last place to look for 20min (120 lines)
e9n10 = en - 59    'the same for 10min (60 lines)
e9n5 = en - 29    'the same for 5min (30 lines)

stch920:    'strt check 20min
s2 = st    'start of day

ch920:
levy = is20min9(d, s2)  'is it a 90% of 20min resef in any level 5 to 1?
If Not (levy = 0) Then    'found 90% 20min same level
avrg = memoza(d, s2, 20)
Else
  GoTo ch910
End If

wh920:
  If avrg > limit(e2) Then
    f = e2 + 1
    ct20lev9(f) = ct20lev9(f) + 1
    s2 = s2 + 119 ' move down 20min=120lines
    GoTo endy9
  End If
  e2 = e2 - 1
  If e2 > 0 Then
    GoTo wh920
  Else
    If avrg < limity And avrg > 0 Then
      ct20lev9(1) = ct20lev9(1) + 1
      s2 = s2 + 119
      GoTo endy9
    End If
  End If
End If

ch910:
levvy = is10min9(d, s2) ' is it a 20min resef in any level 5 to 1 ?
If Not (levvy = 0) Then ' found 20min same level
  avrg = memoza(d, s2, 10)
Else
  GoTo ch95
End If

c2 = 4
wh920: If avrg > limit(e2) Then
  f = e2 + 1
  ct10lev9(f) = ct10lev9(f) + 1
  s2 = s2 + 59 ' move down 10min=60lines
  GoTo endy9
  End If
  e2 = e2 - 1
  If e2 > 0 Then
    GoTo wh910
  Else
    If avrg < limity And avrg > 0 Then
      ct10lev9(1) = ct10lev9(1) + 1
      s2 = s2 + 59
      GoTo endy9
    End If
  End If
End If

ch95:
levvy = is5min9(d, s2) ' is it 90% of 5min resef in any level 5 to 1 ?
If Not (levvy = 0) Then ' found 5min same level
  avrg = memoza(d, s2, 5)
Else
  GoTo endy9
End If

c2 = 4
wh95: If avrg > limit(e2) Then
  f = e2 + 1

ct5lev9(f) = ct5lev9(f) + 1
s2 = s2 + 29 ' move down 20min=120lines
GoTo endy9
End If
e2 = e2 - 1
If e2 > 0 Then
    GoTo wh95
Else
    If avrg < limity And avrg > 0 Then
        ct5lev9(1) = ct5lev9(1) + 1
        s2 = s2 + 29
        GoTo endy9
    End If
End If
End If
endy9: s2 = s2 + 1
If s2 < e9n20 Then GoTo ch920
chlast9:
    If s2 < e9n10 Then
        GoTo ch910
    ElseIf s2 < e9n5 Then
        GoTo ch95
    End If
hadpasot: ' start of write down of the results to sheet2
If (encell - stcell) < 1080 Then ' day was less then 180min = 3 hour so noday
    GoTo noday
End If
Worksheets("sheet2").Cells(1, 1) = "Name"
Worksheets("sheet2").Cells(1, 2) = nm
Worksheets("sheet2").Cells(1, 3) = "Date"
Worksheets("sheet2").Cells(2, 1) = "age=" & gil
If toprec < 50 Then ' if the top value is less then 50 then noday
    GoTo noday
End If
sachkol = encell - stcell + 1 ' time of all day = endof day minus start of day
sachkol = sachkol / 6 ' change from 10s lines to minutes = divide by 6
sumy = sumy / 6
For i = 0 To 5
    resmx(i) = resmx(i) / 6
Next i
If sachkol <= 0 Then ' if the sum of all day is 0 or less then noday
    GoTo Nextday
End If
moz = sumy / sachkol ' average action for this day
Worksheets("sheet2").Cells(k + 1, 3) = dt(d) ' write date , start time & end time
tmst = Format(Cells(stcell, 1), "hh:mm:ss ampm ")
Worksheets("sheet2").Cells(1, 4) = "start time"
Worksheets("sheet2").Cells(k + 1, 4) = tmst
tmed = Format(Cells(encell, 1), "hh:mm:ss ampm ")
Worksheets("sheet2").Cells(1, 5) = "end time"
Worksheets("sheet2").Cells(k + 1, 5) = tmed
Worksheets("sheet2").Cells(1, 6) = "max zero" ' write maximal reseffor all levels
Worksheets("sheet2").Cells(k + 1, 6) = resmx(0)
Worksheets("sheet2").Cells(1, 7) = "max sedentary" ' level 1
Worksheets("sheet2").Cells(k + 1, 7) = resmx(1)
Worksheets("sheet2").Cells(1, 8) = "max light" ' level 2
Worksheets("sheet2").Cells(k + 1, 8) = resmx(2)
Worksheets("sheet2").Cells(1, 9) = "max mod" ' level 3
Worksheets("sheet2").Cells(k + 1, 9) = resmx(3)
Worksheets("sheet2").Cells(1, 10) = "max hard" ' level 4
Worksheets("sheet2").Cells(k + 1, 10) = resmx(4)
Worksheets("sheet2").Cells(1, 11) = "max vhard" ' level 5
Worksheets("sheet2").Cells(k + 1, 11) = resmx(5)

Worksheets("sheet2").Cells(1, 12) = "5 min sedentary" ' write 5min resefIm for all levels
Worksheets("sheet2").Cells(k + 1, 12) = ct5lev(1)
Worksheets("sheet2").Cells(1, 13) = "5 min light"
Worksheets("sheet2").Cells(k + 1, 13) = ct5lev(2)
Worksheets("sheet2").Cells(1, 14) = "5 min mod"
Worksheets("sheet2").Cells(k + 1, 14) = ct5lev(3)
Worksheets("sheet2").Cells(1, 15) = "5 min hard"
Worksheets("sheet2").Cells(k + 1, 15) = ct5lev(4)
Worksheets("sheet2").Cells(1, 16) = "5 min vhard"
Worksheets("sheet2").Cells(k + 1, 16) = ct5lev(5)

Worksheets("sheet2").Cells(1, 17) = "5 min 90% sedentary" ' write 5min resefIm for all levels
Worksheets("sheet2").Cells(k + 1, 17) = ct5lev9(1)
Worksheets("sheet2").Cells(1, 18) = "5 min 90% light"
Worksheets("sheet2").Cells(k + 1, 18) = ct5lev9(2)
Worksheets("sheet2").Cells(1, 19) = "5 min 90% mod"
Worksheets("sheet2").Cells(k + 1, 19) = ct5lev9(3)
Worksheets("sheet2").Cells(1, 20) = "5 min 90% hard"
Worksheets("sheet2").Cells(k + 1, 20) = ct5lev9(4)
Worksheets("sheet2").Cells(1, 21) = "5 min 90% vhard"
Worksheets("sheet2").Cells(k + 1, 21) = ct5lev9(5)

Worksheets("sheet2").Cells(1, 22) = "10 min sedentary" ' write 10min resefIm for all levels
Worksheets("sheet2").Cells(k + 1, 22) = ct10lev(1)
Worksheets("sheet2").Cells(1, 23) = "10 min light"
Worksheets("sheet2").Cells(k + 1, 23) = ct10lev(2)
Worksheets("sheet2").Cells(1, 24) = "10 min mod"
Worksheets("sheet2").Cells(k + 1, 24) = ct10lev(3)
Worksheets("sheet2").Cells(1, 25) = "10 min hard"
Worksheets("sheet2").Cells(k + 1, 25) = ct10lev(4)
Worksheets("sheet2").Cells(1, 26) = "10 min vhard"
Worksheets("sheet2").Cells(k + 1, 26) = ct10lev(5)

Worksheets("sheet2").Cells(1, 27) = "10 min 90% sedentary" ' write 10min resefIm for all levels
Worksheets("sheet2").Cells(k + 1, 27) = ct10lev9(1)
Worksheets("sheet2").Cells(1, 28) = "10 min 90% light"
Worksheets("sheet2").Cells(k + 1, 28) = ct10lev9(2)
Worksheets("sheet2").Cells(1, 29) = "10 min 90% mod"
Worksheets("sheet2").Cells(k + 1, 29) = ct10lev9(3)
Worksheets("sheet2").Cells(1, 30) = "10 min 90% hard"
Worksheets("sheet2").Cells(k + 1, 30) = ct10lev9(4)
Worksheets("sheet2").Cells(1, 31) = "10 min 90% vhard"
Worksheets("sheet2").Cells(k + 1, 31) = ct10lev9(5)

Worksheets("sheet2").Cells(1, 32) = "20 min sedentary" 'write 20min resefim for all levels
Worksheets("sheet2").Cells(k + 1, 32) = ct20lev(1)
Worksheets("sheet2").Cells(1, 33) = "20 min light" 'write 20min resefim for all levels
Worksheets("sheet2").Cells(k + 1, 33) = ct20lev(2)
Worksheets("sheet2").Cells(1, 34) = "20 min mod" 'write 20min resefim for all levels
Worksheets("sheet2").Cells(k + 1, 34) = ct20lev(3)
Worksheets("sheet2").Cells(1, 35) = "20 min hard" 'write 20min resefim for all levels
Worksheets("sheet2").Cells(k + 1, 35) = ct20lev(4)
Worksheets("sheet2").Cells(1, 36) = "20 min vhard" 'write 20min resefim for all levels
Worksheets("sheet2").Cells(k + 1, 36) = ct20lev(5)

Worksheets("sheet2").Cells(1, 37) = "20 min 90% sedentary" 'write 20min resefim for all levels
Worksheets("sheet2").Cells(k + 1, 37) = ct20lev9(1)
Worksheets("sheet2").Cells(1, 38) = "20 min 90% light" 'write 20min resefim for all levels
Worksheets("sheet2").Cells(k + 1, 38) = ct20lev9(2)
Worksheets("sheet2").Cells(1, 39) = "20 min 90% mod" 'write 20min resefim for all levels
Worksheets("sheet2").Cells(k + 1, 39) = ct20lev9(3)
Worksheets("sheet2").Cells(1, 40) = "20 min 90% hard" 'write 20min resefim for all levels
Worksheets("sheet2").Cells(k + 1, 40) = ct20lev9(4)
Worksheets("sheet2").Cells(1, 41) = "20 min 90% vhard" 'write 20min resefim for all levels
Worksheets("sheet2").Cells(k + 1, 41) = ct20lev9(5)

For kj = 0 To 5
  sach(kj) = sach(kj) / 6 ' div. all sums by 6 (change 10s to 1 min)
Next kj

Worksheets("sheet2").Cells(1, 42) = "tot zeros" 'write all total times for all levels
Worksheets("sheet2").Cells(k + 1, 42) = sach(0)
Worksheets("sheet2").Cells(1, 43) = "tot sedetary" 'write all total times for all levels
Worksheets("sheet2").Cells(k + 1, 43) = sach(1)
Worksheets("sheet2").Cells(1, 44) = "tot light" 'write all total times for all levels
Worksheets("sheet2").Cells(k + 1, 44) = sach(2)
Worksheets("sheet2").Cells(1, 45) = "tot mod" 'write all total times for all levels
Worksheets("sheet2").Cells(k + 1, 45) = sach(3)
Worksheets("sheet2").Cells(1, 46) = "tot hard" 'write all total times for all levels
Worksheets("sheet2").Cells(k + 1, 46) = sach(4)
Worksheets("sheet2").Cells(1, 47) = "tot v hard" 'write all total times for all levels
Worksheets("sheet2").Cells(k + 1, 47) = sach(5)
Worksheets("sheet2").Cells(1, 48) = "Total time" 'write all total times for all levels
Worksheets("sheet2").Cells(k + 1, 48) = sachkol

pael = sach(3) + sach(4) + sach(5)
pp = pael * 100

ag = 100 * agg
ahoz(g) = ag
Next g

Worksheets("sheet2").Cells(1, 49) = "%zero" 'write percentage of action in each level
Worksheets("sheet2").Cells(k + 1, 49) = ahoz(0)

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Worksheets("sheet2").Cells(1, 50) = "%sedentary"
Worksheets("sheet2").Cells(k + 1, 50) = ahoz(1)
Worksheets("sheet2").Cells(1, 51) = "%light"
Worksheets("sheet2").Cells(k + 1, 51) = ahoz(2)
Worksheets("sheet2").Cells(1, 52) = "%mod"
Worksheets("sheet2").Cells(k + 1, 52) = ahoz(3)
Worksheets("sheet2").Cells(1, 53) = "%hard"
Worksheets("sheet2").Cells(k + 1, 53) = ahoz(4)
Worksheets("sheet2").Cells(1, 54) = "%v hard"
Worksheets("sheet2").Cells(k + 1, 54) = ahoz(5)

Worksheets("sheet2").Cells(1, 55) = "average"  'write average
Worksheets("sheet2").Cells(k + 1, 55) = moz
Worksheets("sheet2").Cells(1, 56) = "peak"  'write top value
Worksheets("sheet2").Cells(k + 1, 56) = toprec
Worksheets("sheet2").Cells(l, 56) = "peak"
Worksheets("sheet2").Cells(1, 57) = "tot mvh"  'total mod to vhard & it's percentage
Worksheets("sheet2").Cells(k + 1, 57) = pael
Worksheets("sheet2").Cells(1, 58) = "%active"
Worksheets("sheet2").Cells(k + 1, 58) = ap

k = k + 1
GoTo Nextday 'this day is finished go to the next

noday:  ' NO DAY

Nextday:  d = d + 1
          GoTo startingday:
sofy:
End Sub
Function is5min(d, s)  'function to look for FULL 5min resef
Dim v(0 To 50) As Integer
is5min = 0
g = 5  ' start at level 5
clev5:
For j = 0 To 29
  v(j) = 0
Next j
sachy = 0
For i = 0 To 29  ' count how many lines in the same level of action
  v(i) = Worksheets("sheet3").Cells(s + i, d).Value
  If i = 0 And v(i) = 0 Then Exit For
  If v(i) >= g Then sachy = sachy + 1
Next i
If sachy = 30 Then  ' was 30=5min
  is5min = g
  GoTo endof5
End If
  g = g - 1  ' go down from 5 till 1
If g > 0 Then GoTo clev5
endof5:
End Function
Function is5min9(d, s2)  'function to look for FULL 5min resef  'second
Dim v(0 To 50) As Integer
is5min9 = 0
  g = 5  ' start at level 5
clev95:
For \( m = 0 \) To 29
\[ v(m) = 0 \]
Next \( m \)
sac = 0
i = 0
chile:
\[ v(i) = \text{Worksheets("sheet3").Cells}(s2 + i, d).Value \]
If \( i = 0 \) And \( v(i) = 0 \) Then GoTo nelev
If \( v(i) >= g \) Then sac = sac + 1
i = i + 1
If \( i < 30 \) Then GoTo chile
If sac > 26 Then
\[ \text{is}5\text{min}9 = g \]
GoTo endof59
End If

nelev: \( g = g - 1 \) ' go down from 5 till 1
If \( g > 0 \) Then GoTo clev95
endof59:
End Function

Function is10min(d, s) 'function to look for FULL 5min resef
Dim v(0 To 150) As Integer
is10min = 0
\( g = 5 \) ' start at level 5
clev:
For \( j = 0 \) To 120
\[ v(j) = 0 \]
Next \( j \)
sachy = 0
For \( i = 0 \) To 59 ' count how many lines in the same level of action
\[ v(i) = \text{Worksheets("sheet3").Cells}(s + i, d).Value \]
If \( v(i) >= g \) Then sachy = sachy + 1 ' end of counting 20min
Next \( i \)
If sachy = 60 Then ' was 60 = 10min
\[ \text{is}10\text{min} = g \]
GoTo endof10
End If
\( g = g - 1 \) ' go down from 5 till 1
If \( g > 0 \) Then GoTo clev
endof10:
End Function

Function is10min9(d, s2) 'function to look for FULL 5min resef 'second
Dim v(0 To 100) As Integer
is10min9 = 0
\( g = 5 \) ' start at level 5
clev:
For \( j = 0 \) To 59
\[ v(j) = 0 \]
Next \( j \)
sachy = 0
\( i = 0 \)
chile:
\[ v(i) = \text{Worksheets("sheet3").Cells}(s2 + i, d).Value \]
If \( i = 0 \) And \( v(i) = 0 \) Then GoTo newg
If \( v(i) >= g \) Then sachy = sachy + 1
\( i = i + 1 \)
If \( i < 60 \) Then GoTo chline
If sachy > 53 Then ' 54—90% from 60
    is10min9 = g
    GoTo endof109
End If
newg: g = g - 1 ' go down from 5 till 1
If g > 0 Then GoTo clev
endof109:
End Function
Function is20min(d, s) 'function for FULL 20min resef
    Dim v(0 To 150) As Integer
    is20min = 0
    g = 5 ' start at level 5
clev2:
    For j = 0 To 130
        v(j) = 0
    Next j
    sachl = 0
    For i = 0 To 119 ' count how many lines in the same level of action
        v(i) = Worksheets("sheet3").Cells(s + i, d).Value
        If i = 0 And v(i) = 0 Then Exit For
        If v(i) >= g Then sachl = sachl + 1
    Next i
    If sachl = 120 Then
        is20min = g
        GoTo endof20
    Else
        End If
    g = g - 1 ' go down from 5 till 1
    If g > 0 Then GoTo clev2
endof20:
End Function
Function is20min9(d, s2) 'function for FULL 20min resef ' second
    Dim v(0 To 150) As Integer
    is20min9 = 0
    g = 5 ' start at level 5
clev2:
    For j = 0 To 119
        v(j) = 0
    Next j
    sachl = 0
    For i = 0 To 119
        v(i) = Worksheets("sheet3").Cells(s2 + i, d).Value
        If i = 0 And v(i) = 0 Then GoTo newlevg
        If v(i) >= g Then sachl = sachl + 1
        i = i + 1
    Next i
    If sachl > 107 Then
        is20min9 = g
        GoTo endof209
    End If
newlevg: g = g - 1 ' go down from 5 till 1
    If g > 0 Then GoTo clev2
endof209:
End Function
Function memoza(d, lk, x) ' d = day, s=position, x=20min or 10min or 5min
    Dim u(0 To 150) As Integer
Dim valy As Long 'was long
memoza = 0 'initialize all variables
valy = 0
endo = x * 6
For r = 0 To 130
  u(r) = 0
Next r

q = d
i = 0

anoder: w = lk + i
u(i) = Worksheets("sheet1").Cells(w, q * 2).Value
valy = valy + u(i) 'sum up all the values of the counts
i = i + 1
If i < endo Then GoTo anoder 'till the time is up (20min or 10min or 5 min)
aly = valy / endo
endofmem: memoza = aly
End Function

Function findstartcell(d)
For i = 1811 To 8628 'look for start of day after 05:00 AM
  istrt = 1811
  y = Cells(i, 2 * d).Value
  If y > 50 And y < 1500 Then 'find one value between 50 & 1500 then check after
    istrt = i
    y1 = Cells(i + 1, 2 * d).Value
    y2 = Cells(i + 2, 2 * d).Value
    y3 = Cells(i + 3, 2 * d).Value
    If (i > 1992) And Not (y1 = 0) And Not (y2 = 0) Then '5:30am
      Exit For
      End If
    Or If all the next 3 values are non zero then start found
      If Not (y1 = 0) And Not (y2 = 0) And Not (y3 = 0) Then
        Exit For
        End If
    ElseIf y > 1500 Then 'if the first valy is above 1500
      istrt = i
      y1 = Cells(i + 1, 2 * d).Value
      y2 = Cells(i + 2, 2 * d).Value
      y3 = Cells(i + 3, 2 * d).Value
      'if the all the 3 next values are above 1500 start found
      If y1 > 1500 And y2 > 1500 And y3 > 1500 Then
        Exit For
        End If
    End If
Next i
findstartcell = istrt
End Function

Function findendcell(d) 'function to find end of day
Dim iend As Integer
Dim ix As Integer
Dim f1000 As Integer
Dim f100 As Integer
ix = 8628 '23:56= line no. 8628
iend = 8628 '23:56
f1000 = 0
f100 = 0
c last: c = Cells(iend, 2 * d).Value ' check value
If c < 100 Then ' if valy less then 100 keep going up
    iend = iend - 1
    If iend < 4686 Then ' till noon 12:59 = line no. 4686
        ix = 4686
        GoTo sof
    End If
    f100 = 0
    f1000 = 0
    GoTo c last
Else ' valy bigger then 100
    If f1000 > 0 Then GoTo z sof ' if the one before also bigger then 1000 it's end
    If f100 > 0 And f1000 > 1 Then GoTo z sof ' 2 above 1000 & 2 above 100 it's end
    If f100 > 1 And f1000 > 1 Then GoTo z sof ' 3 above 100 & 2 above 1000 it's end
    If f100 = 2 Then GoTo z sof ' 3 above 100 it's end
    If f1000 = 2 Then GoTo z sof ' 3 above 1000 & one above 100 it's end
    If f1000 > 0 And f100 > 0 Then ' 1 above 1000 & 2 above 100 it's end
zesof:               ix = iend + f100 + f1000 ' the end is sum of all checked
    GoTo sof
End If
End If
If c > 100 Then
    If c > 1000 Then
        f1000 = f1000 + 1 ' count the above 1000
    Else:
        f1000 = 0
        f100 = f100 + 1 ' count the above 100
    End If
End Else: f100 = 0
End If
If iend > 4686 Then ' look for end of day till 13:00
    iend = iend - 1
    GoTo c last
Else:
    ix = 4686
    GoTo sof
End If
End If
sof: If ix > 8650 Then ix = 8650 ' if end of day not found it is 24:00
    findendcell = ix
End Function

Private Sub Worksheet_SelectionChange(ByVal Target As Range)
End Sub