BONE AND MUSCLE STRENGTH IN CHILDREN WITH TYPE 1 DIABETES

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By

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Abstract

Introduction: Bone fragility in children and youth with Type 1 Diabetes (DM1) may relate to weaker bones and muscles, but the evidence is limited. The objectives of my thesis were (1) to compare bone and muscle properties and strength between children with and without DM1, and (2) to explore if muscle outcomes are mediators explaining the bone differences between children with and without DM1.

Methods: I included 25 children with DM1 and 168 typically developing children and youth (age 6-15yrs) in my thesis. Their bone properties and muscle size were measured using peripheral quantitative computed tomography (pQCT). Muscle force was assessed using neuromuscular performance measures, including maximal grip force, push-up, countermovement and long jump tests. I compared bone and muscle properties and strength between children with and without DM1 using MANCOVA followed by pairwise comparisons (1st objective). I added muscle size and force into regression models as possible mediators to assess if muscle outcomes are mediators helping explain the potential bone difference between children with and without DM1 (2nd objective).

Results: There were group differences in bone and muscle properties and strength ($p<.05$). Cortical area was 7% and 10% lower and density was 8% higher and 5% higher at radius and tibia shafts, respectively, in children with DM1. Children with DM1 also had 6% lower cortical content at tibia shaft. There was no difference at the distal radius or tibia bone properties and strength between groups. Children with DM1 had 12% higher maximal push-up force. Lower leg muscle area was a mediator for tibia shaft cortical bone content and area difference between children with and without DM1.

Conclusion: Children with DM1 had smaller cortical area but higher density at the radius and tibia shafts. Lower leg muscle area was a mediator explaining the lower tibia shaft cortical bone content and area difference between groups.

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List of Abbreviations

aBMD	Areal Bone Mineral Density (mg/cm^2)
ALP	Alkaline Phosphatase
BMC	Bone Mineral Content (mg/mm)
BMD	Bone Mineral Density (mg/cm^3)
CMJ	Countermovement Jump
BSIc	Bone Strength Index for Compression (mg^2/mm^4)
CoA	Cortical Bone Area (mm^2)
CoC	Cortical Bone Mineral Content (mg/mm)
CoD	Cortical Bone Mineral Density (mg/cm^3)
CTX-1	Carboxy-terminal Crosslinked Telopeptide of Type 1 Collagen
DDK-1	Dickkopf-1
DM1	Type 1 Diabetes
DPD	Deoxypyridinoline
DXA	Dual Energy X-ray Absorptiometry
GF	Maximal Grip Force (N)
HR-pQCT	High Resolution Peripheral Quantitative Computed Tomography
IGF-1	Insulin-like Growth Factor 1
LJ	Standing Long Jump
M	Mediator
MaxPU	Maximal Push-up
MuA	Muscle Cross-sectional Area (cm^2)
MES	Minimal Effective Strain (microstrain, $\mu\varepsilon$)
MRI	Magnetic Resonance Imaging
MO	Maturity Offset (yrs)
OC	Osteocalcin
25-OH-D	25-OH-vitamin D
PAQ-C	Physical Activity Questionnaire for Children
P1CP	Procollagen Type 1 C-terminal Propeptide
P1NP	Procollagen Type 1 N-terminal Propeptide

PTH	Parathyroid Hormone
pQCT	Peripheral Quantitative Computed Tomography
QCT	Quantitative Computed Tomography
QUS	Quantitative Ultrasound
RANKL	Receptor Activator of NF-kB Ligand
SSIp	Polar Strength Strain Index; Density-Weighed Section Modulus (mm^3)
ToA	Total Bone Area (mm^2)
ToC	Total Bone Content (mg/mm)
ToD	Total Bone Density (mg/cm^3)
TrA	Trabecular Bone Area (mm^2)
TrC	Trabecular Bone Content (mg/mm)
TrD	Trabecular Bone Density (mg/cm^3)
X	Independent Variable
Y	Dependent Variable

1. Introduction

Type 1 diabetes (DM1), a life-long disease with insulin deficiency, is predominantly diagnosed in childhood [1,2]. Individuals with DM1 have higher fracture risk throughout their life [2–4], which is potentially associated with lower bone strength [5]. Fracture, especially hip fracture in older age, can seriously affect the life quality and increase the risk of dying [6,7]. Recent research has reported children with DM1 have 14-40% higher fracture risk than their typically developing peers [2], which suggests the increased fracture risk begins at childhood and may relate to poor bone development in children with DM1. Therefore, understanding bone properties and strength is crucial to characterize bone development in children with DM1 [8,9].

Peripheral quantitative computed tomography (pQCT), a commonly used musculoskeletal imaging tool for reliable assessment of bone geometry and estimation of bone strength in children [10]. Previous studies reported various findings comparing bone properties and strength in children with and without DM1 characterized by pQCT. At radius, there have been studies reporting children with DM1 had lower trabecular bone mineral density [11] at the distal site. There were also publications indicating lower total bone area [12], cortical bone area and density [12,13], and bone strength [12] at radius shaft site. For tibia, there was lower total bone area and content [12], trabecular bone area, density and content [14–17] reported in children with DM1 at distal site. There was lower total bone content [12], cortical bone area, density and content [12,14,17], and bone strength [15,17] reported at tibia shaft. However, interestingly, there was one study reporting higher cortical bone mineral density at tibia shaft [17]. Also, the findings at the same measurement site were not consistent across different studies [13,15–17]. Besides pQCT, different imaging tools, dual x-ray absorptiometry (DXA) and magnetic resonance imaging (MRI), have been used to measure bone in children with DM1 in previous studies [12,14,16–21]. Recent research

with MRI reported lower trabecular bone micro-structure parameters at proximal tibia [18]. The results from DXA studies seemed conflicting, while some studies reported normal total body and lumbar spine bone mineral content, area and density [17,20,22–25], and others reported lower lumbar spine bone mineral content and density [12,26]. A metacarpal study using digitized x-ray reported smaller and weaker bone in children with DM1 [27].

Optimal muscle development is essential for bone growth in children with DM1 due to the strong relationship and interaction between muscle contraction force and bone strength [8,9]. The force produced by muscle contraction is one of the primary sources of stimulus for bone strength development [28,29], and the maximal muscle power is an indicator of bone strength in children [30]. Thereby maximal muscle voluntary contraction assessed by neuromuscular performance is especially meaningful to be measured. Fricke et al. reported children with DM1 had lower grip force [31], but Bechtold et al. reported participants with DM1 had higher grip strength than typically developing children reference data [13]. Lukacs et al. reported only younger boys (8-12yrs) with DM1 shew lower grip force [32]. For jumps, Maratova et al. reported adolescents with DM1 had normal maximal muscle force and power but significantly lower maximal relative leg muscle force (maximal force/body mass) and power (maximal power/body mass) during countermovement jump comparing to reference data [15]. On the other side, muscle cross-sectional area (MuA), referred to muscle size, obtained from bone imaging tools is a good surrogate of muscle force [13,28,33,34]. Only few previous studies measured MuA in children with DM1. Moyer-Mileur et al. reported higher MuA in adolescents with DM1 than typically developing reference at baseline using pQCT measures [17]. Bechtold et al. only reported lower MuA in pre-pubertal children with DM1 but not in adolescents [13].

However, to my knowledge, there is no previous study exploring the role of muscle size

or force in the relationship of DM1 and bone outcomes. In particular, the role of muscle outcomes as potential mediators in the relationship between DM1 and bone outcomes has not been explored. Previous research focused more on the interaction between muscle and bone during growth in children with DM1, which the muscle contraction can provide stimulus to bone adaptation and improve bone strength [35]. The findings from previous studies reported diversely on the potential difference of muscle-bone interaction between children with and without DM1. Moyer-Mileur et al. reported DM1 adolescents to have significantly lower total bone content to MuA ratio than typically developing reference (age 12-18yrs) [17]. Bechtold et al. reported a significant correlation between lower MuA and total and cortical area at radius shaft in young DM1 children (mean age 9.7yrs), suggesting that children with DM1 have smaller muscle and bone, which can be explained by muscle-bone interaction [13]. On the other side, Maratova et al. did not report any difference in terms of muscle-bone relationship when comparing to Czech national reference data [15]. However, it is still not well understood the role of muscle in the relationship between DM1 status and possibly differed bone outcomes.

1.0 Background Literature

The scope of this literature review is to understand the musculoskeletal properties, strength, and development in children.

1.1 Bone Physiology

Bone is a dynamic living tissue which is composed of organic and inorganic materials and has several vital functions in our body [36,37]. The first one is to support and connect with skeletal muscle to achieve physical movement [36]. As a dynamic tissue, bone will respond to external forces like muscle contraction and mechanical loading and signaling from, for instance, hormones and growth factor [36]. In addition, bone is a rigid tissue that can protect the organs. Furthermore, bone can serve as a storage of calcium and phosphate for serum homeostasis [36]. Long bone is wider at two ends (epiphyses) and cylindrical shaped in the middle with a medullary cavity at the center (midshaft or diaphysis) [36]. There is also a transition zone in between the epiphysis and diaphysis, which is called metaphysis [36]. There are two types of bone in long bone structure, cortical and trabecular bone, characterized by density and porosity. Cortical bone is denser and more calcified (80-90%) comparing to trabecular bone which is more porous and less calcified (15-25%) [36]. Cortical bone has two surfaces, an outer surface (periosteal) and an inner surface (endosteal) [36].

From the biological view, there are three types of bone cells, osteoblasts, osteoclasts and osteocytes. Osteoblasts and osteocytes work together for bone formation, and osteoclasts are for bone resorption. Osteoblasts will produce the unmineralized bone, osteoid, which will develop into mature bone eventually. Osteocytes are inactive osteoblast cells and able to help with maintaining the tissue produced by osteoblasts. Another main function of osteocytes is to sense

where and how much mechanical strain the bone is experiencing [36–38].

1.1.1 Bone Growth, Modeling and Remodeling

Long bone increases its lengths by endochondral ossification that occurs at the growth plate between the epiphysis and metaphysis [39]. At the growth plate, resting chondrocytes, also known as stem cells, align toward the direction of bone growth and ultimately develop into trabeculae and form trabecular bone [39]. As long as new material is adding into the zone of the growth plate, the bone will calcify and gain length [39]. Along with growth, the metaphysis will get farther from the growth plate. Consequently, the trabecular bone at the center of bone at metaphysis will become thinner and eventually be resorbed. Contradictorily, the trabecular bone at outer metaphysis will thicken and finally build to a cortical cortex [39]. This process is called metaphyseal inwaisting, in which the original metaphysis becomes smaller in size until it attains the size of the diaphysis. In this way, bone grows longer [39,40].

Besides increasing length, bone also needs to expand in width; otherwise, bone is likely to be unstable and prone to fracture [39]. Bone modeling is the process bone enlarged in diameter, and remodeling is the process of bone turnover, in which old bone tissue is removed and replaced by new bone tissue [41,42]. Both modeling and remodeling involve bone cells osteoblast and osteoclast [37,42]. Modeling is a combination of work from osteoblast and osteoclast cells on opposite sides of bone [42]. During growth, osteoclasts resorb bone tissue from the endosteal surface of a bone, and osteoblasts form more bone tissue on the periosteal surface. Thus, there will be more bone formed comparing to the bone removed, by which both the size of the bone and marrow cavity will expand [42].

Bone remodeling formats with continuous bone formation and resorption cycling on the

same bone surface [42]. During remodeling, osteoblasts and osteoclasts work together, which osteoclasts take off some bone tissue which is then later replaced by osteoblasts with a reversal phase in between [42]. The osteoblast and osteoclast cells in this process collectively are called the "basic multicellular unit", and the osteoblasts and osteoclasts working together during the remodeling process are referred to as "coupling" [42]. There is a balance for the "coupling" [41,42]. Remodeling is followed by the "Activation-Resorption-Reversal-Formation" process performed by the "basic multicellular unit" [36]. The osteoclasts are activated first before the resorption process which then is terminated by reversal phase [36]. Afterward, osteoblasts take place to start the formation phase (Figure 1) [36]. During growth, there is more bone formation going on comparing to bone resorption; thereby more bone is gained. On the other hand, during aging, more resorption takes in place, which explains bone loss [42]. For young adults, the remodeling "coupling" is balanced so that the amount of bone is maintained [42].

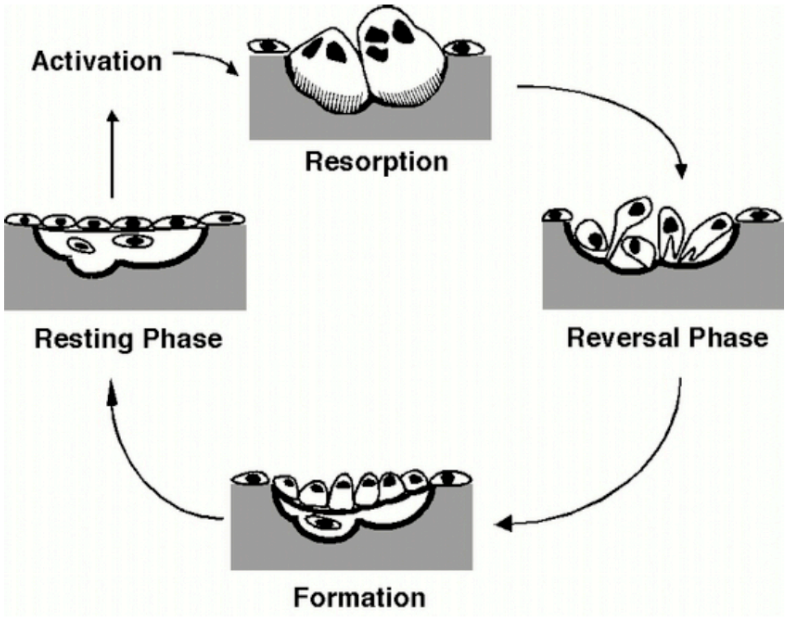

Figure 1: Diagram showing "Activation-Resorption-Reversal-Formation" process of bone remodeling. Adapted from Moreira et al. (2000) [36]

1.1.2 “Mechanostat” and “Mechanotransduction”

“Mechanostat” is a hypothesis that human can maintain bone health from adapting daily load bearing by balancing a combination of modeling and remodeling and their thresholds, bone marrow mediator, signaling mechanisms [43]. Other factors, like hormone, would serve as modulator in the “Mechanostat” model and help carry out this process [43]. If mechanical loading would add more strain to the bone, the modulator will send the signal that extra bone strength is needed to adapt to the higher strain. Then this will trigger the bone modeling and remodeling process to put on more bone tissue to achieve the targeted bone strength (Figure 2) [43]. The lowest strain required to activate bone remodeling, modeling and repair processes is called minimal effective strain (MES) [44]. If the strain that bone experiences does not reach the remodeling MES, there would be no response [44]. If the strain reaches the remodeling MES, the remodeling process would likely take in place, and the net bone mass would be maintained [44]. If the strain exceeds the modeling MES but not repair MES, there would be more bone formed and potentially increase the bone mass and strength [44]. However, if the strain is over repair MES, it would not be beneficial and is likely to cause some microdamage or even fracture to the bone [44]. The bone adaptation responding to mechanical loading is coordinated based on the theory of “Mechanotransduction”, which is a process that bone would turn mechanical stimuli toward bone into biochemical response [45,46]. The steps involve mechanical coupling, biochemical coupling, cell-to-cell signaling and cell response [45].

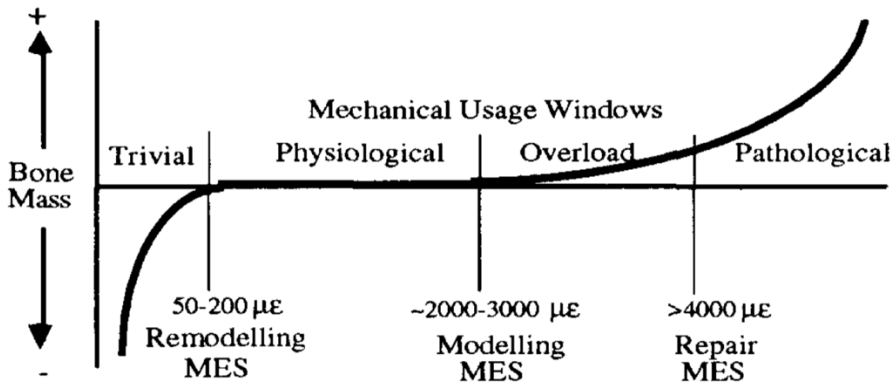

Figure 2: Sample diagram showing with increasing the bone strain to reaching specific MES, bone will go under remodeling, modelling and repair which will either maintain or gain bone mass. With reaching the physiologic zone, remodeling will help keep the overall bone mass and no new bone was gained. After reaching the overload region, modeling will take in place and more bone will be formed. Diagram adapted Forwood & Turner (1995)

1.1.3 Other Biological Factors Influencing Bone Growth

There are also other biological factors associated with muscle and bone development in general children, like sex, age and maturity, anthropometry, physical activity, nutrition intake.

Sex is an essential factor to consider because boys and girls are underline different growth patterns and will develop into different body stature during and after puberty. Boys experience higher bone turnover rate, longer growth duration, and higher peak height velocity than girls [47]; therefore, it is necessary to identify the influence of sex differences in bone and muscle on children.

Age and Maturity are also crucial factors assessing bone in children. However, since the timing of maturation is different in boys and girls, chronological age is not able to fully represent sex-specific maturity [48]. Besides, maturity has been previously indicated as a predictor of bone geometry and strength [49–52]. Thereby, maturity is preferred compared with age when controlling timing in growth and development. During maturation, the maximal linear growth speed is referred to peak height velocity [47]. Instead of using the Tanner stage to characterize secondary

sex maturation, maturity offset (MO), the years from the age at peak height velocity, is more appropriate assessing somatic maturation, or skeletal growth, in children [53]. Moreover, since boys and girls are not underline the same tempo and timing on both sexual and somatic maturation, it is tough to align the sex maturation in boys and girls to somatic maturation [53]. Also, since the Tanner stage is characterized by stages, not continuous number, even children under the same stage of maturity may still be slightly different in maturation progress. MO is a continuous measure and calculated by sex-specific formulae built from regression models based on longitudinal growth data in children [48]. Hence, MO is easier to align with growth and somatic maturity compared to categorical measure Tanner stage. Therefore, MO is preferable when characterizing somatic maturity in children.

Anthropometry, height and body mass specifically, is positively correlated to bone status in children [54–56]. Limb length, a determinant of bone strength, is proportional to body height [57,58], but it is still questionable for the reliability of limb length assessment due to the palpation or measurement errors using a sliding caliper. In addition, heavier children tend to have stronger bones, especially the tibia, and muscles to support their body weight and daily movement [59].

Physical Activity is a critical component during growth and development [60] and associated with bone strength [60,61]. Canadian Physical Activity Guidelines for children and youth (5-17yrs) recommend 60mins moderate-to-vigorous physical activity per day, and at least three times of vigorous physical activity and muscle and bone-strengthening exercise per week [62]. High impact physical activities, like jumps, can provide loading to the bone and help improve the bone strength [60,63].

Nutrition Intake of calcium, Vitamin D and protein also influences bone growth in

children [64–67]. Calcium is associated with areal bone mineral density across different skeletal sites in both boys and girls [64], and is also a determinant of bone strength at the tibia in children [28]. Vitamin D, usually characterized by 25-hydroxyvitamin D (25(OH)D) status, may help to reduce the HbA1c level, the indicator of glycemic control, in children with DM1 [68–70]. However, insufficient (25(OH)D = 50-75nmol/mL) and deficient Vitamin D (25(OH)D < 50nmol/mL) intake will increase the risk of developing osteoporosis and fracture in both children and older adults [65,71,72]. Severe deficiency in Vitamin D (25(OH)D < 10nmol/mL) could develop into ricket, which will cause inadequate bone mineralization and impair bone growth in children [66]. Protein is another factor influencing bone and muscle growth in children. Protein intake is positively associated with bone properties and strength, like bone mineral content, density and area [67,73], and also influences bone development via hormones [74]. However, overtaking of protein may also elevate bone resorption [73]. Accordingly, calcium, Vitamin D, and protein intake can be factor influencing bone growth in both children with and without DM1.

1.1.4 Type 1 Diabetes (DM1)

Type 1 diabetes (DM1), a life-long disease with insulin deficiency, is predominantly diagnosed in childhood [1,2]. This disease can be caused by multiple risk factors, especially genetic and environmental [75]. Worldwide, there are about 0.02% children (0-14yrs) with DM1, and the reported increase of incidence rate is 2-5% per year [76,77]. Canada has one of the highest increases in the rate of pediatric DM1 incidence around the world [76–78], which should draw more attention to the potential effect of DM1 on growth and development in children with DM1.

1.1.4.1 Glycemic Control and Musculoskeletal Growth in Children with DM1

Glycemic control may influence bone development in children with DM1. Glycemic control, i.e., how well DM1 is managed, is usually monitored by glycated hemoglobin (Hemoglobin A1c [HbA1c]) test. Typically HbA1c is tested approximately every three months, and the average HbA1c is assessed by the average of the tests across the year [21]. According to American Diabetes Association guidelines, HbA1c under 58 mmol/mol or 7.5% will be considered under good glycemic control, and HbA1c equal or above 58 mmol/mol or 7.5% will be considered under poor glycemic control [14,21]. The previous studies reported with poor glycemic control, children with DM1 may have or develop into lower bone mineral density compared to children with good glycemic control in both cross-sectional and longitudinal studies [14,21,79–81]. Poor glycemic control also potentially increases the risk of fracture and the development of osteoporosis later in lives [80].

1.1.4.2 Bone Assessment in Children with DM1: Bone Biomarkers

Bone biomarkers, including bone formation and resorption and regulators of bone turnover released during growth, are commonly used to monitor bone remodeling or turnover, which provides information about skeletal growth and development in children. The common biomarkers for bone formation are alkaline phosphatase (ALP), osteocalcin (OC), procollagen type 1 N-terminal propeptide (P1NP), and procollagen type 1 C-terminal propeptide (P1CP). The popular biomarkers for bone resorption are parathyroid hormone (PTH), 25-OH-vitamin D (25-OH-D), (urinary) deoxypyridinoline (DPD), carboxy-terminal crosslinked telopeptide of type 1 collagen (CTX-1) and receptor activator of NF-kB ligand (RANKL). Regulators of bone

turnovers include dickkopf-1 (DDK-1) and sclerostin. Other than bone turnover markers, calcium, phosphorus, growth hormone and insulin-like growth factor 1 (IGF-1), can also indicate bone growth in children.

The emerging evidence has suggested altered bone turnover in children with DM1 from biomarkers [18,19,27,82–84]. However, the findings are not very consistent in terms of higher [27] or lower biomarkers [18,19,83,84]. The potential reason underneath the altered bone turnover rate in children with DM1 may link to the reduced osteoblastic activity and osteoclast signaling associated with DM1 itself [83].

1.2 Bone and Muscle Imaging Tools

There are six types of imaging tools commonly used to measure bone and muscle in children and youth. Below is a brief introduction of each imaging technique previously used in research as well as their corresponding bone and muscle related findings in children or adolescents with DM1.

1.2.1 Dual-Energy X-Ray Absorptiometry (DXA)

DXA is the most widely used two-dimensional bone imaging technique for both clinical and research purposes (Figure 3) [85,86]. Clinically, it is a valid tool to diagnose osteoporosis and estimate fracture risk with good measurement precision (CV% = 1-2%), low radiation dose, relatively low cost and short scan time [85,87]. For research, it can measure bone mineral content (BMC, g), areal bone mineral density (aBMD, g/cm^2), and bone area (BA, cm^2) for total body as well as clinically relevant sites like the lumbar spine and proximal femur [85]. Not only for bones, DXA can also assess body composition and calculate muscle and fat mass [85]. However, the

limitations of DXA are unneglectable owing to the two-dimensional projection on three-dimensional bone structure [85]. Firstly, it is not possible to detect bone geometry and separate cortical and trabecular bone properties [85]. Secondly, the X-ray attenuation is influenced by bone marrow and soft tissue (like subcutaneous fat and muscle) surrounding bone [85]. Especially in children under rapid growth, x-ray penetration might be different if monitoring longitudinally, by which their body composition may change greatly, and potentially affect the consistency and accuracy of measurement results [88].

DXA findings are varible in children with DM1. Children with DM1 have been shown with lower total-body BMC, aBMD and BA [16–18], lumbar spine BMC, aBMD and BA [17,18], femoral neck and head aBMD [12,14,16], great trochanter BMC [12]. However, there were also studies which did not report difference on any of DXA bone parameters comparing children with DM1 to controls or reference [19–21].

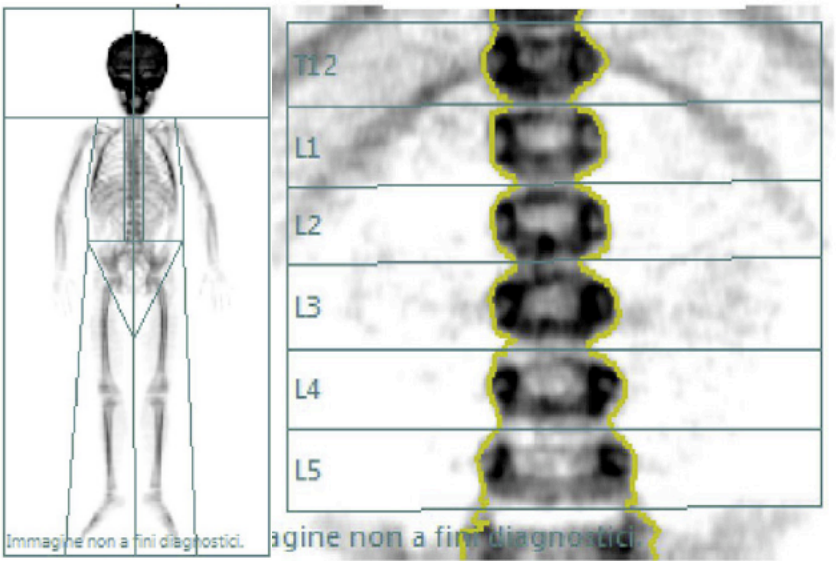

Figure 3: Sample DXA images of total body (left) and lumbar spine 1-4 (right) obtained from 6-year-old child. Adapted from Di Iorgi et al. (2018) [86]

1.2.2 Quantitative Computed Tomography (QCT)

QCT is a bone imaging technology combining computed tomography (CT) and a calibration standard to obtain bone size, density and content [86]. It can scan regions of interest from total body and measure the "true" volumetric bone mineral density (BMD, g/cm^3) with good accuracy and reproducibility (CV% < 3%) [86]. It can also measure bone structure and geometry, like bone mineral content (g) and cross-sectional area (cm^2), and separate trabecular and cortical bone, and the common scanning sites are the lumbar spine (Figure 4) and femoral midshaft [86]. However, QCT (0.8μSV per central area scan in 10yrs old child) has a relatively higher radiation dose and cost compared to DXA (0.02μSV per spine scan in 10yrs old child) [86].

There was one study related to children with DM1, which reported lower cortical bone mineral density at the lumbar spine but not trabecular density [89].

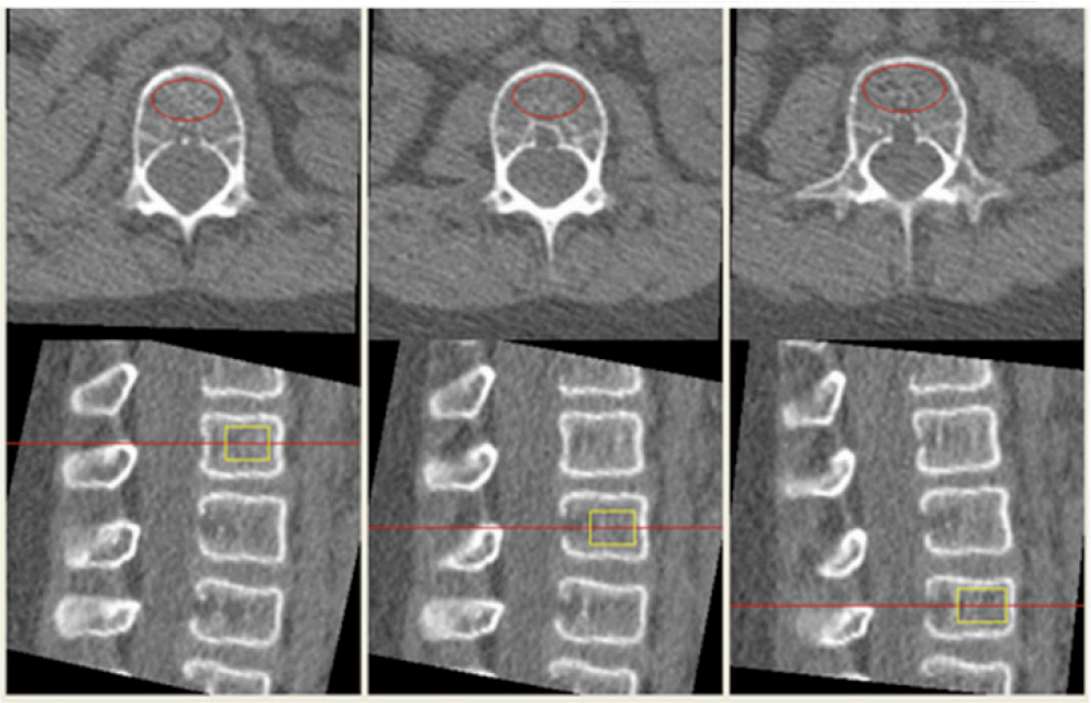

Figure 4: Sample QCT image for lumbar spine 1-3. The red lines in the lower images locate at midplane of each vertebra viewed from sagittal plane. The red oval regions in the upper images are located at the midplane characterized from the lower images, and the circled area is used to calculate the bone mineral density. Adapted from Adams (2016) [87]

1.2.3 Peripheral Quantitative Computed Tomography (pQCT)

Peripheral quantitative computed tomography (pQCT) is a commonly used peripheral CT scanner in pediatric measurement. It is a portable device designed to image the peripheral skeleton, particularly the upper and lower limbs, like radial and tibial bone structure and geometry as well as the surrounding muscle, with relatively low radiation (lower than 0.1μSV per scan including scout view) [10,86,90]. The precision error of pQCT has been determined in bone properties and strength measures in children [10]. The main bone parameters measured by pQCT are total, cortical and trabecular bone area, content and density from different sites on the radius and tibia in children. Cortical and trabecular bone is separated by density thresholds and algorithms [10]. Bone area and geometry (material distribution over the cross-section of bone) are crucial when estimating bone strength at a long bone [10,91]. The formulae for bone strength estimation have been validated at the distal and shaft sites of the radius and tibia [10,91]. At the distal site, the bone strength is estimated against compressive loading, which is calculated by multiplying total bone area and squared total bone density. At shaft site, bone strength is represented by polar sectional modulus against torsional loading [10]. The precision errors of bone properties range from 2-19% for distal sites, and 2-8% for shaft sites [10]. pQCT is also able to measure muscle cross-sectional area (MuA), a surrogate measure of muscle force [92]. It can separate the muscle from bone and subcutaneous tissue by density threshold and algorithm as well [10,93,94]. The precision errors of MuA from our lab range from 3-4%.

However, there is a limitation, partial volume effect, related to all CT scans, since the CT images are constructed by pixels [85]. There are always some pixels which are not fully filled or filled by tissues with different densities, which may underestimate averaged bone density [85]. For

instance, a “red low-density ring” at the periosteal surface of the cortical cortex (Figure 5), which is a combination of cortical bone and muscle tissue in the pixels when calculating total bone density, might result in some underestimation in bone density assessment [85].

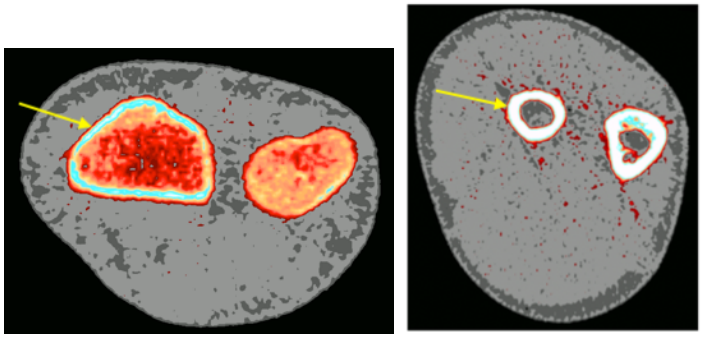

Figure 5: Sample pediatric pQCT images of distal radius (left) and radius shaft (right) at 4% and 65% of ulna length, respectively. The white/blue color structure is with higher density, and red color structure is with lower density. Yellow arrows point at the “red lower-density ring” caused by partial volume effect where pixels contain both cortical bone and muscle tissue.

Previous studies reported various findings comparing bone properties and strength in children with and without DM1 obtained by pQCT. Saha et al. measured dominant radius and right tibia in DM1 adolescents [12]. They reported lower total bone mineral content and area at the distal tibia, and lower total bone mineral content and cortical bone area at the radius and tibia shaft when compared to their typically developing peers. Their findings also suggested lower bone strength (density-weighed polar section modulus, SSI_p) at the radial and tibial shaft, but not for distal sites (cortical to total bone area ratio) (Table 1) [12]. SSI_p is the ability of bone to resist torsional loading, which is related to bone geometry, bone size, bone tissue distribution, and material property and stiffness at the diaphysis [91]. Bechtold et al. measured the non-dominant radius using pQCT from DM1 children with different maturation status [13]. They reported lower trabecular bone density at distal sites in girls, and total and cortical bone area at the shaft in both sexes comparing to typically developing reference. However, the largest difference was detected

in pre-pubertal DM1 participants, not in adolescents (Table 1) [13]. Maratova et al. measured the non-dominant tibia in DM1 adolescents and reported lower trabecular bone density at the distal site and bone strength (SSI_p) at the shaft (Table 1) [15]. Moyer-Mileur et al. scanned non-dominant tibia in DM1 children [17]. They reported lower distal-site trabecular bone and shaft-site cortical bone content, density and area except cortical bone mineral density and shaft bone strength (SSI_p) at baseline (Table 1) [17]. Another study from the same lab reported lower trabecular bone density at the distal tibia (Table 1) [16]. Weber et al. reported lower distal tibia trabecular bone density and tibia shaft cortical bone density in children who were diagnosed with DM1 within one month at baseline, but only detected lower distal tibia trabecular density at 12-month follow-up. However, they did not report any differences on the non-dominant radius bone properties between children with and without DM1 (Table 1) [14]

1.2.4 High Resolution Peripheral Quantitative Computed Tomography (HR-pQCT)

HR-pQCT is an upgrade version of pQCT, and can measure the three-dimensional bone micro-structure from distal radius and tibia with relatively low radiation dose (<4μSV per scan) and precision errors smaller than 7% in children [95,96]. It can separate cortical cortex from the trabecular bone at the distal radius and tibia. HR-pQCT takes 110 slices over a 9.02mm region, which allows 3D visualization of distal radius and tibia (Figure 6) [97]. The trabecular bone outcomes are trabecular number (Tb.N), thickness (Tb.Th) and separation (Tb.Sp) [96]. The common cortical bone outcomes are thickness (Ct.Th), porosity (Ct.Po), bone volume/total volume (BV/TV) [96]. Finite-element models can also be built from the scan images to assess bone biomechanical strength noninvasively, like failure load, which estimates how much force bone can undertake before fracture [81,86]. It can also measure the parameters that pQCT measures,

which are total, cortical and trabecular bone area, and cortical and trabecular bone density at the distal ends of radius and tibia [96]. However, although there is a protocol using HR-pQCT in the pediatric population, the software was initially developed for adults.

There were no previous studies assessing bone micro-structure with HR-pQCT in children with DM1. The adult study reported lower total and cortical bone mineral density, and cortical bone thickness in DM1 group comparing to the control group (mean age 46yrs in both groups) [98].

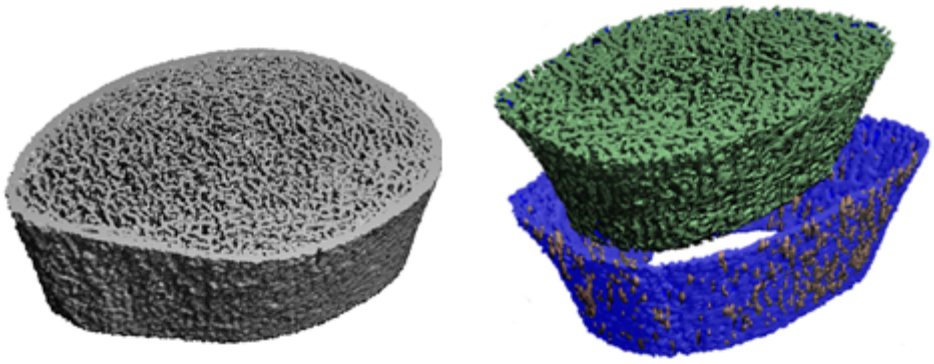

Figure 6: Sample pediatric distal tibia scan from our lab with 3D visualization (left) and separated cortical shell and trabecular bone (right). The outer blue cortex is the cortical cortex with intracortical pores labelled in brown. Trabecular bone is inside the cortical cortex with green color.

1.2.5 Quantitative Ultrasound (QUS)

QUS is a radiation-free and portable device to assess the bone mineral status and stiffness at a low cost [54]. Similar to DXA, QUS is also not able to separate cortical and trabecular bone. The most common scanning site is the heel, but it can also measure fingers, radius and tibia. The bone parameters obtained from QUS scan are speed of sound (m/s) and broadband ultrasound attenuation (dB/MHz) with precision error up to 6% [86,99,100]. Like DXA, one downside of this technique is the measurement results can be interfered by soft tissue thickness [86], since the

ultrasound wave can be scattered and absorbed by not only bone tissue but also bone marrow and the soft tissue surrounding bone [96]. Therefore, the ultrasound is often used at heels since there is less soft tissue. In addition, QUS does not provide a direct conversion to bone density, and the correlation between QUS parameters and densitometry measures from other devices, DXA for instance, remains questionable [100,101].

1.2.6 Magnetic Resonance Imaging (MRI)

To form an image, MRI detects the excitation of hydrogen protons in a high magnetic field [86]. It is a non-invasive technology with no radiation. It can assess the micro-structure for soft tissues and both the trabecular and cortical bone, like HR-pQCT, with good short-term precision errors (CV% = 1-3%) in adults and children [86]. MRI is also able to measure various sites of the body, including both central part of the body and peripheral bones (Figure 7) [86]. However, MRI scanning is costly and noisy, and the accessibility is limited since most MRI devices are for clinical use [86]. The setting of MRI scanning environment is not ideal for children, since parents are not allowed to stay during the scan [86,96].

The recent study in children with DM1 with MR1 reported lower bone volume to total volume, trabecular number and separation at the proximal tibia, which suggests a deficiency in trabecular bone in children with DM1 (Table 1) [18].

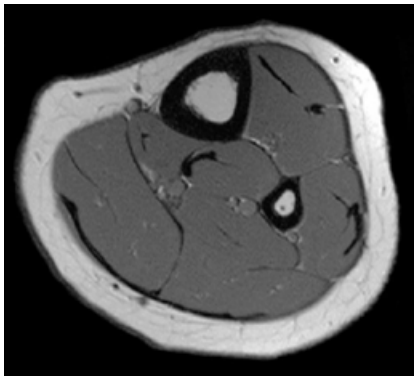

Figure 7: Sample pediatric lower leg MRI scan. Adapted from Whitney et al. (2017) [102]

Table 1: Summary of previous studies assessing bone and muscle with imaging tools on children or adolescents with DM1

Author	Year	Study Design	Participant	Imaging Tools	Findings
Roe et al.	1991	Cross-sectional	48 children with DM1 and 48 controls (mean age 12.8yrs)	QCT	Lower cortical but not trabecular bone mineral density at lumbar spine
Lettgen et al.	1994	Cross-sectional	21 children with DM1 (mean age 12.6yrs) and sex- and age-matched controls (mean age 12.8yrs)	pQCT	Lower trabecular bone mineral density at distal radius
Gunczler et al.	2001	Cross-sectional	23 children with DM1 and 17 age, height, and pubertal status matched controls (mean age 9.5yrs)	DXA	Lower total-body areal bone mineral density *Z*-score in children with DM1 comparing to controls
Heap et al.	2004	Cross-sectional	55 children with DM1 and 95 reference (mean age 15yrs)	pQCT/DXA	Lower distal tibia trabecular bone density Lower femoral neck areal bone mineral density, and total-body areal bone mineral content and density
Moyer-Mileur et al.	2004	Longitudinal, 12-month follow-up	42 children with DM1 (mean age 14-15yrs) and 203 reference (mean age 15yrs)	pQCT/DXA	Lower tibia bone properties and strength, except higher tibia shaft cortical bone density in children with DM1 and lower increase on cortical bone mineral density at 12-month follow-up Lower total-body bone area and area bone mineral content, lumbar spine area, and areal

					bone mineral content and density in children with DM1; lower increase on total-body bone area, and higher increase on lumbar spine bone area and areal bone mineral density at 12-month follow-up Higher muscle cross-sectional area at tibia shaft site at baseline, and higher increase at 12-month follow-up in children with DM1
Bechtold et al.	2007	Cross-sectional	88 children and adolescents with DM1 (mean age 11.7yrs)	pQCT	Lower total and cortical bone area at radius shaft in children with DM1 Lower muscle cross-sectional area at radius shaft in children with DM1
Saha et al.	2009	Cross-sectional	48 adolescents with DM1 (mean age 15yrs) and sex, age, height, weight and pubertal maturity matched control group (mean age 16yes)	pQCT/DXA	Lower distal tibia total bone mineral content and area, radius and tibia shaft total bone mineral content, cortical density and bone strength in participants with DM1 Lower femoral neck and great trochanter areal bone mineral content in participants with DM1
Maggio et al.	2010	Cross-sectional	27 children with DM1 and 32 controls (mean age 10.5yrs)	DXA	No difference on total-body, lumbar spine, femoral neck and greater trochanter areal bone mineral density between children with and without DM1
Maggio et al.	2012	RCT with physical activity intervention	27 children with DM1 and 32 controls (mean age 10.5yrs)	DXA	No difference on total-body, lumbar spine, femoral neck and greater trochanter areal bone mineral density between children with and without DM1 at baseline

Roggen et al.	2013	Cross-sectional	54 adolescents and young adults with DM1 (mean age 18yrs) and 47 controls (mean age 19yrs)	pQCT	Lower total bone area at distal radius in females with DM1 only
Francesci et al.	2018	Cross-sectional	95 children with DM1 (mean age 10.5yrs) and 40 controls (mean age 11.9yrs)	Digitalized X-rays	Lower outer diameter, inner diameter, cortical area and medullary area at 2nd metacarpal in children with DM1
Maratova et al.	2018	Cross-sectional	95 adolescents with DM1 (mean age 16.6yrs)	pQCT	Lower trabecular bone mineral density at distal tibia, lower bone strength (SSI_p) and cortical thickness at tibia shaft
Chen et al.	2019	Cross-sectional	32 children with DM1 and 27 controls (median age 14yrs)	MRI/DXA	Lower apparent bone volume to total volume, trabecular number and separation at proximal tibia in children with DM1 Lower total and lumbar spine areal bone mineral density in children with DM1
Fuusager et al.	2019	Cross-sectional	85 children and adolescents with DM1 (median age 13.2yrs)	DXA	Normal total body areal bone mineral density based on *Z*-score in adolescents with DM1
Weber et al.	2019	Longitudinal, 12-month follow-up	32 children with DM1 (mean age 14.2yrs) at baseline	pQCT/DXA	Lower trabecular and cortical bone mineral density at distal and shaft site tibia at baseline, respectively in children with DM1; lower trabecular bone mineral density at 12-month follow-up as well

	Lower total body less head areal bone mineral content and femoral head areal bone mineral density at baseline and follow-up in children with DM1; lower increase on femoral head areal bone mineral density over 12 months as well

1.3 Neuromuscular Performance

Childhood muscle and bone growth and development are closely related to each other [94,103,104]. Muscle force is a surrogate of bone strength in children [105,106]. However, maximal intrinsic muscle force cannot be assessed in live human [106]. Previous studies have involved isokinetic maximal voluntary contraction tests, providing information like peak torque, as an indicator of maximal muscle force [106]. Still, these movements cannot represent natural movements in daily life [107]. Neuromuscular performance, often referred to as muscle function assessment, including multiple explosive movements, is not merely a muscle strength test. It provides information about not only the estimation of maximal muscle force but also motor performance and body coordination [108]. I will introduce four different explosive movement complexes commonly used to test neuromuscular performance, including maximal push-up, grip force, and countermovement and long jump, to evaluate both upper and lower body muscle extremities. The maximal push-up is an upper body version of countermovement jump [108], and grip force measured by hand dynamometer is a widely used test for upper extremity [109,110]. Jump is frequently used to assess children's physical fitness at field settings, and its ground reaction force is also a predictor for tibia bone strength in children [111].

1.3.1 Upper Extremities: Maximal Push-up and Grip Force

The maximal push-up (MaxPU) is an explosive movement and also the upper body alternative of countermovement jump [108]. Comparing to isometric contraction test, like grip force, push-up is a more complicated functional movement. The pushing action in push-up is related to daily life activities, like a horizontal version of pushing a box. Comparing to repetitive push-up to reach the upper body endurance limits, the maximal push-up is more focus on

maximal and explosive push-off force, represented by vertical ground reaction force, that participants can produce mainly from their upper body. There has been no study tested maximal push-up in children with DM1, but it was tested on youth athletes showing the push-off force is reliable in 10-15yrs old boys [112]. Therefore, ground reaction force, the direct measure from the force platform, is used to characterize maximal push-up in children.

Grip force (GF), measured by hand dynamometer, is a widely used isometric test to assess the upper extremity. Hand dynamometer measures hand grip in kilogram, which is later be converted into newtons to represent grip force. The hand dynamometer used in this study, Jamar dynamometer, has been reported with good reliability in children [113]. Although handgrip is a simple isometric test, its force is an indicator of upper body muscle strength and distal radius bone strength in children [105,114]. Overall, the grip force measure is a commonly-used and reliable upper extremity measure indicating both muscle and bone strength in children. Previous research provided various findings comparing grip strength in children with and without DM1. Fricke et al. suggested children with DM1 tended to develop lower grip force comparing to typically developing children [31], but Bechtold et al. reported participants with DM1 had higher grip strength comparing with reference data (Table 2) [13]. Lukacs et al. reported only younger boys (8-12yrs) with DM1 showed lower grip force (Table 2) [32].

1.3.2 Lower Extremities: Jumps

Jump mechanography, using the force platform to assess muscle function from dynamic movement, is a relatively new way to assess lower-body muscle extremity with better reproducibility and accuracy when compared to isokinetic maximal voluntary muscle contraction tests [106,109,115]. Although grip force is a widely used and inexpensive test to estimate muscle

force, it can only evaluate maximal isometric contraction of upper body [109,110]. Countermovement jump (CMJ) maximal take-off ground reaction force and power have been shown with good reproducibility in typically developing children [109]. Also, countermovement jump force and power are indicators of lower leg muscle size and tibia bone strength in children and adults [30,106]. Standing long jump (LJ) is a common school-based fitness test in children to measure musculoskeletal fitness [116], especially for jump length due to little equipment required. Jump length has been measured with good reliability in school-age children [116,117]. However, although standing long jump is widely tested in children, force and related output, power and impulse, have been barely studied in standing long jump.

There were only two studies comparing jump force in children with DM1. Both Fricke et al. and Maratova et al. reported adolescents with DM1 had normal maximal jump force and power comparing to reference data, but Maratova et al. also reported significantly lower maximal relative leg muscle force (maximal force/body mass) and power (maximal power/body mass) during countermovement jump (Table 2) [15,31]. In addition, there was one study testing long jump length in children with DM1 (8-18yrs) but it did not report difference compared to controls (Table 2) [32].

Table 2: Summary of previous studies assessing neuromuscular performance on children or adolescents with DM1

Author	Year	Study Design	Participant	Measurement	Findings
Bechtold et al.	2007	Cross-sectional	88 children and adolescents with DM1 (mean age 11.7yrs)	Grip force	Lower grip force comparing to reference data
Fricke et al.	2008	Cross-sectional	40 children with DM1 (mean age 13.0yrs)	Grip Force, Countermovement jump ground reaction force and power	Lower grip force, but no difference on countermovement jump force and power in children with DM1 comparing to reference data
Lukacs et al.	2012	Cross-sectional	106 children and adolescent with DM1 and 130 controls (8-18yrs)	Grip force, Long jump length	No difference on grip force and long jump length between children with and without DM1
Maratova et al.	2018	Cross-sectional	95 adolescents with DM1 (mean age 16.6yrs)	Countermovement jump ground reaction force and power	No difference on countermovement jump force and power comparing to reference data, but lower relative force and power after divided by body mass or weight

1.4 Muscle-Bone Interaction

The theory of muscle-bone interaction between muscle loading and bone strength was derived from the "Mechanostat" model since muscle contraction would provide the largest load to the bones [43]. Voluntary muscle contraction can produce up to 10 times of external loading on bones due to the short moment arm, which potentially helps stimulate bone adaptation [9,103,118]. Especially in childhood, muscle and bone growth and development are closely related to each other [94,103,104]. With increased loading from muscle or other external sources, bone has to withstand more strain, and, consequently, will become stronger to adapt to the strain [39,43,119].

In terms of muscle-bone interaction between children with and without DM1, there is limited literature with discrepant findings assessing the relationship between muscle and bone outcomes in DM1 children. Moyer-Mileur et al. reported that adolescents with DM1 had higher muscle area but lower bone mineral content, as well as lower ToC/MuA ratio, which suggested bone properties might not adapt to muscle size (surrogate of muscle force/stimulus) as much as in typically developing children [17]. On the other hand, Maratova did not report differences in muscle-bone interaction between DM1 adolescents and reference data [15].

However, the role of muscle plays in bone outcomes is challenging to identify by just ratio or correlation. It is important to assess if muscle outcomes have a mediating role which explains possible bone difference in children and youth with and without DM1. This potentially supports future intervention aiming to improve muscle size and strength to optimize bone development in children with DM1.

1.5 Summary and Research Gap

The literature has reported that children with DM1 have higher fracture risk [2], but the

findings regarding bone and muscle in children with DM1 were disparate across studies. There are various tools that can image bone and muscle in children, but pQCT is a reasonable choice as it can measure bone geometry and density, and muscle size, as well as estimate bone strength at various sites of the radius and tibia [10,86]. Neuromuscular performance, including maximal push-up, grip force and jumps, are maximal and explosive tests that can assess upper and lower body muscle extremities [108–110]. pQCT scans and neuromuscular performance can provide the opportunity to look into the potential difference in bone and muscle properties and strength for both upper and lower body limbs between children with and without DM1.

One downside of previous studies involving pQCT was that they only measured one aspect over another (e.g., either bone or muscle, or either radius or tibia). This study would measure both bone and muscle properties and strength for both upper and lower body limbs using pQCT and neuromuscular performance in children with and without DM1. Additionally, the measurement of muscle also allows the exploration of the role of muscle size and force in the possible bone differences between DM1 and typically developing children, while previous studies only provided information on the relationship between bone and muscle characteristics in children with DM1.

2. Research Objectives, Questions and Hypotheses

In order to explore the research gap, my thesis focused on the following two research objectives:

2.1 Research Objective 1

My first research objective is to assess the bone and muscle properties and strength difference between children with and without DM1.

In order to address the first research objective, I asked the following research question: Do bone and muscle properties and strength differ between children with DM1 and typically develop children?

I hypothesized bone and muscle properties and strength would differ between children with DM1 and typically developing children.

2.2 Research Objective 2

My second research objective is to explore the potential mediating role of muscle size and neuromuscular performance in explaining the differences in bone outcomes between children with and without DM1.

In order to address the second research objective, I asked the following research question: Are muscle area and neuromuscular performance mediators explaining the differences in bone outcomes between children with and without DM1?

I hypothesized muscle outcomes would be mediators explaining the bone differences between children with and without DM1.

3. Methods

3.1 Study Design and Participant Recruitment

We recruited 38 children with DM1 from the local community, Saskatchewan diabetes camp (summer activity camp mainly for children with diabetes (age 6-15yrs) in Saskatchewan) and diabetes-related events for this cross-sectional investigation. A database of 170 typically developing children and youth, recruited from local schools and community programs, served as controls [95]. In my thesis analyses, I included 25 children with DM1 as the DM1 group and 168 typically developing children as the control group. Participants included two groups were with valid peripheral quantitative computed tomography (pQCT) and neuromuscular performance test data (Figure 8). The age range for both groups was 6-15 years old, but participants in the DM1 group were 1 years older and more mature, and 18% less physically active on average when compared to participants in the control group (Table 3).

This study has been approved by University of Saskatchewan Biomedical Research Ethics Board. Parental consent and child assent were obtained prior to testing.

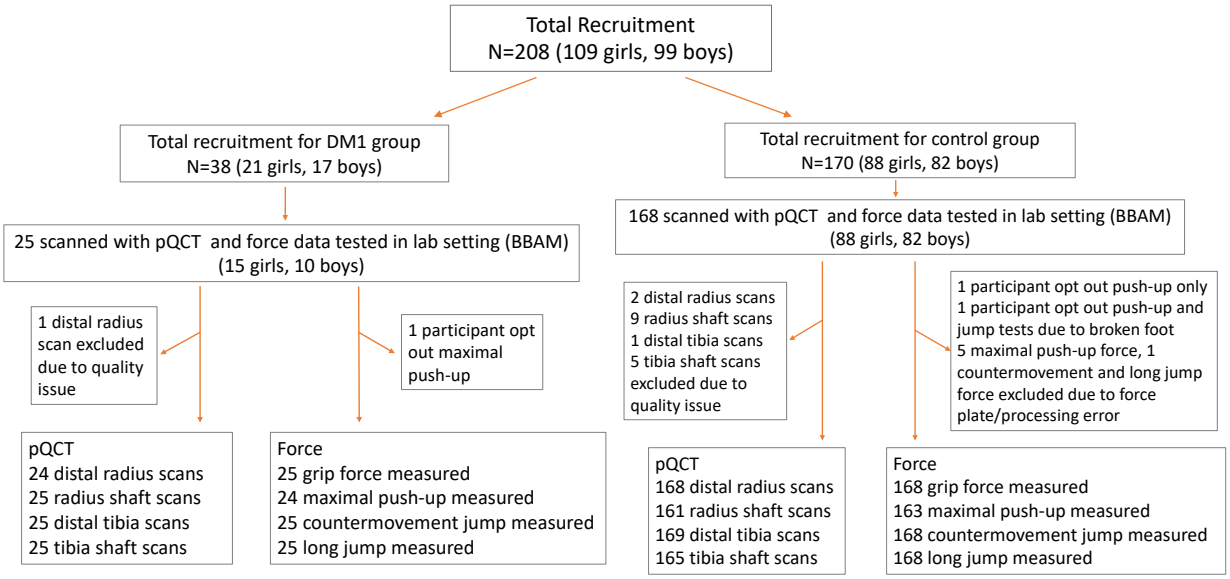

Figure 8: Flowchart of DM1 and control group participants recruited and included in this study

Table 3: Mean, standard deviation (SD) and difference of background characteristics in DM1 and control groups. The significance of groups differences was set at $p<.05$. The significantly differed characteristics between groups were bolded.

	DM1		Control		
Number	25		168		
	Mean	SD	Mean	SD	*p*-value
Chronological Age (yrs)	**12.3**	**2.2**	**10.8**	**1.8**	**0.001***
Maturity Offset (yrs)	**-0.4**	**1.9**	**-1.5**	**1.6**	**0.002***
Height (cm)	150.9	13.9	146.2	12.0	0.079
Body Mass (kg)	**48.0**	**17.7**	**40.7**	**12.7**	**0.042***
PAQ-C	**2.5**	**0.6**	**3.0**	**0.6**	**<0.001**
Protein (g)	75.0	36.5	66.0	34.9	0.100*
Calcium (mg)	908.5	339.8	916.0	440.2	0.684*
Vitamin D (IU)	135.6	98.3	191.7	162.1	0.174*
Years after diagnosis	5.2	2.4			
HbA1c (%)	7.8	0.8			

*Significance between groups tested with Mann-Whitney U test. The rest characteristics was tested with independent t-test

3.2 Measurement Procedure

3.2.1 Questionnaires

There were three questionnaires in this study, "Physical Activity Questionnaire for Children", "Limb Dominance, Medical History and Health Questionnaire" and "Food Frequency Questionnaire". Participants and their parents/guardians had the choice to complete them before, during, or after the measurement session.

Physical Activity Questionnaire for Children (PAQ-C) (Appendix 1) is a 7-day self-reported recall of physical activity level for children. The questions include what sports participants play, physical activity at different time in a day, and potential barriers for physical activity. The focus of this questionnaire is the frequency and types of activity instead of intensity. PAQ-C has good validity and internal consistency reported in previous studies [120,121]. The PAQ-C score is based on a 1 (low activity level) to 5 scale (high activity level) and was

considered as a potential covariate in statistical analysis.

Limb Dominance, Medical History and Health Questionnaire (Appendix 2) helped determine which limb/side to measure in pQCT and to analyze in neuromuscular performance testing. We typically measured the dominant limb; however, if children had previous fracture on their dominant limbs, their non-dominant limbs were measured instead. Medical history and health condition helped with the determination of participant eligibility. If there were any medication and diseases besides DM1 that participant had, which would influence bone and muscle health and growth, this child would be excluded from our study. This questionnaire also asked for DM1 durations, which is a confounding factor potentially influencing musculoskeletal growth and reported in background characteristic table [122].

Food Frequency Questionnaire (Appendix 3) is a validated self-report dietary questionnaire to assess nutrition intake over past six months (NutritionQuest, 1998 BDDS) [123,124]. It requires recall for a variety of food, including fruit, diary, cereal, vegetable, meat and fish, carbohydrates, as well as beverage and supplements. The finished questionnaires were then sent to NutritionQuest for dietary analysis to provide detailed information on calories and nutrient intake per day.

3.2.2 Anthropometry Measurement

Body height was measured in centimeters (cm) by a stadiometer on the wall (Holtain Limited, Crymych, UK), which can be accurate to the millimeter. When measuring height, participants stood straightly against the wall with shoes off and feet together. To measure seated height, participants sat on a box with back against stadiometer on the wall. The measurement was in centimeter scale and was then accurate to the millimeter. I subtracted the readings to box

height for seated height. Both body height and seated height were measured three times, and the median value was taken. Body mass was measured in kilogram (kg) by weight scale (Toledo Scale Company of Canada Ltd, Windsor, ON, Model 2830) to the nearest 0.5kg.

3.2.3 Maturation Assessment

The maturity status of participants was assessed by maturity offset (MO), which was the number of years at the measurement date away from the age of peak height velocity (aPHV). aPHV marks the age children will experience the highest rate of stature growth [125,126]. MO is the estimated maturity in years calculated from sex-specific formulae considering both children's chronological age and height or seated height [48], and the formulae are shown below (Equations 3.1 & 3.2):

$$\text{Maturity Offset for Boys} = -8.128741 + 0.0070346 \times \text{age} \times \text{seated height} \quad (3.1)$$

$$\text{Maturity Offset for Girls} = -7.709133 + 0.0042232 \times \text{age} \times \text{height} \quad (3.2)$$

Where the "age" is the participant's chronological age (yrs).

3.2.4 Medical Record Review

I also reviewed the medical record to obtain the background characteristics of long-term blood glucose level and disease duration. Long-term blood glucose level was assessed by mean annual hemoglobin level (HbA1c, %). Disease duration in years was calculated by subtracting testing date and date of diagnosis.

3.2.5 Bone and Muscle Properties Assessment

Bone and muscle properties were measured from participant's dominant forearm and

lower leg using the peripheral quantitative computed tomography (pQCT) (Stratec XCT 2000) with a slice thickness of 2.4mm and pixel size of 0.4mm*0.4mm [127]. Previous studies reported the precision error of pQCT on children's bone properties and strength measures (2-19%) [10] and adult muscle cross-sectional area (MuA) (1-4%) [128,129]. Prior to testing, the participant's forearm and lower leg length were measured. The forearm length was based on ulna length. During the measurement, participants were required to put their elbows on the table and to flex their elbows to make their dominant (or non-fractured) forearms perpendicular to the table. We took the measurement from the bottom of the elbow (olecranon process) to the most distal and lateral point of the styloid process of the radius using an anthropometric sliding caliper [10]. The lower leg length was based on tibia length. Children sat on a chair with their ankle of the dominant (or non-fractured) leg on their thigh. Then, we measured the length from proximal border of the medial epicondyle to the most distal point of medial malleolus as lower leg length [10]. The limb dominancy was determined by preferred writing hand and ball kicking foot for dominant arm and leg, respectively [10]. After measuring limb length, we pre-scaned participants for scout view to determine the distal end of the ulna and tibia as a reference line, which was placed above the growth plate and distal to the proximal edge of epiphysis (Figure 9). pQCT took the scans at 4% and 65% sites of ulna from the most distal edge of the styloid process of ulna and 4% and 66% sites of the tibia from the most distal edge of the medial malleolus (Figure 10 & 11) [10]. At distal sites, the threshold was set at 480mg/cm^3 to separate cortical and trabecular bone, and 200mg/cm^3 to classify bone tissue. At shaft sites, the threshold was set at 480 mg/cm^3 to separate cortical and trabecular bone, 280mg/cm^3 to separate bones and soft tissues, and 40mg/cm^3 to separate muscle and subcutaneous fat [10,93,94]. At distal sites, total bone properties (content (ToC, mg/mm) density (ToD, mg/cm^3) and area (ToA, mm^2)) and trabecular bone properties (content

(TrC, mg/mm), density (TrD, mg/cm^3) and area (TrA, mm^2),) were measured. At shaft sites, total bone properties (content (ToC) density (ToD) and area (ToA)) and cortical bone properties (content (CoC, mg/mm), density (CoD, mg/cm^3) and area (CoA, mm^2)) were measured. The precision errors of bone properties range from 2-19% for distal sites, and 2-8% for shaft sites in our lab [10]. Participants were required to keep still during the whole scan to achieve the best quality of the scan images. Unlike bone properties, muscle property, and muscle cross-sectional area (MuA, cm^2) was only measured at shaft sites. The unpublished precision errors of MuA from our lab range from 3-4%.

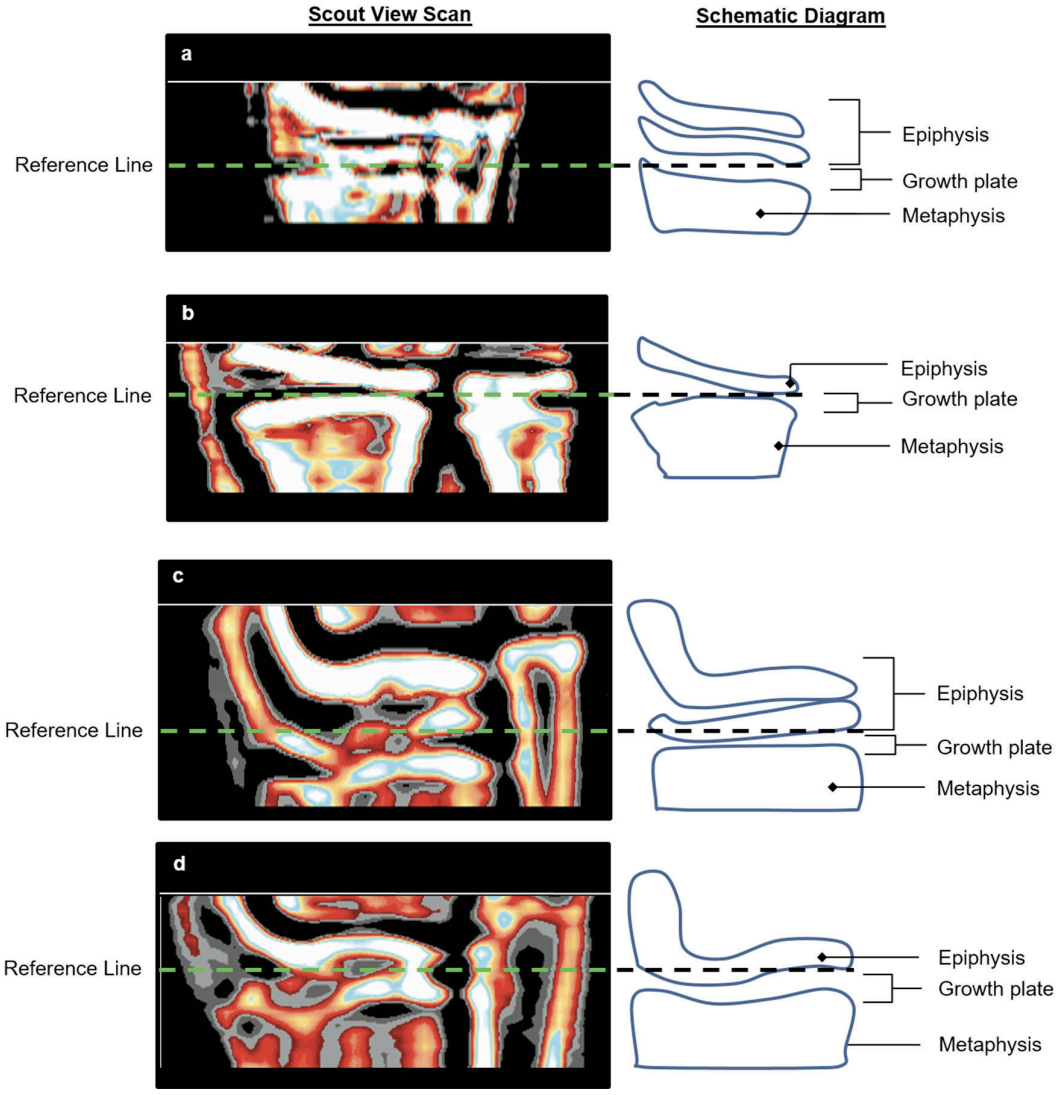

Figure 9: Sample reference line placement for radius (a,b) and tibia (c,d) from scout view scans during pQCT scan. Adapted from Duff et al. (2017) [10]

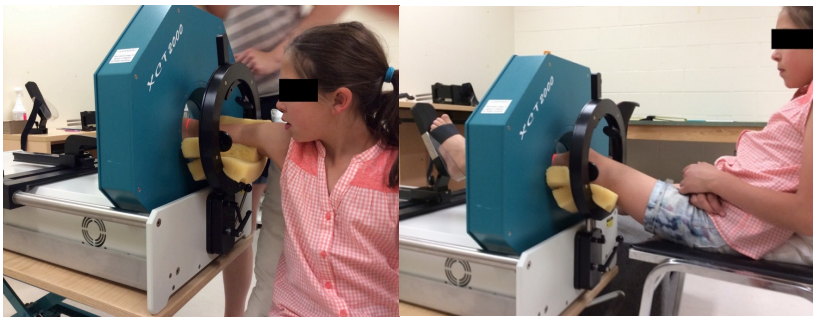

Figure 10: One participant was receiving arm (left) and leg (right) scans with pQCT

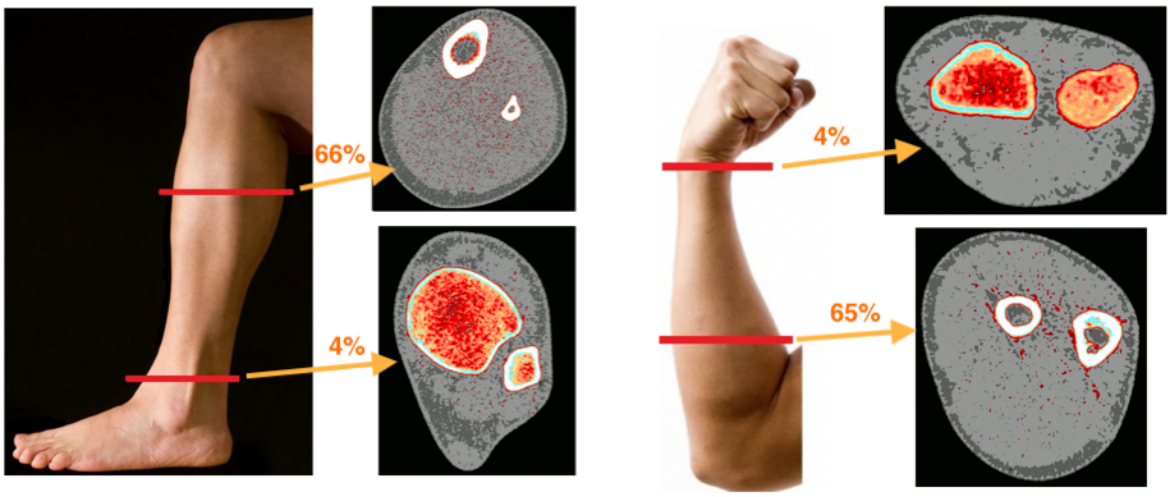

Figure 11: Sample lower leg (left) and forearm (right) scans at distal and shaft sites with pQCT

3.2.6 Bone Strength Estimation

Bone strength was determined by bone strength index for compression (BSI_c, mg^2/cm^4) at distal sites and density-weighed section modulus (SSI_p, mm^3) at shaft sites. BSI_c and SSI_p were calculated by the following equations (Equations 3.3 & 3.4) [28,91]:

$$BSI_c = ToA \times ToD^2 \quad \text{.....(3.3)}$$

$$SSI_p = \sum_i \frac{(a_i \times d_i^2)(\frac{CoD}{ND})}{d_{max}} \quad \text{.....(3.4)}$$

Where a represents the cross-section area of one voxel (mm^2), d is the distance from the voxel to center of gravity (mm), and d_{max} is the distance from the farthest voxel to the center of gravity (mm). CoD is the cortical bone density (mg/mm^3) at the shaft, and ND is the normal physiological density (mg/mm^3) [28].

The bone strength index for compression (BSI_c) is able to explain 85% of the variance in bone failure load at the distal site based on validation study, and had a precision error of 8% in children from our lab [10,91]. Density-weighed polar sectional modulus (SSI_p) considers not only the bone density but also the bone size and material distribution to assess the ability that bone resists torsional loading at the radial and tibial shaft [91]. The precision error for SSI_p is 6% in children from our lab [10]. Therefore, BSI_c and SSI_p were used to represent bone strength in this study.

3.2.7 Neuromuscular Performance

Maximal Push-up (MaxPU) is an upper-body explosive test, which requires participants to push themselves off from the ground as high as possible without bending elbows after hands leaving the ground until reaching the top of the movement [108]. Before performing MaxPU, participants were required to place their hands shoulder-width apart on two force platforms (Figure 12 A). During the test, participants started from a full plank position and elbow fully extended. Then they lowered down their bodies by bending the elbows. After the elbows ware bent at least 90 degrees or reached their limit, participants pushed their bodies up as fast as they could. The highest ground reaction force during push-off phase was selected based on kinematic data collected by an eight-camera motion capture system (Vicon Nexus, Vicon Motion Systems, CO) and then processed by Matlab code (R2006b). Reflective tracking markers were placed on

participants' two shoulder joints (acromioclavicular joints) and on the top of their backs (centered between superior scapulae) prior to the testing. These three markers could help capture vertical movement of upper body. The maximal push-off ground reaction force was selected during the "upward" phase of the push-up. Three trials were performed, and the largest push-off ground reaction force (Newton, N) from the dominant arm among all three trials was used for statistical analysis. The unpublished precision error of MaxPU force is 9% in our lab.

Maximal Grip Force (GF) is a common test to assess children's hand and wrist strength, which was measured by JAMAR 200 hand dynamometer (Sammon Preston Inc., Boldingbrook, IL) (Figure 12 B) in kilogram and then converted into Newton. While lab technician was saying "Squeeze as hard as you can. Squeeze. Squeeze. Squeeze", participants squeezed the hand dynamometer as hard as they could with elbow flexing 90 degrees and arm away from the body [113]. Participants performed this test three times on each hand with alternating hands to eliminate the potential muscle fatigue. Only the maximal force (N) from the dominant hand, one representation of upper body muscle force, was recorded for further analysis. The unpublished precision error of GF is 14% in our lab.

Countermovement Jump (CMJ) is an explosive jumping test to assess children's lower body muscle extremity. Participants started by standing upright on one force platform, then performed a countermovement by jumping as high as they could (Figure 12 C). Arm swing was allowed during the movement. Knee angles during countermovement and jumping were not specifically controlled. This test involved three trials. The maximal vertical ground reaction force (N), power (Watts, W) and impulse (Newton second, Ns) during take-off phase were measured for each trial to represent lower body muscle force. Only the data from the trial with the highest impulse was used for further statistical analysis. The unpublished precision errors of CMJ

outcomes range from 11-23% in children in our lab.

Long Jump (LJ) is the other explosive jumping test to assess children's lower extremity. Participants started from standing on one force platform behind a marked take-off line, then jumped as far as they could (Figure 12 D,E). The LJ length was measured from the take-off line to the back of the participants' heel closest to the take-off line. Arm swing was allowed during the movement. Knee angle before and during the jump was not specifically controlled, either. This test was performed three times. The maximal vertical and horizontal ground reaction force, power and impulse and jumping length (cm) were measured for each trial during take-off phase to represent lower body muscle force, but only values from the trial with the longest jumping length was used for further statistical analysis. The unpublished precision errors of LJ outcomes range from 6-25% in children in our lab.

Figure 12: Neuromuscular performance tests in biomechanics lab: A) Maximal Pushup force was measured based on two force platforms. B) Maximal grip strength was measured by a handgrip dynamometer. C) Maximal countermovement jump force was measured on the single force platform. D,E) Maximal Long jump horizontal and vertical force was measured based on the single force platform.

3.3 Statistical Analysis

I separated measurement into two sets of outcomes, bone and muscle outcomes. I analyzed radius, tibia, upper body, and lower body muscle outcomes separately. Upper-body muscle outcomes included forearm muscle area, grip force and maximal push-up force. Lower body muscle outcomes included lower leg muscle area and countermovement and long jump outcomes. Both sexes were combined in the analyses.

3.3.1 MANCOVA Assumptions

Bone Outcomes: I checked (1) the normality of all pQCT outcomes in both DM1 and control groups by visual inspection with normal Q-Q plots, (2) independence of observation with Durbin-Watson test, which all values were close to 2, (3) outliers with boxplots, and there were two outliers for distal radius total bone density (ToD) and one for tibia shaft cortical content (CoC) in control group. However, I retained the outliers in statistical analysis since exclusion did not influence my results. I also checked (4) homogeneity of variance and covariance matrices by Levene's test ($p<.05$) and Box's test ($p<.001$), respectively. Homogeneity of regression coefficients was checked by scatter plots. There was significance on the homogeneity of variance on distal radius total bone content (ToC), trabecular content (TrC), and radius shaft cortical content (CoC), tibia shaft total bone area (ToA). Also, there was violation on homogeneity of covariance in both radius and tibia. I checked (5) linearity between all pairs of dependent variables (DVs, bone outcomes) and independent variable (IV, diabetes status), and DVs and covariates using scatter plots. (6) Multicollinearity and singularity in between DVs and IV were checked by VIF (<10) or tolerance (>0.1) values and bivariate correlation ($r < 0.9$), respectively.

Muscle Outcomes: I checked (1) the normality of all force and related outputs in both DM1 and control groups by visual inspection with normal Q-Q plots, (2) independence of observation with Durbin-Watson test, which all values were close to 2, (3) outliers with boxplots. There were two outliers for forearm muscle area (MuA) and one for lower leg MuA, one for countermovement jump vertical impulse and one for long jump horizontal impulse in the control group. However, I retained the outliers in statistical analysis since exclusion did not influence my results. I also checked (4) homogeneity of variance and covariance matrices by Levene's test ($p<.05$) and Box's test ($p<.001$), respectively. Homogeneity of regression coefficients was checked by scatter plots. There was significance on homogeneity of variance on maximal push-up ground reaction force and countermovement jump vertical power and long jump vertical force. Also, there was violation on the homogeneity of covariance in upper body muscle outcomes. I checked (5) linearity between all pairs of dependent variables (DVs, muscle outcomes) and independent variable (IV, diabetes status), and DVs and covariates using scatter plots. (6) Multicollinearity and singularity in between DVs and IV were checked by VIF (<10) or tolerance (>0.1) values and bivariate correlation ($r < 0.9$), respectively.

In the case of assumption violation, I transformed all bone and muscle outcomes, height, body mass, PAQ-C score and nutrition intake, based on *lg10* algorithm, and then ran the transformed variables in MANCOVA again for the same models. The same covariates stayed for each MANCOVA model but in the transformed form, except for maturity offset. I included the results after data transformation Appendices 4 and 5 (Table 6 & 7). However, I still reported the results and discussed the findings based on the data before transformation, since they are easier for interpretation and the results are comparable before and after transformation.

3.3.2 Covariate Determination

The potential covariates were sex, maturity offset, height, body mass, PAQ-C score and nutrition factors (calcium, vitamin D and protein) for bone and muscle outcomes [47,49–52,54–56,61,64–67]. I first determined if any of the potential covariates differed between the groups using either independent *t*-test or Mann-Whitney U test depending on the normality of distribution for each factor in both control and DM1 groups ($p<.05$). The sex difference between groups was tested using chi-square ($p<.05$). Maturity offset, body mass and PAQ-C score differed between groups; hence they were applied into MANCOVA model. However, PAQ-C score only contributed to the model for lower body muscle outcomes, determined by the significance of multivariate significance ($p<.05$) in the model ($p<.05$). Therefore, for radius and tibia bone outcomes and upper body muscle outcomes, the covariates I selected were maturity offset and body mass for the MANCOVA models to test hypothesis 1. For lower body muscle outcomes, the covariates were maturity offset, body mass and PAQ-C score.

3.3.3 Mediation

Mediation is a hypothesis that one factor could intermediate during the process which one variable affects another variable, which the factor is usually labeled in "M" as mediator (Figure 13) [130]. The independent variable is labeled as "X", and the dependent variable is labeled as "Y" (Figure 13) [130]. The amount of mediation is referred to "indirect effect" [130]. Pathway c in Figure 13 is "total effect" and c' is "direct effect", which total effect is the sum of direct and indirect effect [130]. Pathway a and b are both "direct effect" from X to M and M to Y, respectively (Figure 13) [130]. The "indirect effect" can be calculated by c subtracting c' [130]. The "effects" are characterized by the unstandardized beta coefficient in each regression model [130].

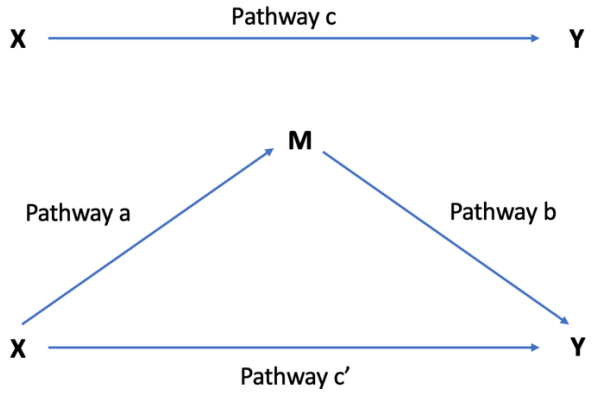

Figure 13: Diagram showing total effect (c) between an independent variable (X) and dependent variable (Y) (upper figure), and direct effect (a, b, c') from X to M, M to Y and X to Y without M, respectively

The analysis of mediation is usually based on regression analysis with a four-step analysis [131]. The first step is to test the relationship between X and Y as pathway c, followed by testing the relationship between X and M, as pathway a, and M and Y, as pathway b, by bivariate regression [131]. The last step is to run a multiple regression including both X and M predicting Y as pathway c' [131]. The popular method to evaluate mediation is by using bootstrap [130], since bootstrap does not require the assumption of normality and works for small to large sample sizes [130,132,133]. The independent variable (X), DM1 and control groups, in this study was binary and not continuous. Therefore, bootstrapping is an ideal choice when exploring mediation in this study. Bootstrapping assumes a non-parametric way relying on random resampling with replacement for a large number of times, like 5000 times [130]. Bootstrapping provides a confidence interval for calculating indirect effect; a confidence interval without crossing zero implies the significance of mediation, or indirect effect [130].

3.3.3 Hypothesis Testing: Research Objective 1 and 2

Objective 1: I used multiple analysis of covariance (MANCOVA) to determine if there was a significant difference in bone outcomes, including radius and tibia properties and strength, and upper and lower body muscle outcomes between groups ($p<.05$). The corresponding covariates I adjusted in MANCOVA models were maturity and body mass for radius, tibia and upper body muscle outcomes, and maturity, body mass and PAQ-C score for lower body muscle outcomes. I reported the omnibus effect in each bone or muscle MANCOVA model. I also reported the mean and standard deviations of each outcome for both groups, as well as between-group adjusted mean differences and % difference with 95% confidence interval.

Objective 2: I tested mediation using macro code for SPSS (PROCESS, Hayes, 2018) by adding MuA or neuromuscular performance outcomes as a possible mediator one by one into the regression models. In the models, I included groups as X and differed bone outcomes between groups determined in Objective 1 as Y as well as the same covariates, maturity and body mass. Significance of mediation was determined by 95% confidence interval of indirect effect calculated with bootstrap (5000 bootstrap samples).

4. Results

4.1 Research Objective 1: Bone and Muscle Outcomes

4.1.1 Radius

There was a significant group difference (omnibus effect) in radius bone outcomes, $F(12,168) = 4.705$, $p<.001$. At the distal radius, there were no significant differences in bone properties and strength between DM1 and control groups (Table 4). At radius shaft sites, there were 8% higher cortical bone density (CoD) and 7% lower cortical bone area (CoA) in DM1 group (Figure 14, Table 4). None of the other radius shaft bone properties nor strength parameters differed between groups (Table 4).

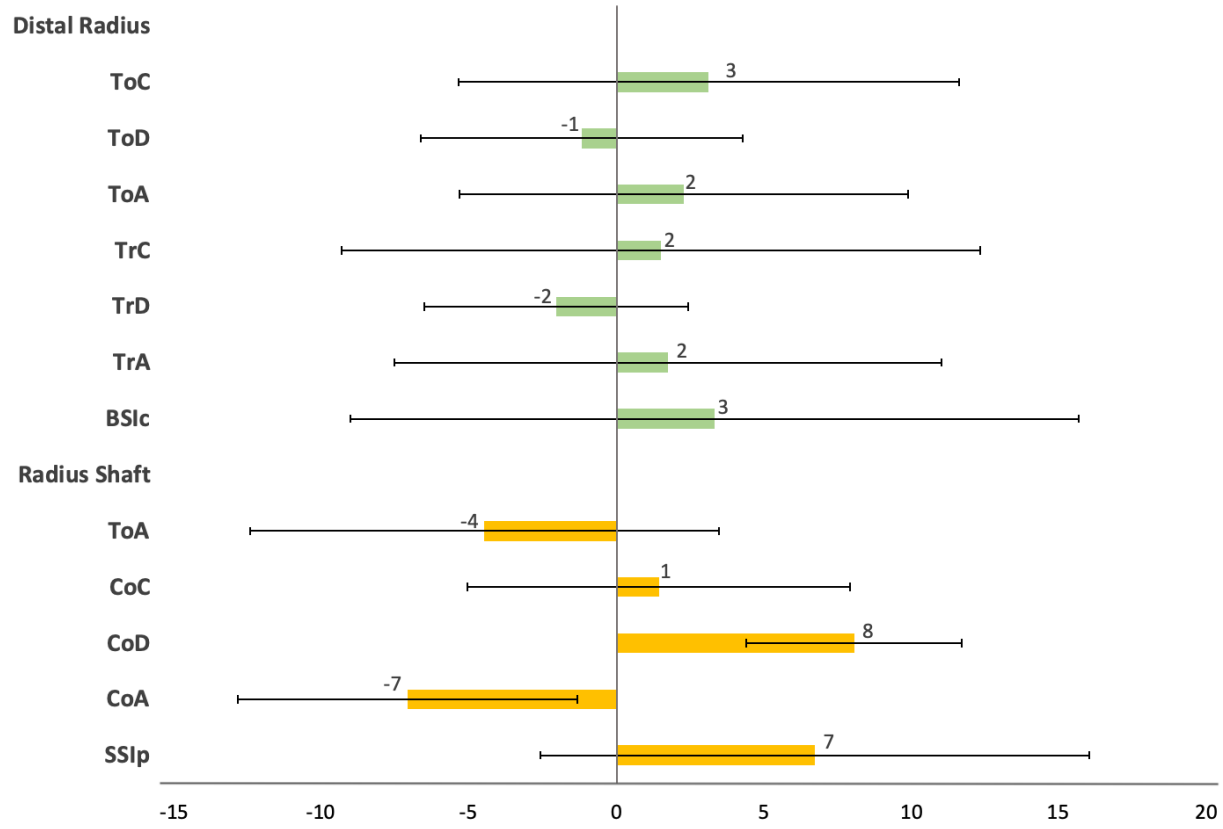

Figure 14: Bar graph showing adjusted mean % difference of radius properties and strength with 95% confidence intervals comparing DM1 group with the control group

4.1.2 Tibia

There was a significant group difference (omnibus effect) in tibia bone outcomes, $F(12,173) = 3.881$, $p<.001$. At distal tibia, there was no significant difference between DM1 and control groups on bone properties and strength (Table 4). At the tibia shaft, children with DM1 had 6% lower cortical bone area content (CoC), 5% higher cortical bone density (CoD) and 10% lower cortical bone area (CoA) (Figure 15, Table 4). The rest of tibia shaft bone properties and strength parameters did not have significant difference between groups (Table 4).

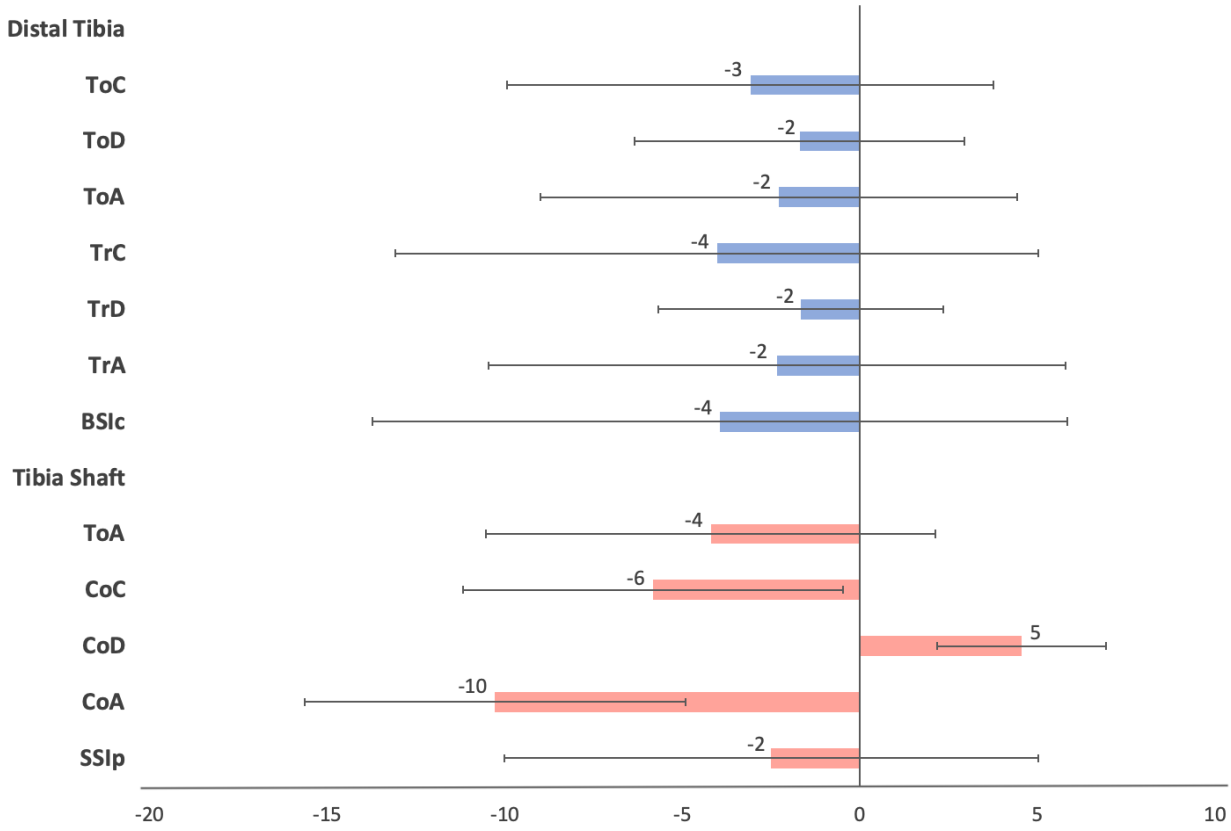

Figure 15: Bar graph showing adjusted mean % difference of tibia properties and strength with 95% confidence intervals comparing DM1 group with the control group

Table 4: Mean, standard deviation (SD), adjusted mean difference and % difference of bone properties and strength between DM1 and control groups. The significance of groups differences was set at $p<.05$. The significant different bone outcomes between groups were bolded.

	DM1		Control				
	Mean	SD	Mean	SD	*p*-value	Difference	% Difference
Distal Radius							
ToC (mg/mm)	76.0	14.4	73.7	14.0	0.470	2.3	3.1
ToD (mg/cm^3)	285.2	36.3	288.6	35.3	0.668	-3.4	-1.2
ToA (mm^2)	262.8	45.0	256.9	43.7	0.555	5.9	2.3
TrC (mg/mm)	59.8	14.7	58.9	14.3	0.784	0.9	1.5
TrD (mg/cm^3)	248.3	26.1	253.5	25.4	0.366	-5.2	-2.1
TrA (mm^2)	236.3	49.7	232.3	48.3	0.712	4.0	1.7
BSIc (mg^2/mm^4)	22.2	6.1	21.5	5.9	0.596	0.7	3.3
Radius Shaft							
ToA (mm^2)	121.9	23.4	127.6	22.7	0.268	-5.7	-4.5
CoC (mg/mm)	68.2	10.1	67.2	9.8	0.664	1.0	1.4
CoD (mg/cm^3)	**903.8**	**70.5**	**836.5**	**68.6**	**<0.001**	**67.3**	**8.1**
CoA (mm^2)	**74.5**	**10.7**	**80.2**	**10.4**	**0.016**	**-5.7**	**-7.1**
SSIp (mm^3)	211.6	42.5	198.3	41.4	0.156	13.3	6.7
Distal Tibia							
ToC (mg/mm)	211.7	35.2	218.4	34.4	0.375	-6.7	-3.1
ToD (mg/cm^3)	284.0	31.5	288.9	30.9	0.473	-4.9	-1.7
ToA (mm^2)	741.8	120.0	759.2	117.3	0.503	-17.3	-2.3
TrC (mg/mm)	164.8	36.6	171.7	35.8	0.381	-6.9	-4.0
TrD (mg/cm^3)	245.6	23.6	249.8	23.1	0.411	-4.2	-1.7
TrA (mm^2)	669.5	130.9	685.4	128.1	0.572	-16.0	-2.3
BSIc (mg^2/mm^4)	61.1	14.6	63.6	14.3	0.427	-2.5	-4.0
Tibia Shaft							
ToA (mm^2)	476.4	73.9	497.3	72.3	0.191	-20.9	-4.2
CoC (mg/mm)	**224.3**	**30.0**	**238.2**	**29.3**	**0.033**	**-13.9**	**-5.8**
CoD (mg/cm^3)	**932.9**	**49.9**	**892.3**	**48.8**	**<0.001**	**40.6**	**4.6**
CoA (mm^2)	**238.8**	**33.6**	**266.2**	**32.9**	**<0.001**	**-27.4**	**-10.3**
SSIp (mm^3)	1466.1	265.8	1503.6	260.1	0.513	-37.6	-2.5

4.1.3 Muscle Area and Neuromuscular Performance

There were significant group differences (omnibus effect) in upper (F(3,172) = 3.111, p= .028) and lower body (F(11,125) = 2.100, p=.025) muscle outcomes. Participants in DM1 group had higher maximal push-up group reaction force (12%) compared to control group (Figure 16 &

17, Table 5). There were no differences between the groups in forearm and lower leg muscle cross-sectional area, grip force and all jump outcomes (Table 5).

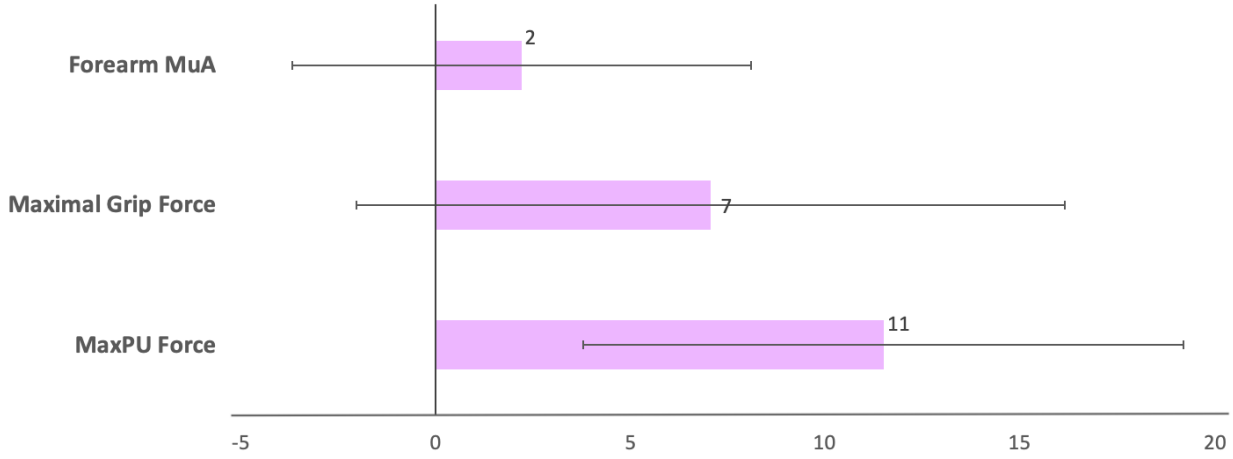

Figure 16: Bar graph showing adjusted mean % difference of upper body muscle outcomes with 95% confidence intervals comparing DM1 group to the control group

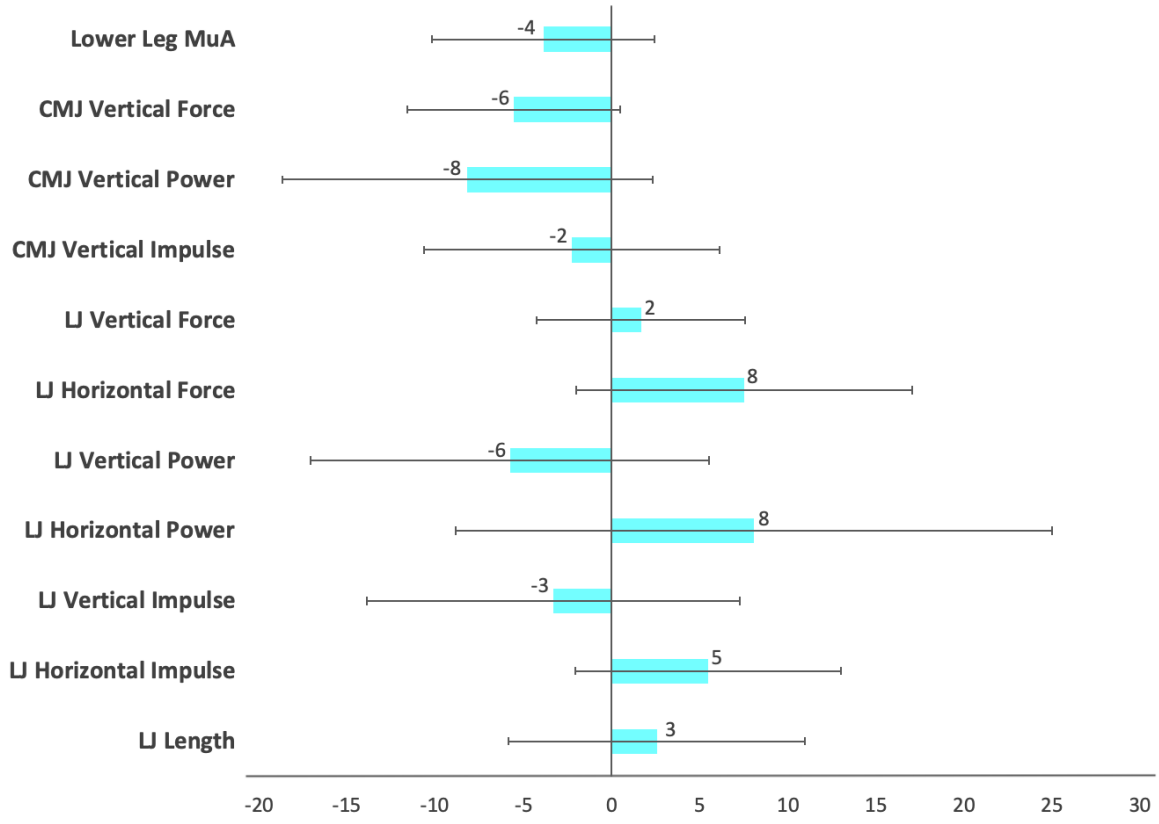

Figure 17: Bar graph showing adjusted mean % difference of lower body muscle outcomes with 95% confidence intervals comparing DM1 group to control group

Table 5: Mean, standard deviation (SD), adjusted mean difference and % difference of muscle area and neuromuscular performance outcomes between DM1 and control groups. The significance of groups differences was set at $p<.05$. The significant different muscle outcomes between groups were bolded.

	DM1		Control				
	Mean	SD	Mean	SD	*p*-value	Difference	% Difference
Upper Body							
Forearm MuA (cm^2)	22.6	3.0	22.1	2.9	0.462	0.5	2.2
Maximal Grip Force (N)	201.2	39.3	188.0	38.6	0.128	13.2	7.1
Maximal Push-up Force (N)	**203.2**	**32.3**	**182.2**	**31.7**	**0.004**	**20.9**	**11.5**
Lower Body							
Lower Leg MuA (cm^2)	41.6	5.7	43.3	5.3	0.227	-1.7	-3.9
Countermovement Jump							
Vertical Force (N)	831.4	110.3	880.5	103.6	0.071	-49.1	-5.6
Vertical Power (W)	1626.6	385.4	1772.1	362.0	0.125	-145.5	-8.2
Vertical Impulse (Ns)	90.8	16.1	92.9	15.1	0.591	-2.1	-2.3
Long Jump							
Vertical Force (N)	846.4	101.9	832.5	95.7	0.577	13.9	1.7
Horizontal Force (N)	308.7	56.7	287.2	53.2	0.122	21.6	7.5
Vertical Power (W)	993.4	246.8	1054.3	231.9	0.314	-60.9	-5.8
Horizontal Power (W)	564.4	183.0	522.3	171.9	0.348	42.1	8.1
Vertical Impulse (Ns)	55.8	12.6	57.7	11.9	0.535	-1.9	-3.3
Horizontal Impulse (Ns)	90.6	13.4	85.9	12.6	0.154	4.7	5.5
Length (cm)	137.9	23.4	134.5	22.0	0.549	3.4	2.6

4.2 Research Objective 2: Mediation of Muscle Outcomes on Bone Outcomes

Lower leg muscle area was a mediator in predicting the tibia shaft cortical bone content (indirect effect = -5.1, 95%CI = -10.76, -0.03) and area (indirect effect = -5.5, 95%CI = -11.82, -0.00) differences between DM1 and control groups (Figure 18 & 19), as the 95% confidence interval (CI) of indirect effect does not cross zero. Other muscle outcomes did not have significant indirect effect due to the 95% CI across zero.

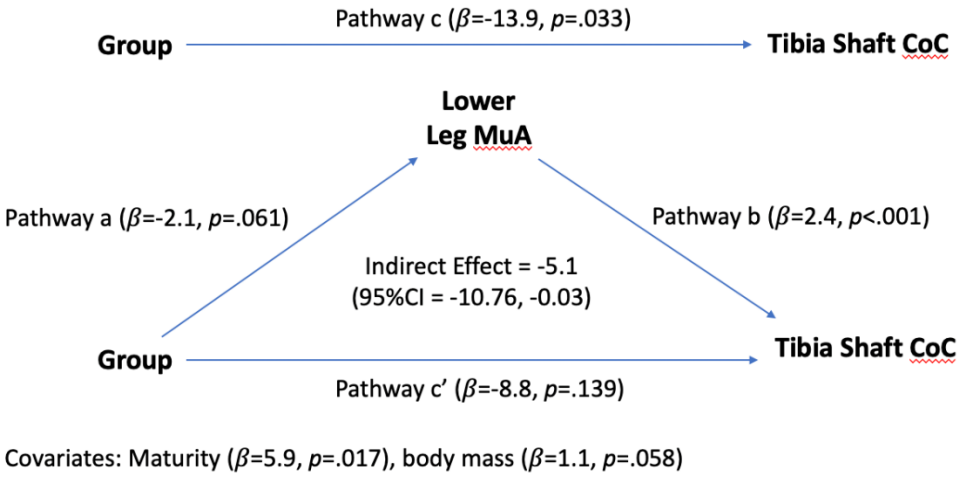

Figure 18: Pathway diagram showing group (X) predicting tibia shaft CoC (Y) without considering possible mediator (M) lower leg MuA (pathway c), and with considering lower leg MuA as mediator (pathway c'). Pathway a represents the group (X) predicting mediator, lower leg MuA (M). Pathway b represents the mediator, lower leg MuA (M), predicting tibia shaft CoC (Y). Unstandardized beta (β) and significance of prediction of individual pathway and covariates ($p<.05$), as well as the indirect effect of the mediator, lower leg MuA, and its 95% confidence interval (CI).

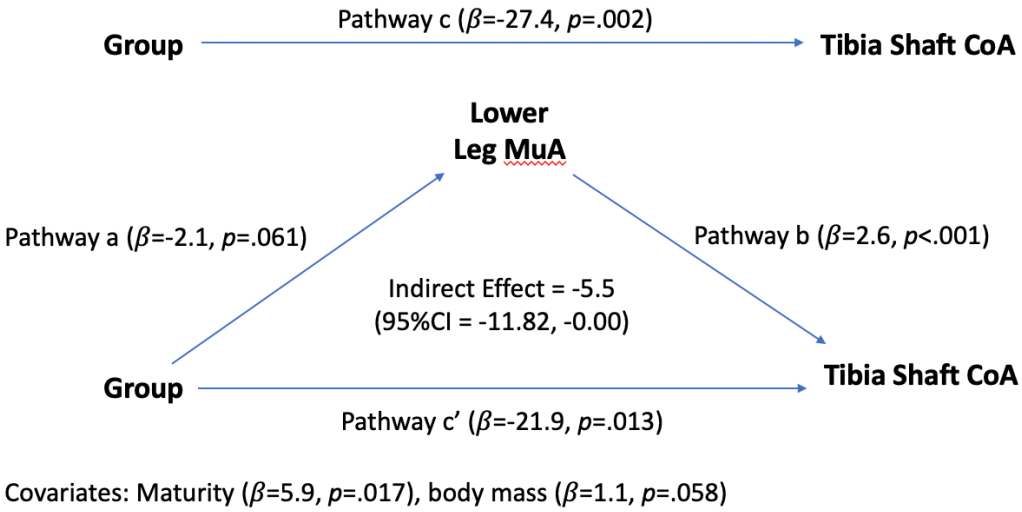

Figure 19: Pathway diagram showing group (X) predicting tibia shaft CoA (Y) without considering possible mediator (M) lower leg MuA (pathway c), and with considering lower leg MuA as mediator (pathway c'). Pathway a represents the group (X) predicting mediator, lower leg MuA (M). Pathway b represents the mediator, lower leg MuA (M), predicting tibia shaft CoA (Y). Unstandardized beta (β) and significance of prediction of individual pathway and covariates ($p<.05$), as well as the indirect effect of the mediator, lower leg MuA, and its 95% confidence interval (CI).

5. Discussion

My findings suggested group difference in bone and muscle outcomes between children with DM1 and their typically developing peers. Children in DM1 group had 7% lower cortical bone mineral area, and 8% higher density at the radius shaft. My thesis findings added evidence to previous literature measuring radius with pQCT, in which children with DM1 had lower shaft-site cortical bone area [12,13]. I did not detect between-group difference in radius shaft SSI_p while Saha et al. reported 5-9% lower radius shaft SSI_p in children with DM1 [12]. However, there was no difference at the distal radius between the DM1 and control groups, which agreed with two previous studies with radius measurement [12,13].

For tibia, we observed 6% and 10% lower shaft-site cortical bone content and area, respectively, and 5% higher cortical bone density, which supported previous findings [17]. However, we did not find any difference at distal tibia outcomes and tibia shaft bone strength (SSI_p), which contrasted with the previous literature reporting 5-10% lower trabecular bone outcomes [15,17] and 9-12% lower SSI_p [12,15]. Our findings on the tibia were similar to the radius, both suggesting lower cortical bone area and higher density at both upper and lower body limbs. Furthermore, although there was no difference found at distal sites of the radius and tibia, it did not represent there was no difference if looking into bone micro-structure.

For muscle size, there was no difference in muscle cross-sectional area between groups in our study. In terms of previous literature, Bechtold et al. reported 0.2-0.3 standard deviation below the mean of German reference population on forearm muscle cross-sectional area [13], and Moyer-Mileur reported 1% higher at lower leg muscle area comparing to their non-diabetic control group [17]. The findings on muscle size seemed inconsistent. However, muscle area did not appear to have big difference between children with and without DM1 even if the difference was

significant, since the observed change was smaller than the unpublished precision error of pQCT measured muscle area (3-4%) from our lab.

The children with DM1 had an average HbA1c of 7.8% , which suggested a good glycemic control when compared to previous literature with values ranging from 8.2 to 9.2% [12,15,17]. Our findings on cortical bone area and distal radius were comparable one previous paper with similar average HbA1c level (7.7%), but they also reported lower total bone area and muscle cross-sectional area at radius shaft as well as higher grip strength [13]. There was a study comparing the pQCT results between children with good (<7.5%) and poor (≥7.5%) glycemic control, and reported the gains in distal tibia trabecular bone density and tibia shaft total and cortical bone area were less in children with poor glycemic control when compared to those children with good glycemic control [14]. However, even if the growth differed after 12 months, they did not report differences in tibia shaft total and cortical bone area between children with good and poor glycemic control and between children with DM1 and reference [14]. Therefore, the glycemic control may play a role on bone outcomes, but more evidence is needed before determining the exact role of glycemic control in bone and muscle development.

For nutrition intake, only Moyer-Mileur et al. reported calcium intake in children with DM1 [17]. Participants in their study appeared to have higher daily calcium intake in both DM1 and reference groups comparing to ours, and there was no group difference in calcium intake [17]. Their findings at tibia shaft matched with our findings, but they also reported lower trabecular bone area, density and content at distal tibia and lower leg muscle area [17]. However, the disparate findings on distal-site bone outcomes and muscle area between their and our studies cannot be explained by calcium intake, since calcium intake did not differ between groups in these two studies.

In terms of neuromuscular performance, maximal push-up force was 12% greater in children with DM1. The potential explanation for higher MaxPU force in children with DM1 was the greater relative number of children (data not shown) participating in sports including upper body training, like gymnastics and taekwondo [134,135]. We did not observe differences in grip force which agreed with a previous study [31] but disagrees with others [12,27]. We did not observe differences in jump force and power, and long jump length between the groups, which agreed with previous literature suggesting normal jump take-off force and power in children with DM1 when compared to reference values based on z-scores [15,31].

In terms of the mediation role of muscle, our findings suggested lower leg muscle area as a mediator in between the relationship of DM1 status and tibia shaft cortical bone area and content. The calculated "indirect effect" implied that the between-group difference in cortical area and content might be lower by increasing muscle size at the lower leg in children with DM1. There was no previous literature assessing the role of muscle in bone outcomes in DM1 children. Other muscle outcomes, like neuromuscular performance, were not significant when testing for mediation. However, some of them might still be mediators with a larger sample size as the boundaries of 95% CI of some neuromuscular performance outcomes were close to zero. Also, neuromuscular performance testing appeared to have larger precision errors (6-25%) than muscle area (3-4%) based on unpublished precision error from our lab. As a result, a larger sample size might be required to detect its role as a possible mediator.

5.1 Clinical relevance

These findings provided evidence that children with DM1 had lower bone cortical bone area but higher density at both radius and tibia shaft, and lower cortical bone content at tibia

shaft compared to typically developing children. Although the bone strength did not differ between groups, the smaller but denser cortical bone at shaft sites may suggest a lower bone turnover rate in children with DM1 during growth [18,19,83]. During growth, the bone grows in length, and the trabeculae close to the periosteal surface will thicken and develop into cortical bone [136]. The cortical bone formation cannot match the speed of resorption during rapid growth, which is likely to leave more pores inside the cortical bone and reduces the cortical bone density [136]. However, when the bone turnover rate or bone formation is lower, the cortical bone may not form as much as in typically developing children, and there would not be as much porosity as well [136]. Therefore, the potential lower bone turnover might be the reason underlying both the higher cortical bone density and smaller cortical bone area. This is clinically important because it may relate to the development of weaker skeleton in the future, and contribute to the reported higher fracture risk in individuals with DM1 [2,106].

Our findings also suggest if children with DM1 have larger lower leg muscle size, the difference in tibia shaft cortical area and content between children with and without DM1 might be smaller. An intervention focusing on enlarging lower body muscle size might be beneficial for developing larger size cortical bone at tibia shaft in children with DM1. High impact exercise, like jumps, might be an ideal choice of exercise improving muscle size, and also jumps can help improve bone strength in children [20,63].

5.2 Strengths and Limitations

This study had a few strengths and limitations that warranted discussion. The first strength related to the bone imaging tool, pQCT, which facilitated the investigation of bone and muscle in children with and without DM1. pQCT allowed me to separate cortical and trabecular

bone and to measure their size and "true" volumetric bone mineral density. Furthermore, the study design involved an actual control group instead of comparing to reference data like some of the previous studies [13–15]. The control group data was also collected from our lab with the same measurement tools and procedures within the same lab space with known measurement precision errors. In addition, we also evaluated muscle size and force produced in neuromuscular performance to assess how strong the muscle is. Muscle cross-sectional area was a relatively precise way and good surrogate of muscle strength [92]. Neuromuscular performance had a focus on maximizing muscle force output in a more direct way, and was measured from movements involving motor performance and body coordination [108]. Previous studies usually only focused on one way or another. In this way, we were able to assess the role of both muscle size and force in bone properties and strength in children with and without DM1.

There are also some limitations in this study. Firstly, there was a relatively small sample size in DM1 group and the uneven sample size between two groups; if there would be more participants in DM1 group, the power of analysis would be stronger [137]. Secondly, we were not able to obtain PAQ-C score (133 in control group, 20 in DM1 group) and nutrition data (103 in control group, 23 in DM1 group) from all participants, which reduced our sample size in both groups and the power during analysis when including PAQ-C score as a covariate. Thirdly, there were limitations related to the voluntary muscle contraction during the neuromuscular performance test, which could be influenced by skill level as well as motivation, like how hard they wanted to push themselves, at the testing day [109,138]. The skill level was not controlled since most of these tests were commonly used in general children [110,139]. Although the practice trials were provided and checked by researchers, the skill level nor motivation was not recorded and thus not addressed in the analyses. As a result, neuromuscular performance appeared to be higher

in precision error and had more variability during testing comparing to muscle area obtained from pQCT scans.

5.3 Future Directions

A larger sample of children with DM1 is required for the future for a sex-specific analysis. Matching sex, maturity, height and body mass between the DM1 and control groups would be ideal for identifying disease influence on bone and muscle. Another direction could be exploring growth in children with DM1, by one-year or longitudinal follow-up, and then comparing bone and muscle growth with typically developing children. Follow-up study will be meaningful to see if bone growth and development in children with DM1 differs from children without DM1, owning to suspicion of the altered bone turnover rate in children with DM1 [19,27,84,140]. Also, there is no previous study linking bone formation and resorption biomarkers to long bone growth in geometry, properties and strength in children with DM1, which makes it a meaningful direction to be explored. Bone formation and resorption biomarkers could be measured along with bone scans in a longitudinal study to monitor the long-term change in bone turnover, which would help build a potential linkage between biomarkers and bone strength. In addition, future research could include subgroup analysis taking disease duration into consideration when analyzing bone properties and strength in children with DM1, since a previous study suggested an early manifestation could alter bone growth [13].

In this study, we suggested muscle area could mediate and explain the cortical bone content and area difference between groups. A future study could look into the relationship between muscle and bone in children with DM1 in a clinical way through a "functional muscle-bone unit". This is an analysis of bone properties with consideration of muscle function, which

was developed for clinical assessment of the bone deficits for individual children [33,35]. It could be valuable to look into the type of potential bone deficit in children with DM1 from a clinical perspective.

5.4 Conclusion

There was group difference (omnibus effect) in bone and muscle outcomes between children with and without DM1. Children with DM1 had 7-10% lower cortical bone area and 5-8% higher density at the radial and tibial shaft, and 6% lower cortical bone content at the tibial shaft compared to typically developing children. Children with DM1 also produced 12% higher maximal push-up force when comparing to their typically developing peers. Lower leg muscle area was a mediator explaining the tibia shaft cortical bone area and content difference between children with and without DM1.

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Appendix 1 Physical Activity Questionnaire for Children (PAQ-C)

Physical Activity Questionnaire
For Bone Strength Study

Name_____ Age_____

Sex M_____ F_____

We are trying to find out about your level of physical activity from ***the last 7 days*** (in the last week). This includes sports or dance that make you sweat or make your legs feel tired, or games that make you breathe hard, like tag, skipping, running, climbing, and others.

Remember:
There are no right and wrong answers — this is not a test.
Please answer all the questions as honestly and accurately as you can — this is very important.

---

1. Physical activity in your spare time: Have you done any of the following activities in the past 7 days (last week)? If yes, how many times? (Mark only one circle per row.)

	No	1-2	3-4	5-6	7 or more times
Skipping	○	○	○	○	○
Rowing/canoeing	○	○	○	○	○
In-line skating	○	○	○	○	○
Tag	○	○	○	○	○
Walking for exercise	○	○	○	○	○
Bicycling	○	○	○	○	○
Jogging or running	○	○	○	○	○
Aerobics	○	○	○	○	○
Swimming	○	○	○	○	○
Baseball, softball	○	○	○	○	○
Dance	○	○	○	○	○
Football	○	○	○	○	○
Badminton	○	○	○	○	○
Skateboarding	○	○	○	○	○
Soccer	○	○	○	○	○
Street hockey	○	○	○	○	○
Volleyball	○	○	○	○	○
Floor hockey	○	○	○	○	○
Basketball	○	○	○	○	○
Ice skating	○	○	○	○	○
Cross-country skiing	○	○	○	○	○
Ice hockey/ringette	○	○	○	○	○
Gymnastics	○	○	○	○	○
Martial Arts	○	○	○	○	○
Wrestling	○	○	○	○	○
Other: _____	○	○	○	○	○
_____	○	○	○	○	○

2. In the last 7 days, during your physical education (PE) classes, how often were you very active (playing hard, running, jumping, throwing)? (Check one only.)

I don't do PE ..... ○
Hardly ever ..... ○
Sometimes ..... ○
Quite often ..... ○
Always ..... ○

3. In the last 7 days, when you were active, how often did you use your hands for pushing, climbing, or throwing? (Check only one.)

I only use my legs ..... ○
Hardly ever..... ..... ○
Sometimes ..... ○
Quite often ..... ○
Always ..... ○

4. In the last 7 days, what did you normally do *at lunch* (besides eating lunch)? (Check one only.)

Sat down (talking, reading, doing schoolwork)…..... ○
Stood around or walked around ..... ○
Ran or played a little bit ..... ○
Ran around and played quite a bit ..... ○
Ran and played hard most of the time ..... ○

5. In the last 7 days, on how many days *right after school*, did you do sports, dance, or play games in which you were very active? (Check one only.)

None ..... ○
1 time last week ..... ○
2 or 3 times last week ..... ○
4 times last week ..... ○
5 times last week ..... ○

6. In the last 7 days, on how many *evenings* did you do sports, dance, or play games in which you were very active? (Check one only.)

None ..... ○
1 time last week ..... ○
2 or 3 times last week ..... ○
4 or 5 last week ..... ○
6 or 7 times last week ..... ○

7. *On the last weekend*, how many times did you do sports, dance, or play games in which you were very active? (Check one only.)

None ..... ○
1 time ..... ○
2 — 3 times ..... ○
4 — 5 times ..... ○
6 or more times ..... ○

8. Which *one* of the following describes you best for the last 7 days? Read *all five* statements before deciding on the *one* answer that describes you.

F. All or most of my free time was spent doing things that involve little physical effort

G. I sometimes (1 — 2 times last week) did physical things in my free time (e.g. played sports, went running, swimming, bike riding, did aerobics)

H. I often (3 — 4 times last week) did physical things in my free time

I. I quite often (5 — 6 times last week) did physical things in my free time

J. I very often (7 or more times last week) did physical things in my free time

9. Mark how often you did physical activity (like playing sports, games, doing dance, or any other physical activity) for each day last week.

	None	Little Bit	Medium	Often	Very Often
Monday	○	○	○	○	○
Tuesday	○	○	○	○	○
Wednesday	○	○	○	○	○
Thursday	○	○	○	○	○
Friday	○	○	○	○	○
Saturday	○	○	○	○	○
Sunday	○	○	○	○	○

10. Were you sick last week, or did anything prevent you from doing your normal physical activities? (Check one.)

Yes ..... ○
No ..... ○

If Yes, what prevented you? _____

11. Please list any sports or physical activities that involve using your hands or arms you have participated in regularly. Please tick the boxes to indicate how old you were for each sport/activity and how many years you participated for.

	Age:																	
Activities:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

12. Please list any sports or physical activities that involve using your hands or arms you have participated in regularly during the **last 12 months** and indicate the average frequency of the activity (sessions/week).

Activity:______________________________ Sessions/week: ______

Activity:______________________________ Sessions/week: ______

Activity:______________________________ Sessions/week: ______

Activity:______________________________ Sessions/week: ______

Activity:______________________________ Sessions/week: ______

Activity:______________________________ Sessions/week: ______

Activity:______________________________ Sessions/week: ______

Appendix 2 Limb Dominance Questionnaire, Medical History and Health Questionnaire

Name: _____ **Date:** ____/____/_____ (DD/MM/YY)

Limb Dominance, Medication, and Health Questionnaire

Please answer the following questions to the best of your ability. You may also choose not to answer any of these questions.

1. **Which is your dominant hand (e.g., which hand do you write with)?**
   - Right
   - Left
   - I can write with both hands
   - I don't know

2. **Which is your dominant leg (e.g., which leg do you use to kick a ball)?**
   - Right
   - Left
   - I can kick a ball with both legs
   - I don't know

3. **Have you been diagnosed with type 1 diabetes?**
   - Yes
   - No
   - Not Sure

   If *yes*, at what age you were diagnosed with type 1 diabetes? _____

   If *yes*, what format of insulin and how many units do you take daily?

   Format _____ Units/day_____

   Format _____ Units/day_____

   Format _____ Units/day_____

4. **Are you taking any prescription medications (other than insulin)?**
   - Yes
   - No
   - Not Sure

   If *yes*, how many prescription medications are you taking? _____

Name: _____ Name: _____ Name: _____

Dosage: _____ Dosage: _____ Dosage: _____

5. Are you taking any over-the-counter medications?

Pain killers, antacids, allergy pills, and hydrocortisone creams are all examples of over the-counter medications.

Yes
No
Not Sure

If *yes*, how many over-the-counter medications are you taking? _____

Name: _____ Name: _____ Name: _____
Dosage: _____ Dosage: _____ Dosage: _____

6. Have you ever smoked?

Yes
No

If *yes*, please indicate how often you smoke.

Daily (number of cigarettes): _____
Weekly (number of cigarettes): _____

7. Have you ever had a wrist fracture?

Yes
No
Not Sure

If *yes*, please indicate the side and date of the fracture:

Left or Right (Please circle) Date: (MM/YY): _____/_____

8. Have you ever had any other broken bones or stress fractures?

Yes
No
Not Sure

If *yes*, please indicate the bone, the side and the date of break/stress fracture:

Bone:_____ Left or Right (Please circle) Date: (MM/YY): _____/_____

Bone:_____ Left or Right (Please circle) Date: (MM/YY): _____/_____

9. Have you ever been treated or diagnosed with arthritis or other joint or bone disease?

Yes
No
Not Sure

If *yes*, please explain:

_____

The following question is for female participants only.

10. Have you started menstruating?

Yes
No
Not Sure

If *yes*, what age was your first menstrual period? _____

The following questions are for the parent/guardian of the participant.

10. Where were you born?

Mother: _____ Father: _____

11. Where were your parents born?

Maternal Mother: _____ Maternal Father: _____

Paternal Mother: _____ Paternal Father: _____

12. How long has your family lived in North America? Years: _____ Months: _____

13. Where did your family live before moving to North America? _____

14. How would classify your family ethnically? (I.e., Caucasian-Canadian, Japanese-Canadian, etc.) _____

Thank you for taking the time to complete this questionnaire.

For Research Purposes Only:

ID:_____

Date received: ____/____/____
(DD/MM/YY)

Checked by: _____

Appendix 3 Food Frequency Questionnaire

RESPONDENT ID NUMBER

TODAY'S DATE

○ Jan ○ Feb ○ Mar ○ Apr ○ May ○ Jun ○ Jul ○ Aug ○ Sep ○ Oct ○ Nov ○ Dec

DAY

YEAR ○ 2004 ○ 2005 ○ 2006 ○ 2007 ○ 2008 ○ 2009 ○ 2010 ○ 2011 ○ 2012 ○ 2013 ○ 2014 ○ 2015 ○ 2016 ○ 2017 ○ 2018 ○ 2019 ○ 2020 ○ 2021 ○ 2022 ○ 2023

FOOD QUESTIONNAIRE

University of Saskatchewan

This form is about the foods you usually eat.
It will take about 30 - 40 minutes to complete.

- **Please answer each question as best you can. Estimate if you aren't sure.**
- **Use only a No. 2 pencil.**
- **Fill in the circles completely, and erase completely if you make any changes.**

Please print your name in this box.

SEX
○ Male
○ Female

AGE

WEIGHT pounds

HEIGHT ft. in.

First, a few general questions about what you eat.	AVERAGE USE IN THE PAST 6 MONTHS								
	LESS THAN ONCE per WEEK	1-2 per WEEK	3-4 per WEEK	5-6 per WEEK	1 per DAY	1 1/2 per DAY	2 per DAY	3 per DAY	4+ per DAY
About how many servings of vegetables do you eat, per day or per week, not counting salad or potatoes?	○	○	○	○	○	○	○	○	○
About how many servings of fruit do you eat, not counting juices?	○	○	○	○	○	○	○	○	○
How often do you eat cold cereal?	○	○	○	○	○	○	○	○	○
How often do you use fat or oil in cooking?	○	○	○	○	○	○	○	○	○

What kinds of fat or oil do you usually use in cooking? MARK ONLY ONE OR TWO

○ Don't know, or Pam
○ Stick margarine
○ Soft tub margarine
○ Butter
○ Butter/margarine blend
○ Low-fat margarine
○ Corn oil, vegetable oil
○ Olive oil or canola oil
○ Lard, fatback, bacon fat
○ Shortening

PLEASE DO NOT WRITE IN THIS AREA

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During the past 6 months, have you taken any vitamins or minerals regularly, at least once a month?

○ No, not regularly ○ Yes, fairly regularly →

(IF YES) WHAT DID YOU TAKE FAIRLY REGULARLY?

VITAMIN TYPE	HOW OFTEN					FOR HOW MANY MONTHS?					
	DIDN'T TAKE	A FEW DAYS per MONTH	1-3 DAYS per WEEK	4-6 DAYS per WEEK	EVERY DAY	LESS THAN 1 MONTH	1 MONTH	2 MONTHS	3-4 MONTHS	5-9 MONTHS	10+ MONTHS
Multiple Vitamins. Did you take...											
Regular Once-A-Day, Centrum, or Thera type	○	○	○	○	○	○	○	○	○	○	○
Stress-tabs or B-Complex type	○	○	○	○	○	○	○	○	○	○	○
Antioxidant combination type	○	○	○	○	○	○	○	○	○	○	○
Single Vitamins (not part of multiple vitamins)											
Vitamin A (not beta-carotene)	○	○	○	○	○	○	○	○	○	○	○
Beta-carotene	○	○	○	○	○	○	○	○	○	○	○
Vitamin C	○	○	○	○	○	○	○	○	○	○	○
Vitamin E	○	○	○	○	○	○	○	○	○	○	○
Folic acid, folate	○	○	○	○	○	○	○	○	○	○	○
Calcium	○	○	○	○	○	○	○	○	○	○	○
Vitamin D	○	○	○	○	○	○	○	○	○	○	○
Iron	○	○	○	○	○	○	○	○	○	○	○
Selenium	○	○	○	○	○	○	○	○	○	○	○

If you took Once-a-day, Centrum or Thera-type multiple vitamins, did you usually take types that ○ contain minerals, iron, zinc, etc. ○ do not contain minerals ○ don't know

If you took vitamin C or vitamin E:

How many milligrams of **vitamin C** did you usually take, on the days you took it?
○ 100 ○ 250 ○ 500 ○ 750 ○ 1000 ○ 1500 ○ 2000 ○ 3000+ ○ Don't know

How many IUs of **vitamin E** did you usually take, on the days you took it?
○ 100 ○ 200 ○ 300 ○ 400 ○ 600 ○ 800 ○ 1000 ○ 2000+ ○ Don't know

Did you take any of these supplements at least once a month?

○ Ginkgo ○ Ginseng ○ St. John's Wort ○ Kava Kava ○ Echinacea ○ Flax seed oil ○ Flax seeds
○ Glucosamine/Chondroitin ○ Something else ○ Didn't take these

---

The next section is about your <u>usual</u> eating habits in the past 6 months or so. This includes all meals or snacks, at home or in a restaurant or carry-out. There are two kinds of questions to answer for each food:

HOW OFTEN, on average, did you eat the food during the past 6 months?
*Please DO NOT SKIP any foods. Mark "Never" if you didn't eat it.

HOW MUCH did you usually eat of the food?

*Sometimes we ask how <u>many</u> you eat, such as 1 egg, 2 eggs, etc., ON THE DAYS YOU EAT IT.
*Sometimes we ask "how much" as A, B, C or D. LOOK AT THE ENCLOSED PICTURES. For each food, pick the picture (bowls or plates) that looks the most like the serving size you usually eat.
(If you don't have pictures: A=1/4 cup, B=1/2 cup, C=1 cup, D=2 cups.)
*Sometimes we made the "D" column a darker color. This is just to remind you to make sure you really eat that large a serving.

EXAMPLE: This person drank apple juice twice a week, and had one glass each time. Once a week he ate a "C" sized serving of rice (about 1 cup).

HOW OFTEN IN PAST 6 MONTHS	NEVER	A FEW TIMES per 6 MOS.	ONCE per MON.	2-3 TIMES per MON.	ONCE per WEEK	TWICE per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH EACH TIME SEE PORTION SIZE PICTURES FOR A-B-C-D				
Apple juice	○	○	○	○	○	●	○	○	○	How many glasses each time	● 1	○ 2	○ 3	○ 4
Rice	○	○	○	○	●	○	○	○	○	How much each time	○ A	○ B	● C	○ D

HOW OFTEN IN PAST 6 MONTHS	NEVER	A FEW TIMES per 6 MOS.	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK		HOW MUCH EACH TIME How many glasses on the days you drink it?
How often do you drink the following beverages?										
Tomato juice or V-8 juice	○	○	○	○	○	○	○	○	○	How many glasses **each time** ○1 ○2 ○3 ○4
Real 100% orange juice or grapefruit juice, including fresh, frozen or bottled	○	○	○	○	○	○	○	○	○	How many glasses **each time** ○1 ○2 ○3 ○4
Other real fruit juices like apple juice, prune juice, lemonade	○	○	○	○	○	○	○	○	○	How many glasses ○1 ○2 ○3 ○4
Hi-C, SoBe, or other drinks with added vitamin C	○	○	○	○	○	○	○	○	○	How many glasses ○1 ○2 ○3 ○4
Drinks with some juice in them, like Sunny Delight, Fruitopia, 5-Alive	○	○	○	○	○	○	○	○	○	How many bottles ○1 ○2 ○3 ○4
Instant breakfast milkshakes like Carnation, diet shakes like SlimFast, or liquid supplements like Ensure	○	○	○	○	○	○	○	○	○	How many glasses or cans ○1 ○2 ○3 ○4
Glasses of milk (any kind)	○	○	○	○	○	○	○	○	○	How many glasses ○1 ○2 ○3 ○4

When you drink orange juice, how often do you drink a calcium-fortified brand?
○ Usually calcium-fortified ○ I don't know
○ Sometimes calcium-fortified ○ I don't drink orange juice
○ Hardly ever calcium-fortified

When you drink glasses of milk, what kind do you usually drink? **MARK ONLY ONE:**
○ Whole milk ○ Reduced-fat 2% milk ○ Low-fat 1% milk ○ Non-fat milk
○ Rice milk ○ Soy milk ○ I don't drink milk or soy milk

HOW OFTEN IN PAST 6 MONTHS	NEVER	FEW/ 6 MOS.	ONCE/ MONTH	2-3 TIMES/ MONTH	ONCE/ WEEK	TWICE/ WEEK	3-4 TIMES/ WEEK	5-6 TIMES/ WEEK	EVERY DAY	HOW MUCH EACH TIME
Regular soft drinks, or bottled drinks like Snapple (not diet drinks)	○	○	○	○	○	○	○	○	○	How many bottles or cans ○1 ○2 ○3-4 ○5+
Beer or non-alcoholic beer	○	○	○	○	○	○	○	○	○	How many bottles or cans ○1 ○2 ○3-4 ○5+
Wine or wine coolers	○	○	○	○	○	○	○	○	○	How many glasses ○1 ○2 ○3-4 ○5+
Liquor or mixed drinks	○	○	○	○	○	○	○	○	○	How many drinks ○1 ○2 ○3-4 ○5+
Glasses of water, tap or bottled	○	○	○	○	○	○	○	○	○	How many glasses ○1 ○2 ○3-4 ○5+
Coffee, regular or decaf	○	○	○	○	○	○	○	○	○	How many cups ○1 ○2 ○3-4 ○5+
Tea or iced tea (not herb teas)	○	○	○	○	○	○	○	○	○	How many cups ○1 ○2 ○3-4 ○5+

What kind? **MARK ONLY ONE:** ○ Regular beer ○ Light beer ○ Non-alcoholic beer ○ I don't drink beer

What do you usually add to coffee? **MARK ONLY ONE:** ○ Cream or half & half ○ Nondairy creamer ○ Milk ○ None of these

What do you usually add to tea? **MARK ONLY ONE:** ○ Cream or half & half ○ Nondairy creamer ○ Milk ○ None of these

Do you usually add sugar (or honey) to coffee? ○ No ○ Yes IF YES, how many teaspoons each cup? ①②③④⑤

Do you usually add sugar (or honey) to tea? ○ No ○ Yes IF YES, how many teaspoons each cup? ①②③④⑤

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HOW OFTEN IN PAST 6 MONTHS	NEVER	A FEW TIMES per 6 MOS.	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH EACH TIME SEE PORTION SIZE PICTURES FOR A-B-C-D				
How often do you eat each of the following fruits, just during the 2-3 months when they are in season?														
Raw peaches, apricots, nectarines, while they are in season	○	○	○	○	○	○	○	○	○	How many each time	○ 1/2	○ 1	○ 2	○ 3
Cantaloupe, in season	○	○	○	○	○	○	○	○	○	How much	○ 1/8	○ 1/4	○ 1/2	○ 1
Strawberries, in season	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Watermelon, in season	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Any other fruit in season, like grapes, honeydew, pineapple, kiwi	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
How often do you eat the following foods all year round? Estimate your average for the whole year.														
Bananas	○	○	○	○	○	○	○	○	○	How many each time	○ 1/2	○ 1	○ 2	○ 3
Apples or pears	○	○	○	○	○	○	○	○	○	How many each time	○ 1/2	○ 1	○ 2	○ 3
Oranges or tangerines	○	○	○	○	○	○	○	○	○	How many each time	○ 1/2	○ 1	○ 2	○ 3
Grapefruit	○	○	○	○	○	○	○	○	○	How much	○ 1/2	○ 1	○ 2	○ 3
Canned fruit like applesauce, fruit cocktail, or dried fruit like raisins	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D

HOW OFTEN IN PAST 6 MONTHS	NEVER	FEW/ 6 MOS.	ONCE/ MONTH	2-3 TIMES/ MONTH	ONCE/ WEEK	TWICE/ WEEK	3-4 TIMES/ WEEK	5-6 TIMES/ WEEK	EVERY DAY	HOW MUCH EACH TIME				
Eggs, including egg biscuits or Egg McMuffins (Not egg substitutes)	○	○	○	○	○	○	○	○	○	How many eggs each time	○ 1	○ 2	○ 3	○ 4
Bacon	○	○	○	○	○	○	○	○	○	How many pieces	○ 1	○ 2	○ 3	○ 4
Breakfast sausage, including sausage biscuits	○	○	○	○	○	○	○	○	○	How many pieces	○ 1	○ 2	○ 3	○ 4
Pancakes, waffles, French toast, Pop Tarts	○	○	○	○	○	○	○	○	○	How many pieces	○ 1	○ 2	○ 3	○ 4
Breakfast bars, granola bars, Power bars	○	○	○	○	○	○	○	○	○	How many	○ 1	○ 2	○ 3	○ 4
Cooked cereals like oatmeal, cream of wheat or Red River Cereal	○	○	○	○	○	○	○	○	○	Which bowl		○ B	○ C	○ D
High-fiber cereals like All Bran, Raisin Bran, Fruit-n-Fiber	○	○	○	○	○	○	○	○	○	Which bowl		○ B	○ C	○ D

Which high-fiber cereal do you eat most often? **MARK ONLY ONE:** ○ All Bran or Bran Buds ○ Raisin Bran ○ Fiber One, Fruit-n-Fiber, etc. ○ Something else ○ I don't know ○ I don't eat it

Vector or Vive cereal	○	○	○	○	○	○	○	○	○	Which bowl		○ B	○ C	○ D
Any other cold cereal, like Corn Flakes, Cheerios, Special K	○	○	○	○	○	○	○	○	○	Which bowl		○ B	○ C	○ D
Milk or milk substitutes on cereal	○	○	○	○	○	○	○	○	○	How many oz. on cereal	○ 3 oz.	○ 4-5 oz.	○ 6-7 oz.	○ 8+ oz.
Yogurt or frozen yogurt	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Cheese, sliced cheese or cheese spread, including on sandwiches	○	○	○	○	○	○	○	○	○	How many slices	○ 1	○ 2	○ 3	○ 4

When you eat cheese, is it ○ Usually low-fat ○ Sometimes low-fat ○ Hardly ever low-fat ○ Don't know/don't eat

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HOW OFTEN IN PAST 6 MONTHS	NEVER	A FEW TIMES per 6 MOS.	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH EACH TIME SEE PORTION SIZE PICTURES FOR A-B-C-D				
How often do you eat the following vegetables, including fresh, frozen, canned or in stir-fry, at home or in a restaurant?														
Broccoli	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Carrots, or mixed vegetables or stews containing carrots	○	○	○	○	○	○	○	○	○	How much carrot	○ A	○ B	○ C	○ D
Corn	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Green beans or green peas	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Spinach	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Mustard greens, beet greens, collards	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
French fries, fried potatoes or hash browns	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
White potatoes not fried, incl. boiled, baked, mashed & potato salad	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Sweet potatoes, yams (Not in pie)	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Cole slaw, cabbage	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Green salad	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Raw tomatoes, including in salad	○	○	○	○	○	○	○	○	○	How much	○ 1/4	○ 1/2	○ 1	○ 2
Salad dressing	○	○	○	○	○	○	○	○	○	How many Tbsp.	○ 1	○ 2	○ 3	○ 4

Is your salad dressing ○ Usually low-fat ○ Sometimes low-fat ○ Hardly ever low-fat ○ Don't know/don't use

HOW OFTEN IN PAST 6 MONTHS	NEVER	FEW/ 6 MOS.	ONCE/ MONTH	2-3 TIMES/ MONTH	ONCE/ WEEK	TWICE/ WEEK	3-4 TIMES/ WEEK	5-6 TIMES/ WEEK	EVERY DAY	HOW MUCH EACH TIME				
Any other vegetable, like okra, squash, cooked green peppers	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Refried beans or bean burritos	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Chili with beans (with or without meat)	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Baked beans, chick peas, pintos, any other dried beans	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Vegetable stew	○	○	○	○	○	○	○	○	○	Which Bowl		○ B	○ C	○ D
Vegetable soup, vegetable beef, chicken vegetable, or tomato soup	○	○	○	○	○	○	○	○	○	Which Bowl		○ B	○ C	○ D
Split pea, bean or lentil soup	○	○	○	○	○	○	○	○	○	Which Bowl		○ B	○ C	○ D
Any other soup, like chicken noodle, chowder, mushroom, instant soups	○	○	○	○	○	○	○	○	○	Which Bowl		○ B	○ C	○ D
Spaghetti, lasagna or other pasta with tomato sauce	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Cheese dishes without tomato sauce, like macaroni and cheese	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Pizza, including carry-out	○	○	○	○	○	○	○	○	○	How many slices	○ 1	○ 2	○ 3	○ 4

HOW OFTEN IN PAST 6 MONTHS	NEVER	A FEW TIMES per 6 MOS.	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH EACH TIME SEE PORTION SIZE PICTURES FOR A-B-C-D				
Do you ever eat chicken, meat or fish? ○ Yes ○ No **IF NO, SKIP TO NEXT PAGE**														
Hamburgers, cheeseburgers, meat loaf, at home or in a restaurant	○	○	○	○	○	○	○	○	○	How much meat	○ 1/8 lb.	○ 1/4 lb.	○ 1/2 lb.	○ 3/4 lb.
Tacos, burritos, enchiladas, tamales, etc. with meat or chicken	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Beef steaks, roasts, pot roast, or in frozen dinners or sandwiches	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
How do you like beef cooked? ○ Rare ○ Medium ○ Well done ○ I don't eat beef														
Pork chops, pork roasts, or dinner ham	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
When you eat meat, do you ○ Avoid eating the fat ○ Sometimes eat the fat ○ Often eat the fat ○ I don't eat meat														
Veal, lamb or deer meat	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Ribs, spareribs	○	○	○	○	○	○	○	○	○	How many ribs	○ 3-4	○ 5-6	○ 7-8	○ 9+
Liver, including chicken livers or liverwurst	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Gizzard, pork neckbones, pigs feet, etc.	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Mixed dishes with beef or pork, like stew, corned beef hash, stuffed cabbage, meat dish with noodles	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Mixed dishes with chicken, like chicken casserole, chicken & noodles, pot pie or in stir-fry	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Fried chicken, at home or in a restaurant	○	○	○	○	○	○	○	○	○	# medium pieces	○ 1	○ 2	○ 3	○ 4
Chicken or turkey not fried, such as baked, grilled, or on sandwiches	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
When you eat chicken, do you ○ Avoid eating the skin ○ Sometimes eat the skin ○ Often eat the skin														

HOW OFTEN IN PAST 6 MONTHS	NEVER	FEW/ 6 MOS.	ONCE/ MONTH	2-3 TIMES/ MONTH	ONCE/ WEEK	TWICE/ WEEK	3-4 TIMES/ WEEK	5-6 TIMES/ WEEK	EVERY DAY	HOW MUCH EACH TIME				
Oysters	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Other shellfish like shrimp, scallops, crabs	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Tuna, tuna salad, tuna casserole	○	○	○	○	○	○	○	○	○	How much of the tuna	○ A	○ B	○ C	○ D
Fried fish or fish sandwich, at home or in a restaurant	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Other fish, not fried	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Hot dogs, or sausage like Polish, Italian or chorizos	○	○	○	○	○	○	○	○	○	How many	○ 1	○ 2	○ 3	○ 4
Are your hot dogs ○ Usually low-fat ○ Sometimes low-fat ○ Hardly ever low-fat ○ Don't know/don't eat them														
Boloney, sliced ham, turkey lunch meat, other lunch meat	○	○	○	○	○	○	○	○	○	How many slices	○ 1	○ 2	○ 3	○ 4
Are your lunch meats ○ Usually low-fat or turkey ○ Sometimes low-fat ○ Hardly ever low-fat														

HOW OFTEN IN PAST 6 MONTHS	NEVER	A FEW TIMES per 6 MOS.	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH EACH TIME — SEE PORTION SIZE PICTURES FOR A-B-C-D				
Noodles, macaroni, pasta salad	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Tofu, bean curd	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Meat substitutes, such as veggie burgers, Gardenburgers	○	○	○	○	○	○	○	○	○	How many patties	○ 1	○ 2	○ 3	○ 4
Chinese food, Thai or other Asian food, not counted above	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Snacks like potato chips, corn chips, popcorn (not pretzels)	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Are these snacks ○ Usually low-fat ○ Sometimes low-fat ○ Hardly ever low-fat ○ Don't know/don't eat														
HOW OFTEN IN PAST 6 MONTHS	**NEVER**	**FEW/ 6 MOS.**	**ONCE/ MONTH**	**2-3 TIMES/ MONTH**	**ONCE/ WEEK**	**TWICE/ WEEK**	**3-4 TIMES/ WEEK**	**5-6 TIMES/ WEEK**	**EVERY DAY**	**HOW MUCH EACH TIME**				
Peanuts, other nuts or seeds	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Crackers	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Doughnuts, Danish pastry	○	○	○	○	○	○	○	○	○	How many	○ 1	○ 2	○ 3	○ 4
Cake, sweet rolls, coffee cake	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Are they ○ Usually low-fat ○ Sometimes low-fat ○ Hardly ever low-fat ○ Don't know/don't eat														
Cookies	○	○	○	○	○	○	○	○	○	How many	○ 1-2	○ 3-5	○ 6-7	○ 8+
Are your cookies ○ Usually low-fat ○ Sometimes low-fat ○ Hardly ever low-fat ○ I don't know/don't eat														
Ice cream, ice milk, ice cream bars	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Is your ice cream ○ Usually low-fat ○ Sometimes low-fat ○ Hardly ever low-fat ○ I don't know/don't eat														
Pumpkin pie, sweet potato pie	○	○	○	○	○	○	○	○	○	How many slices	○ 1/2	○ 1	○ 2	○ 3
Any other pie or cobbler	○	○	○	○	○	○	○	○	○	How many slices	○ 1/2	○ 1	○ 2	○ 3
Chocolate candy, candy bars	○	○	○	○	○	○	○	○	○	How many bars	○ 1 small	○ 1 medium	○ 1 large	○ 2 large
Other candy, not chocolate, like hard candy, caramel, jelly beans	○	○	○	○	○	○	○	○	○	How many pieces	○ 1-2	○ 3-5	○ 6-7	○ 8+

PLEASE DO NOT WRITE IN THIS AREA

HOW OFTEN IN PAST 6 MONTHS	NEVER OR A FEW TIMES IN PAST 6 MONTHS	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	2+ TIMES per DAY	HOW MUCH EACH TIME SEE PORTION SIZE PICTURES FOR A-B-C-D				
Biscuits or muffins	○	○	○	○	○	○	○	○	○	How many **each time**	○ 1	○ 2	○ 3	○ 4
Rolls, hamburger buns, English muffins, bagels	○	○	○	○	○	○	○	○	○	How many **each time**	○ 1/2	○ 1	○ 2	○ 3
Dark bread like rye or whole wheat, including in sandwiches	○	○	○	○	○	○	○	○	○	How many slices **each time**	○ 1	○ 2	○ 3	○ 4
White bread or toast, including French, Italian, or in sandwiches	○	○	○	○	○	○	○	○	○	How many slices **each time**	○ 1	○ 2	○ 3	○ 4
Corn bread, corn muffins	○	○	○	○	○	○	○	○	○	How many pieces	○ 1	○ 2	○ 3	○ 4
Tortillas	○	○	○	○	○	○	○	○	○	How many **each time**	○ 1	○ 2	○ 3	○ 4
Rice, or dishes made with rice	○	○	○	○	○	○	○	○	○	How much	○ A	○ B	○ C	○ D
Margarine (not butter) on bread or on potatoes or vegetables, etc.	○	○	○	○	○	○	○	○	○	How many pats (tsp.)	○ 1	○ 2	○ 3	○ 4
Butter (not margarine) on bread or on potatoes or vegetables, etc.	○	○	○	○	○	○	○	○	○	How many pats (tsp.)	○ 1	○ 2	○ 3	○ 4
Gravy	○	○	○	○	○	○	○	○	○	How many Tbsp.	○ 1	○ 2	○ 3	○ 4
Peanut butter	○	○	○	○	○	○	○	○	○	How many Tbsp.	○ 1	○ 2	○ 3	○ 4
Jelly, jam, or syrup	○	○	○	○	○	○	○	○	○	How many Tbsp.	○ 1	○ 2	○ 3	○ 4
Mayonnaise, sandwich spreads	○	○	○	○	○	○	○	○	○	How many Tbsp.	○ 1	○ 2	○ 3	○ 4
Ketchup, salsa or chile peppers	○	○	○	○	○	○	○	○	○	How many Tbsp.	○ 1	○ 2	○ 3	○ 4
Mustard, soy sauce, steak sauce, barbecue sauce, other sauces	○	○	○	○	○	○	○	○	○	How many Tbsp.	○ 1	○ 2	○ 3	○ 4

A few final questions:

If you eat Vector or Vive cereal, is it usually ○ Vector ○ Vive ○ I don't eat these cereals

If you eat cooked cereal is it usually ○ Red River cereal ○ Other cooked cereal ○ I don't eat cooked cereal

Not including shellfish, tuna or any fried fish, what kind of fish do you usually eat?
○ Oily fish like salmon, arctic char, trout, sardine ○ Don't know/Not applicable
○ Cod, pickerel or other lean fish

If you eat ice cream, ice milk, or ice cream bars, is your ice cream
○ Usually cow milk ○ Usually soy milk ○ I don't eat ice cream

If you eat dark bread like whole wheat or rye does it
○ Always contain flax or rye ○ Sometimes contain flax or rye
○ Never contain flax or rye ○ Don't know/I don't eat it

If you drink fruit juice is it highly fortified Vita Burst
○ Always ○ Never ○ Sometimes ○ Don't know/I don't eat it

Did you use the pictures to choose your serving size on this form? ○ Yes ○ No ○ I didn't have any pictures.

Thank you very much for filling out this questionnaire. Please take a minute to go back and fill in anything you may have skipped.

PLEASE DO NOT WRITE IN THIS AREA

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Appendix 5 Transformed Bone Outcomes

Table 6: Mean, standard deviation (SD) of transformed bone properties and strength of DM1 and control groups. The significance of groups differences was set at $p<.05$.

	DM1		Control		
	Mean	SD	Mean	SD	*p*-value
Distal Radius					
ToC	1.861	0.08	1.856	0.08	0.791
ToD	2.454	0.06	2.457	0.05	0.775
ToA	2.407	0.08	2.399	0.08	0.629
TrC	1.753	0.12	1.754	0.11	0.979
TrD	2.393	0.05	2.402	0.04	0.358
TrA	2.36	0.10	2.352	0.09	0.693
BSIc	1.314	0.12	1.313	0.11	0.956
Radius Shaft					
ToA	2.074	0.08	2.094	0.08	0.239
CoC	1.819	0.07	1.815	0.06	0.778
CoD	2.955	0.04	2.921	0.04	<0.001
CoA	1.864	0.06	1.894	0.05	0.015
SSIp	2.293	0.09	2.273	0.09	0.310
Distal Tibia					
ToC	2.317	0.07	2.326	0.06	0.530
ToD	2.452	0.04	2.458	0.05	0.563
ToA	2.864	0.06	2.868	0.06	0.803
TrC	2.209	0.09	2.216	0.09	0.728
TrD	2.39	0.04	2.396	0.04	0.506
TrA	2.82	0.08	2.821	0.08	0.954
BSIc	1.769	0.10	1.784	0.10	0.471
Tibia Shaft					
ToA	2.662	0.06	2.687	0.06	0.065
CoC	2.34	0.05	2.365	0.05	0.035
CoD	2.969	0.02	2.95	0.03	<0.001
CoA	2.371	0.05	2.415	0.05	<0.001
SSIp	3.133	0.07	3.156	0.08	0.148

Appendix 5 Transformed Muscle Outcomes

Table 7: Mean, standard deviation (SD) of transformed muscle area and neuromuscular performance outcomes of DM1 and control groups. The significance of groups differences was set at $p<.05$.

	DM1		Control		
	Mean	SD	Mean	SD	*p*-value
Upper Body					
Forearm MuA	1.34	0.05	1.33	0.05	0.450
Maximal Grip Force	2.27	0.11	2.25	0.10	0.420
Maximal Push-up Force	2.28	0.09	2.24	0.09	0.045
Lower Body					
Lower Leg MuA	1.60	0.06	1.62	0.05	0.314
Countermovement Jump					
Vertical Force	2.89	0.05	2.92	0.05	0.038
Vertical Power	3.16	0.08	3.21	0.08	0.015
Vertical Impulse	1.91	0.07	1.94	0.05	0.169
Long Jump					
Vertical Force	2.89	0.05	2.90	0.05	0.603
Horizontal Force	2.45	0.08	2.43	0.08	0.392
Vertical Power	2.95	0.10	2.99	0.09	0.146
Horizontal Power	2.68	0.13	2.67	0.12	0.790
Vertical Impulse	1.71	0.09	1.73	0.09	0.505
Horizontal Impulse	1.92	0.06	1.91	0.05	0.374
Length	2.13	0.08	2.12	0.08	0.800