Adolescent fractures and vitamin D status: The Birth to Twenty cohort

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Declaration

I, Morakane Violet Selebeleng, student number: 1537907, declare that this research report is my own work. It it is being submitted for partial fulfillment in the degree of Master of Medicine in Paediatrics at the University of the Witwatersrand Johannesburg. It has not been submitted for any degree or examination at any other university.

Signature of candidate……  ……..

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A special thank you to my supervisors, Prof K Thandrayen and Prof J.M Pettifor, for their guidance and support and to the Birth to Twenty cohort.

Dedication

I dedicate this work to my beloved family… who have been my support system and to my departed loved ones…. May your light continue to shine brighter.

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Abbreviations

AAP	American Academy of Pediatrics
BH	Bone health sub-cohort
BMC-HA	Bone mineral content height adjusted
BMD-HA	Bone mineral density height adjusted
BMI	Body mass index
Bt20	Birth to twenty cohort
CI	Confidence interval (95%)
DXA	Dual energy X-ray absorptiometry
GH	Growth hormone
25(OH)D	25-hydroxyvitamin D
IOM	Institute of Medicine
IQR	Interquartile range
MET	Metabolic equivalent of task
PA	Physical activity
SD	Standard deviation
TBLH	Total body less head
OR	Odds ratio
WHO	World Health Organisation

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Abstract

Background: Fractures are common in childhood and previous studies on the Birth to twenty cohort (Bt20) found that adolescent males had higher fracture rates than the females and white males had the highest fracture rates. The association between fractures and 25-hydroxyvitamin-D (25(OH)D) levels has not been investigated in this cohort.

Objectives: The association between 25(OH)D levels at 10 years and fracture risk within the first 10 and 15 years of life was assessed.

Methods: Data analysis of the Bone Health sub-cohort of the Bt20 cohort (children born in 1990 and residing in Johannesburg) was done and children with 25(OH)D levels at 10 years were included in the study.

Body mass index (BMI), dual-energy X-ray absorptiometry scan measurements and physical activity (PA) scores performed at 10 and 15 years were converted to Z-scores. Fracture risk (within 10 and 15 years of life) and 25(OH)D were assessed in relation to the above measurements.

Results: Data from 385 children was analysed; 72% were black children, 58% were males. At 10 years, 13% of the children had fractured and by 15 years 21%. The median 25(OH)D level was 96.9 nmol/L (interquartile range 73.3;120.5) with insufficient levels (<50 nmol/L) in 6.5% and deficiency (<30 nmol/L) in 0.25%. No differences were noted in 25(OH)D levels between the fractured and non-fractured groups with only 4 of the 50 who fractured having insufficient levels. The levels were not influenced by gender, ethnicity, BMI or PA.

A greater proportion of white than black children fractured at both 10 (24% vs 9%; p<0.001) and 15 years of age (36% vs 15%; p<0.001). White children who fractured had higher formal PA

scores at 15 years compared to black children. An increase in lean mass was associated with increased fracture risk at 15 years (adjusted OR 1.23 (CI 1.00;1.51)).

Conclusion

The risk of fracturing in children is multifactorial and 25(OH)D levels did not affect risk of fracturing, although only a small proportion had low 25(OH)D levels. Higher lean mass increased the risk for fracturing at 15 years and white children were two times more likely to fracture compared to black children.

Introduction

Fractures are common in childhood with fracture rates peaking during adolescence, during the period of maximum growth velocity (11-15 years of age).[1] There is relative under-mineralization of the skeletal system during the pubertal growth spurt, rendering the adolescent child at a higher risk for fractures.[2] Thandrayen et al. reported adolescent fracture rates during the first 15 years of life as 18.5/1000 children/year in the Birth to Twenty (Bt20) cohort.[1] Similar rates between 12 to 36/1000/year have been reported by other studies.[3,4] Upper limb fractures are the most frequently reported fractures in children and adolescents.[1,5] In the Bt20 cohort, male South African children and adolescents were more likely to fracture than females (27.5% versus 16.3% respectively) and white South African children fractured more frequently than the black children (41.5% versus 19%).[1]

Many modifiable factors such as nutrition, physical activity (PA), sedentary lifestyle, body habitus, hormonal factors and sunlight exposure play a role in establishing the integrity of the skeletal system. The non-modifiable factors are genetics, sex and ethnicity.[2] The major contributors to skeletal integrity are calcium intake and vitamin D status. Skin exposure to ultraviolet B rays from the sun is the main contributor to vitamin D synthesis, with only small amounts found in foods such as oily fish. Sunlight exposure for 5-15 minutes to the arms and legs twice a week will produce about 3000 IU vitamin D. Dark skinned individuals require longer sun exposure compared to light skinned individuals and the use of sunscreen and long clothing also impedes vitamin D synthesis.[2]

The half-life of serum 25(OH)D is 3-4 weeks, and it is a good marker of vitamin D stores.[2] Vitamin D is important in intestinal absorption of calcium, mineral homeostasis and in the maintenance of skeletal integrity. Calcium plays a pivotal role in the mineralization of bone matrix and 99% of total body calcium is found within the skeletal system. Ninety per cent of the calcium ingested is

dependent on the active metabolite of vitamin D,1,25-dihydroxyvitamin-D, for absorption.[2,6] Despite its clear importance in bone accretion, a meta-analysis of randomized controlled trials in children reported that calcium supplementation increased the bone mineral content (BMC) by only 1.7% and had no effect on reduction of fracture risk.[7] The recommended daily calcium intake by the Institute of Medicine (IOM) depends on age: 0-6 months is 200 mg/day, 6-12 months is 260 mg/day, 1-3 years is 700 mg/day, 4-8 years is 1000 mg/day, 9-18 years is 1300 mg/day and the recommended intake of vitamin D for children under 1 year is 400 IU and for children between 1 year to 18 years of age is 600 IU/day.[8]

Poopedi et al. assessed 25(OH)D levels in the bone health (BH) sub-cohort of the Bt20.[6] Vitamin D deficiency (< 50 nmol/L) and insufficiency (50 – 75 nmol/L) were noted in 7% and 19% of the cohort.[6] There is no universal consensus on the definitions of vitamin D status. A level of ≥50 nmol/L has been reported to be sufficient by the American Academy of Pediatrics (AAP) (deficient levels ≤ 37.5 nmol/L),[9] Pediatric Endocrine Society (PES) (deficient levels <30 nmol/L)[9] and the IOM.[8] The global evidence-based consensus guidelines recommended that 25(OH)D levels of >50 nmol/L as being sufficient, 30 to 50 nmol/L as insufficient and <30 nmol/L as deficient.[10] A review by Green et al. looking at Africa and Middle East, also recommended the same defining levels as the AAP.[11] A sufficient level ≥50 nmol/L is the level at which adequate bone health is maintained in >97.5% of the general population.[10] Parathyroid hormone (PTH) starts to rise when 25(OH)D levels are below 30-34 nmol/L and the risk of developing nutritional rickets increases.[10,12] There's conflicting evidence regarding the effect of 25(OH)D levels on risk of fracture as some studies have not shown an association between 25(OH)D levels with fracture risk,[4,13] but Ryan et al. reported that children with forearm fractures were more likely to have deficient levels with OR 3.46 (CI 1.09; 10.94).[14] James et al. also found higher rates of deficiency in children with upper limb fractures.[15] Yang et al. did a systemic review of 23 studies and concluded that children with low vitamin D status were at a higher risk of fractures\.[16]

Osteogenesis and bone formation are stimulated by PA to the areas that are mechanically loaded during exercise and is associated with an increase in bone mineral density (BMD), therefore strengthening the skeletal system.[2,17] However, PA can have a contradictory effect and increase the risk of fracturing, due to increased mechanical forces applied to the skeletal system, which are related to the impact and intensity of the exercise, and which might influence oestrogen levels in those adolescents excessively exercising.[17,18] Children with forearm fractures have a high likelihood of being overweight and mechanisms hypothesized for this include poor motor co-ordination and relative weakness of the bone for patient's weight,[14] but this effect has not been universally observed.[15] Lower levels of 25(OH)D are also reported in children with increased adiposity due to sequestration in adipose tissues.[19]

The aim of this study was to determine the fracture risk in the first 10 and 15 years of life in relation to vitamin D status (at 10 years) in the BH sub-cohort as this has not been done before and to determine if there are other associated factors contributing to fracture risk. We hypothesize that due to the previously reported low prevalence of insufficient or deficient vitamin D status in Johannesburg, there may be no association with increased fracture risk in this population.

Methods

This is a retrospective, secondary analysis of the BH sub-cohort of the Bt20 cohort. The Bt20 cohort comprised of 3273 children born between April 23rd and June 8th in 1990 in public sector health services and living in Johannesburg and Soweto. The sub-cohort had a total of 475 children. The cohort and sub-cohort are described in detail by Richter et al.[20]

The total sample for our study comprised of 385 children who had 25(OH)D levels measured at 10 years, children without levels were excluded. The levels were measured and then categorized as follows: sufficient ≥50 nmol/L, insufficient 30-50 nmol/L, and deficient <30 nmol/L. Blood sampling and analysis are explained in detail by Poopedi et al.[6] Fractures were self-reported by means of skeletal diagram retrospectively with/without radiological confirmation and children were classified as white/black if both parents were white/black respectively.[17]

Body mass index (BMI) z-scores were classified according to the World health organization (WHO) criteria as follows: severe thinness ≤ -3 z-score, thinness ≤ -2 z-score, normal between +1 and -2 z-score, overweight ≥ +1 z-score, obesity ≥ +2 z-score, and severe obesity ≥ +3 z-score.[21] The WHO software Anthroplus was used to convert BMI values into age related Z scores.

Metabolic equivalent of task (MET) score was used to measure PA scores. Questionnaires quantifying PA for the previous 12 months were administered at 10 and 15 years via interview. The score assessed intensity, duration and frequency of all formal and informal activity and sedentary lifestyle. The PA was scored in minutes/week multiplied by MET of a task. One MET is defined as energy expenditure for sitting quietly (1kcal/kg/hour). MET scores were converted to Z-scores and compared between fractured and non-fractured groups at 10 and 15 years. Black females who did not sustain any fractures were used as controls to create MET Z-scores and whole-body subtotal lean and fat mass for the other groups.

Dual energy X-ray absorptiometry (DXA) scan (Hologic Inc, Marlborough, Mass, USA) measurements (BMC, BMD) were calculated for the total hip, 1/3 radius, lumbar spine as well as total body less head (TBLH) at 10 and 15 years of age. The values were then converted to Z-scores and compared between the fractured and non-fractured group. BMD and BMC height adjusted (HA) Z-scores were used for the bone measurements and calculated using the

Paediatric bone density calculator (https://zscore.research.chop.edu) and based on white Caucasian children; as the South African black population have similar bone measurements to the reference population.

Statistical analysis

Data were entered into a Microsoft Excel spreadsheet and statistical analyses were performed using Stata version 17. Frequencies and percentages were used to describe categorical variables. Continuous variables were analysed as Z scores, therefore means and standard deviation (SD) were used to summarise data. Medians and interquartile ranges (IQR) were used to summarise 25(OH)D levels as it was not normally distributed.

The chi-squared test was used to assess associations between categorical variables. The two-sample unpaired t-test was used to compare the means between two groups. Non-parametric Wilcoxon rank sum test was used for comparisons of 25(OH)D levels between different groups. A p-value less than 0.05 was regarded as significant.

Univariate (unadjusted) and multivariate (adjusted) logistic regression analyses were done to find factors associated with fracturing at 10 and 15 years. A stepwise regression was used in the multivariate analysis for variable selection and only variables with p-value < 0.1 were entered into the model. Odds ratios (OR) were used to measure the effect size at 95% confidence intervals (CI), where values less than 1 were protective against fracturing and more than 1 increased the risk of fracturing.

Results

There was a total of 385 children who met the inclusion criteria and the characteristics of the children in the cohort are listed in Table I. Reflecting population demographics, black children

constituted the majority of 72% (n=279/385) while white children made up 28% (n=106/385). There were 225 males (58%) and 160 (42%) females in the cohort. At 10 years of age, 13% (n=50/385) of the children had fractured and 21% (n=80/385) by 15 years of age. Eighteen percent (n=9/50) of the children who had fractured by 10 years had multiple fractures by 15 years, making up 2.3% (n=9/385) of our cohort. The median 25(OH)D level at 10 years of age was 96.9 nmol/L (IQR 73.3;120.5). Levels were sufficient in 93.2% (n=359/385) of the sub-cohort, insufficient in 6.5% (n=25/385) and deficient in 0.25% (n=1/385) of the children. The one child with deficient level was included in the insufficient group for analysis. Ninety two percent (n=46/50) of the children who fractured by 10 years, had sufficient levels while 8% (n=4/50) had insufficient levels. In the group that did not fracture, 93% (n=313/335) had sufficient levels and 7% (n=22/335) had insufficient levels. By 15 years, 80 children had fractured and 93% (n=74/80) had sufficient levels and 7% (n=6/80) had insufficient levels measured at 10 years. No differences were noted in vitamin D status between the fractured and non-fractured groups at 10 years (p= 0.71) or at 15 years of age (p=0.77) (Table II). Vitamin D status was not influenced by sex, age, ethnicity, BMI Z-scores and PA scores. There were no differences in DXA measurements between those with sufficient and insufficient 25(OH)D levels at 10 years and 15 years (Table III).

White children were noted to fracture more frequently than black children by 10 years (24% vs 9%, $p<0.001$) and 15 years (36% vs 15%, $p<0.001$). There were no significant differences in fracture risk between boys and girls at 10 years (p=0.39) or 15 years of age (p=0.95). White males had more fractures 22% (n=20/89) than black males 9% (n=12/136) by 10 years (p=0.004). White females had more fractures by 10 years, 29% (n=5/17) compared to black females 9% (n=13/143) (p=0.012) but there was poor representation of white females, 4% (n=17/385) in the group. A similar pattern was noted at 15 years (Table IV).

Children who fractured at 10 years had significantly lower BMD-HA Z-scores when compared to the non-fractured group at the total hip (p=0.002), lumbar spine (p= 0.034), 1/3 radius (p=0.011) and total body less head (p=0.001). At 15 years, BMD-HA Z-score differences were also noted at the total hip (p=0.003), lumbar spine (p=0.016) and TBLH (p=0.028) between the two groups. Significant differences were also noted in BMC-HA Z-scores at 10 and 15 years with those who fractured having lower BMC-HA Z-scores (Table II). Whole-body subtotal lean mass Z-scores were higher in those who fractured (1.17 (±1.51) vs 0.77 (±1.37); p=0.042) compared to the non-fractured group. White children who fractured had higher whole-body subtotal lean mass Z-scores (0.82 (±1.18) vs -0.30 (±0.46); p<0.001) at 10 years and at 15 years (1.93 (±1.73) vs 0.78 (±1.25); p=0.004) compared to black children who fractured (Table V). White children who fractured at 10 years, had lower hip BMD-HA Z-scores compared to their black counterparts (-1.03 (±0.84) vs -0.15 (±0.66); p=0.002) but had higher 1/3 radius BMC-HA Z-score at 15 years (0.02 (±0.68) vs -0.92 (±1.37); p=0.015). Higher whole-body subtotal lean mass Z-scores were noted in white children who fractured when compared to black children who fractured (Table V).

No differences were noted in physical activity (MET Z-scores) and whole-body subtotal fat mass Z-score measurements between the fractured and non-fractured groups at 10 and 15 years. Black children who fractured at 10 years had higher total and informal MET Z-scores compared to white children who fractured. White children who fractured had significantly higher formal MET Z-scores (1.38 (±1.87) vs 0.46 (±1.26); p=0.03) at 15 years when compared to blacks. No differences in BMI Z-scores were noted between the white and black children who fractured at 10 and 15 years (Table V).

The univariate logistic regression results at 10 and 15 years are listed in table VI. Multivariate logistic regression analysis showed that white children had higher risk of fracturing at 10 years of age (OR 2.72 (CI 1.25; 5.94); p=0.012) and at 15 years (OR 2.33 (CI 1.21; 4.47); p=0.01) compared to black children and that an increase in whole-body subtotal lean mass Z-score is also

associated with higher risk of fracturing (OR 1.23 (CI 1.00; 1.51); p=0.04). Factors shown to be protective against fracture risk in the multivariate logistic regression analysis at 10 years of age were a higher TBLH BMD-HA Z-score (OR 0.55 (CI 0.34; 0.88); p=0.015) and at 15 years a higher TBLH BMC-HA Z-score (OR 0.56 (CI 0.39; 0.89); p=0.003).

Discussion

The association between 25(OH)D levels and fracture risk has not been studied in the Bt20 cohort previously. Most studies reviewed originated from Europe or North America (high income countries) and used higher cut-offs for deficiency (<50 nmol/L) with prevalence rates between 12% and 24%.[4,13,15] Studies by Ceroni et al. and Contreras et al. did not show any association between Vitamin D and fracture risk.[4,13] The prevalence of vitamin D deficiency in our study was 0.25% and insufficiency was 6.5%, which are similar to a recent large study comprising of 4509 children from five African countries, including South Africa, where 0.6% of the children had levels <30 nmol/L and 7.8 % had levels <50 nmol/L.[22] Johannesburg has ample sunshine throughout the year and children can replenish their vitamin D stores from sun exposure and this probably explains the low prevalence of insufficient or deficient vitamin D status in the cohort.

Individuals with dark skin require longer exposure in the sun to get enough vitamin D,[2] however our study showed no differences in the incidence of Vitamin D insufficiency between black and white children who fractured (7.5% vs 6.5%, p=0.70). An analysis by Poopedi et al. on the same cohort found that white children had significantly higher levels of 25(OH)D than black children in autumn (p=0.01) and summer (p=0.0001).[6] These findings are supported by James et al. in North Carolina, who reported white children to have higher levels compared to black and hispanic children (p<0.001) as well as higher levels during autumn and summer.[15] High levels of 25(OH)D

do not seem to be protective against fracturing as white males in our study, fractured more than blacks. Wren et al. and Clark et al. have also reported higher rates of fracturing in white children compared to other races.[23,24]

Our study showed no differences in 25(OH)D levels between the different BMI z-score categories, between black and white children or in those who fractured. Ryan et al. in a case control study compared fractured and non-fractured black African American children, found an increased risk of fractures amongst those with higher BMI (49.3% vs 31.4%; p=0.03).[14] Wren et al. and Lyon did not support these findings.[24,25] Thandrayen et al. had larger numbers in the original cohort (n=533) and showed that white girls who fractured had higher BMI and similar findings have been noted by Goulding et al.[17,26]

Osteogenesis is stimulated by PA and it strengthens the skeletal system but this benefit may be outweighed by the increased fracture risk associated with vigorous exercise.[18,23] A prospective study in the UK by Clark et al. showed that high impact PA is associated with a higher fracture risk (OR 2.06 (95% CI 1.39; 3.05).[27] Participation in formal sports by white children at 15 years increased their fracture risk, they had higher formal MET Z-scores compared to black children in our study with a mean 1.38 (±1.87) vs 0.46 (±1.2); (p=0.03). Black children who fractured were noted to have significantly higher informal (p=0.009) and total (p=0.014) MET Z-scores compared to the white children. This could be due to over-estimation of time spent with informal activities or that informal activity is associated with less vigorous PA and therefore no increase in fracture risk as white children had higher risk of fracturing overall.

A previous fracture in childhood doubles the risk for further fractures and studies report that 1.9% to 2.7% of children will experience multiple fractures, a similar rate was noted in our study of 2.3%.[24,25,28] Clark et al. reviewed 6000 participants and reported that low bone mass is associated with an increased fracture risk (OR 1.89 (CI 1.18- 3.04); p=0.009).[29] This was also observed in our study as children who fractured at 10 years had significantly lower BMD-HA Z-scores (all

sites) and lower BMD-HA Z-scores at all sites except 1/3 radius at 15 years when compared with non-fractured group. Black children who fractured in our study had higher hip BMD-HA Z-scores than white children who fractured. Another study reported that Hispanic and African American children had higher BMD at the radius and tibia compared to white children, but fracture risk was not assessed.[30]

The small sample size of the BH sub-cohort and thus the small number of fractures sustained are limitations to adequately answering the research question. Further, the small number of children with a insufficient vitamin D status makes it difficult to assess the role of vitamin D insufficiency or deficiency on fracture risk. A larger sample size is needed to make meaningful comparisons and to assess the effect of 25(OH)D levels on fracture risk.

Conclusion

The factors associated with an increased fracture risk are multifactorial. Most children (93%) had sufficient levels >50nmol/L with only 6.5% with insufficiency (<30nmol/l). Insufficient vitamin D status was not associated with increased fracture risk in the BH sub-cohort. White children were two times more likely to fracture compared to black children and noted to have higher lean mass with an associated increased fracture risk. Due to abundant sunshine in Johannesburg and the low prevalence of deficient and insufficient 25(OH)D levels, routine testing and supplementation of Vitamin D is not necessary for the general population except where it is clinically indicated.

Ethics

Ethical approval was obtained from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg, before commencement of the study. The ethics clearance number is M 2211118.

Permission was requested and granted by the guardian of the database.

Conflict of interest

The author declares that there are no conflicts of interest that are directly or indirectly related to the research.

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Author Contributions

The first author contributed to the writing of manuscript, data analysis and reporting. The two senior authors equally contributed as above and additionally assisted with the conceptualization of the research question and supervision.

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Tables

Table I. Characteristics of the Bone Health sub-cohort at 10 and 15 years (males and females have been combined)

Characteristic	**10 YEARS N=385 n (%)**	**15 YEARS N=385 n (%)**
Vitamin D category		
Deficient	1 (0.25)	
Insufficient	25 (6.5)	
Sufficient	359 (93.2)	
BMI z-score category		
Severe thinness ≤ -3	0 (0)	6 (2)
Thinness ≤ -2	0 (0)	7 (2)
Normal >-2<+1	256 (66)	266 (69)
Overweight ≥ +1	22 (6)	45 (11)
Obese ≥ +2	7 (2)	12 (3)
Severe Obesity ≥ +3	7 (2)	4 (1)
Missing	93 (24)	45 (12)
	Mean (±SD)	**Mean (±SD)**
MET Z-score		
Total	-0.01 (0.98)	0.32 (1.34)
Formal	0.73 (2.81)	0.55 (1.60)
Informal	-0.11 (0.91)	0.09 (1.17)
BMC-HA Z-score		
TBLH	-0.69 (0.96)	0.30 (0.82)
Total hip	-0.64 (0.88)	-0.02 (0.97)
Lumbar spine	0.03 (0.87)	0.11 (1.33)
1/3 Radius	-0.21 (0.89)	0.21 (1.00)
BMD-HA Z-scores		
TBLH	-0.13 (0.82)	0.09 (0.98)
Total hip	-0.12 (1.23)	0.07 (1.05)
Lumbar spine	-0.52 (0.93)	-0.05 (1.76)
1/3 Radius	-1.01 (1.02)	-0.36 (1.22)
Whole-body subtotal Z-score		
Fat mass	-0.26 (0.90)	-0.50 (1.06)
Lean mass	0.09 (0.91)	0.84 (1.40)

BMC-HA=bone mineral content height adjusted,
BMD-HA=bone mineral density height adjusted,
MET=metabolic equivalent of task,
TBLH=total body less head

Table II. Association between different exposures and fracture risk at 10 and 15 years (males and females have been combined)

Characteristics	**Fracture at 10 years**		**p-value**	**Fracture at 15 years**		**p-value**
	No n=335 n (%)	**Yes n=50 n (%)**		**No n=305 n (%)**	**Yes n=80 n (%)**	
Vitamin D category			0.706			0.765
Insufficient/Deficient	22 (7)	4 (8)		20 (7)	6 (7)	
Sufficient	313 (93)	46 (92)		285 (93)	74 (93)	
BMI z- score category			0.863			0.685
Thinness and severe thinness ≤ -2	0 (0)	0 (0)		10 (3)	3 (4)	
Normal >-2<+1	225 (67)	31 (62)		216 (71)	50 (63)	
Overweight and obese ≥ +1	32 (10)	4 (8)		52 (17)	9 (11)	
Missing	78 (23)	15 (30)		27 (9)	18 (22)	
	Mean (±SD)	**Mean (±SD)**		**Mean (±SD)**	**Mean (±SD)**	
MET Z-score						
Total	0.02 (1.00)	-0.23 (0.79)	0.143	0.30 (1.38)	0.45 (1.17)	0.434
Formal	0.73 (2.94)	0.71 (1.59)	0.969	0.50 (1.61)	0.76 (1.53)	0.271
Informal	-0.08 (0.92)	-0.33 (0.80)	0.114	0.08 (1.22)	0.14 (0.98)	0.753
BMC-HA Z-score						
TBLH	-0.66 (0.97)	-0.98 (0.77)	0.062	*0.37 (0.79)*	*0.01 (0.88)*	*0.002**
Total hip	*-0.59 (0.89)*	*-0.94 (0.67)*	*0.029**	-0.01 (0.97)	-0.05 (0.96)	0.769
Lumbar spine	*0.07 (0.88)*	*-0.27 (0.74)*	*0.029**	*0.19 (1.33)*	*-0.27 (1.27)*	*0.015**
1/3 Radius	*-0.16 (0.89)*	*-0.55 (0.80)*	*0.015**	0.24 (0.98)	0.07 (1.11)	0.264
BMD-HA Z-scores						
TBLH	*-0.07 (0.81)*	*-0.54 (0.78)*	*0.001**	*0.15 (0.94)*	*-0.16 (1.12)*	*0.028**
Total hip	*0.20 (1.26)*	*-0.48 (0.84)*	*0.002**	*0.15 (1.04)*	*-0.30 (1.05)*	*0.003**
Lumbar spine	*-0.48 (0.95)*	*-0.84 (0.77)*	*0.034**	*0.06 (1.75)*	*-0.55 (1.74)*	*0.016**
1/3 Radius	*-0.96 (1.00)*	*-1.42 (1.04)*	*0.011**	-0.30 (1.21)	-0.65 (1.28)	0.058
Whole-body subtotal Z-score						
Fat mass	-0.26 (0.92)	-0.23 (0.74)	0.848	-0.47(1.08)	-0.62 (0.95)	0.319
Lean mass	0.08 (0.90)	0.11 (0.96)	0.856	*0.77 (1.37)*	*1.17 (1.51)*	*0.042**

BMC HA=bone mineral content height adjusted, BMD HA=bone mineral density height adjusted, MET=metabolic equivalent of task, SD=standard deviation, TBLH=total body less head

Table III. Association between vitamin D levels at 10 years and different exposures at 10 and 15 years of age (males and females combined)

Characteristics	**YEAR 10 N=385**		**p-value**	**YEAR 15 N=385**		**p-value**
	Insufficient n=26	**Sufficient n=359**		**Insufficient n = 26**	**Sufficient n=359**	
	Mean (±SD)	**Mean (±SD)**		**Mean (±SD)**	**Mean (±SD)**	
Ethnicity			0.702			
Black [n=279] n (%)	18 (6.5)	261 (93.5)				
White [n=106] n (%)	8 (7.5)	98 (92.5)				
MET Z-score						
Total	0.13 (0.82)	-0.02 (0.99)	0.503	0.51 (2.01)	0.31 (1.28)	0.494
Formal	0.79 (1.98)	0.72 (2.86)	0.911	0.81 (2.25)	0.53 (1.54)	0.419
Informal	0.02 (0.73)	-0.12 (0.92)	0.498	0.10 (1.28)	0.09 (1.17)	0.973
BMC-HA Z-score						
TBLH	-0.52 (0.72)	-0.71 (0.97)	0.430	0.38 (0.80)	0.30 (0.82)	0.626
Total hip	-0.25 (0.78)	-0.66 (0.88)	0.061	0.12 (0.89)	-0.03 (0.98)	0.496
Lumbar spine	0.28 (0.86)	0.02 (0.87)	0.222	-0.15 (1.53)	0.13 (1.31)	0.342
1/3 Radius	0.00 (0.99)	-0.22 (0.88)	0.308	0.37 (1.26)	0.20 (0.98)	0.459
BMD-HA Z-score						
TBLH	0.08 (0.70)	-0.14 (0.83)	0.283	0.13 (0.91)	0.09 (0.99)	0.841
Total hip	0.24 (1.08)	0.11 (1.24)	0.678	0.05 (0.88)	0.07 (1.06)	0.923
Lumbar spine	-0.33 (0.82)	-0.54 (0.94)	0.378	-0.32 (1.78)	-0.03 (1.76)	0.452
1/3 Radius	-1.04 (0.91)	-1.01 (1.02)	0.927	-0.37 (1.02)	-0.36 (1.24)	0.978
Whole-body subtotal Z-score						
Fat mass	-0.33 (0.90)	-0.25 (0.90)	0.747	-0.59 (0.82)	-0.49 (1.07)	0.646
Lean mass	0.15 (0.93)	0.08 (0.91)	0.765	0.85 (1.29)	0.84 (1.41)	0.971

BMC HA=bone mineral content height adjusted, BMD HA= bone mineral density height adjusted, MET=metabolic equivalent of task, SD=standard deviation, TBLH=total body less head

Table IV. Fractures by gender and ethnicity at 10 and 15 years

	Total male N=225 n (%)	**Total female N=160 n (%)**	**p-value**	**White male n (%) 89 (23)**	**Black male n (%) 136 (35)**	**p-value**	**White female n (%) 17 (4)**	**Black female n (%) 143 (37)**	**p-value**	**Total N (%) 385 (100)**
YEAR 10			0.393			*0.004**			*0.012**	
Fracture	32 (14)	18 (11)		20 (22)	12 (9)		5 (29)	13 (9)		50 (13)
No fracture	193 (86)	142 (89)		69 (78)	124 (91)		12 (71)	130 (91)		335 (87)
YEAR 15			0.950			*<0.001**			*0.027**	
Fracture	47 (21)	33 (21)		31 (35)	16 (12)		7 (41)	26 (18)		80 (21)
No fracture	178 (79)	127 (79)		58 (65)	120 (88)		10 (59)	117 (82)		305 (79)

Table V. Characteristics of the children who fractured from birth to 10 and 15 years based on ethnicity and comparing between the different exposures.

Characteristics	**Year 10 Fractures (N=50)**		**p-value**	**Year 15 Fractures (N=80)**		**p-value**
	Black = 25 n (%)	**White = 25 n (%)**		**Black = 42 n (%)**	**White = 38 n (%)**	
Sex			*0.018**			*<0.001**
Male	12 (48)	20 (80)		16 (39)	31 (83)	
Female	13 (52)	5 (20)		26 (61)	7 (17)	
Vitamin D			1			0.899
Insufficient/Deficient	2 (8)	2 (8)		3 (7)	3 (8)	
Sufficient	23 (92)	23 (92)		39 (93)	35 (92)	
BMI z-score category			0.572			0.056
Thinness and severe thinness ≤ -2	0 (0)	0 (0)		2 (5)	1 (3)	
Normal > -2 < +1	20 (80)	11 (44)		37 (88)	13 (34)	
Overweight and obese ≥ +1	2 (8)	2 (8)		3 (7)	6 (16)	
Missing	3 (12)	12 (48)		0 (0)	18 (47)	
MET Z-score	**Mean (±SD)**	**Mean (±SD)**		**Mean (±SD)**	**Mean (±SD)**	
Total	*-0.00 (0.77)*	*-0.65 (0.66)*	*0.014**	0.33 (1.14)	0.70 (1.22)	0.251
Formal	0.40 (1.59)	1.29 (1.47)	0.102	*0.46 (1.26)*	*1.38 (1.87)*	*0.030**
Informal	*-0.09 (0.77)*	*-0.78 (0.65)*	*0.009**	0.15 (1.05)	0.10 (0.82)	0.859
BMC-HA Z-score						
TBLH	-1.07 (0.67)	-0.82 (0.92)	0.364	-0.06 (0.94)	0.14 (0.76)	0.430
Total hip	-0.93 (0.60)	-0.96 (0.81)	0.894	-0.08 (0.95)	-0.00 (1.01)	0.770
Lumbar spine	-0.15 (0.55)	-0.47 (0.98)	0.227	-0.40 (1.31)	0.00 (1.17)	0.255
1/3 Radius	-0.60 (0.86)	-0.47 (0.72)	0.635	0.04 (1.24)	0.14 (0.73)	0.771
BMD-HA Z-scores						
TBLH	-0.39 (0.66)	-0.80 (0.92)	0.132	-0.24 (1.17)	0.01 (1.00)	0.412
Total hip	*-0.15 (0.66)*	*-1.03 (0.84)*	*0.002**	-0.26 (1.06)	-0.39 (1.03)	0.657
Lumbar spine	-0.89 (0.69)	-0.75 (0.91)	0.620	-0.81 (1.78)	-0.01 (1.55)	0.101
1/3 Radius	-1.43 (1.13)	-1.41 (0.90)	0.966	*-0.92 (1.37)*	*0.02 (0.68)*	*0.015**
Whole-body subtotal Z-score						
Fat mass	-0.38 (0.53)	0.03 (0.97)	0.112	-0.73 (0.85)	-0.40 (1.13)	0.198
Lean mass	*-0.30 (0.46)*	*0.82 (1.18)*	*<0.001**	*0.78 (1.25)*	*1.93 (1.73)*	*0.004**

BMC HA=bone mineral content height adjusted, BMD HA= bone mineral density height adjusted, MET=metabolic equivalent of task, SD=standard deviation, TBLH=total body less head

Table VI. Univariate logistic regression on fracture risk in the first 10 and 15 years of life

Characteristics	**10 YEARS**		**15 YEARS**	
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**
Sex				
Male vs Female [a]	1.35 (0.73; 2.5)	0.33	0.98 (0.60; 1.60)	0.95
Ethnicity				
White vs Black [a]	*3.3 (1.81; 6.04)*	*<0.001**	*3.15 (1.88; 5.28)*	*<0.001**
Vitamin status				
Insufficient/Deficient vs Sufficient [a]	1.2 (0.40; 3.66)	0.740	1.16 (0.45; 2.98)	0.48
MET Z-score				
Total	0.71 (0.47; 1.08)	0.11	1.08 (0.89; 1.31)	0.44
Formal	1.00 (0.88; 1.13)	0.961	1.09 (0.93; 1.28)	0.27
Informal	0.67 (0.43; 1.06)	0.08	1.04 (0.83; 1.30)	0.75
BMC-HA Z-score				
TBLH	0.70 (0.49; 1.01)	0.06	*0.58 (0.41; 0.82)*	*0.002**
Total hip	*0.61 (0.41; 0.92)*	*0.018**	1.03 (0.92; 1.14)	0.63
Lumbar spine	*0.62 (0.41; 0.99)*	*0.04**	0.97 (0.89; 1.05)	0.44
1/3 Radius	*0.56 (0.37; 0.85)*	*0.007**	0.84 (0.62; 1.14)	0.56
BMD-HA Z-score				
TBLH	*0.48 (0.30; 0.77)*	*0.002**	*0.72 (0.54; 0.97)*	*0.03**
Total hip	*0.62 (0.46; 0.85)*	*0.003**	*0.66 (0.50; 0.87)*	*0.004**
Lumbar spine	*0.76 (0.59; 0.98)*	*0.034**	*0.82 (0.70; 0.94)*	*0.017**
1/3 Radius	*0.61 (0.43; 0.86)*	*0.005**	*0.80 (0.63; 1.01)*	0.06
Whole-body subtotal Z-score				
Lean mass	1.01 (0.70; 1.48)	0.95	*1.21 (1.01; 1.47)*	*0.044**
Fat mass	1.04 (0.72; 1.52)	0.82	0.86 (0.66; 1.14)	0.32

[a] = reference category

BMC-HA=bone mineral content height adjusted, BMD-HA= bone mineral density height adjusted, CI=confidence interval, MET=metabolic equivalent of task, OR=odds ratio, TBLH=total body less head

Table VII. Multivariate (adjusted) logistic regression analysis on fracture risk in the first 10 and 15 years of life.

Characteristics	**10 YEARS**		**15 YEARS**	
	Adjusted OR (95% CI)	**p-value**	**Adjusted OR (95% CI)**	**p-value**
Ethnicity				
White vs Black [a]	*2.72 (1.25; 5.94)*	*0.012**	*2.33 (1.21; 4.47)*	*0.01**
TBLH BMD-HA Z-scores	*0.55 (0.34; 0.88)*	*0.015**		
TBLH BMC-HA Z-scores			*0.56 (0.39; 0.89)*	*0.003**
Whole-body subtotal Lean mass Z-scores			*1.23 (1.00; 1.51)*	*0.04**

[a]=reference category
BMC-HA=bone mineral content height adjusted, BMD-HA=bone mineral density height adjusted, CI=confidence interval, OR=odds ratio

Appendix 1 Approved research protocol

Adolescent fractures and vitamin D status: The Birth to Twenty cohort (Bt20).

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1. Introduction

Fractures in adolescents have been on a steady increase over the last 30 years in the USA (1). This obviously has a tremendous impact on financial implications for hospitals and Departments of Health, especially in developing countries such as South Africa, given the financial challenges that these countries face.

Fractures are common in childhood and the most common site of fracture in adolescents is the upper limb (1, 2). A South African study by Thandrayen et al, indicated upper limb fractures to be the most frequent with rates as high as 57% of all fractures in children (3).

Fracture rates peak in adolescents around the period of maximal growth velocity between ages of 11 to 15 years (3). There is relative under mineralization of skeleton during the pubertal growth spurt rendering the adolescent child at a higher risk for fractures (4).

In a study by Ceroni et al, vitamin D insufficiency was shown to be prevalent in adolescents irrespective of presence or absence of fractures (5).

Factors affecting vitamin D status and bone mineralization

Bone consists of a non-mineralized matrix of collagen and non-collagenous proteins. The structure is then mineralized and strengthened as calcium and phosphate are deposited. The period of maximal bone mineralization and bone mass accretion is in adolescence and this is the period where bone formation exceeds bone resorption (4). It is estimated that 40-60% of adult bone mass is accrued during adolescence, with 25% of that bone mass being acquired during the 2 year period of peak height growth (6).

There are a number of factors that affect bone mineral density. These are non-modifiable factors such as genetics, gender and ethnicity. The modifiable factors are nutrition, physical activity or exercise, sedentary lifestyle, body habitus, sunlight exposure and hormonal factors (4).

Nutrition

1. Calcium

Calcium is important in bone accretion and about 99% of calcium is found within the skeleton. The main source of calcium is diet and about 90% of dietary calcium is dependent on vitamin D for absorption The majority of calcium ingested is actively absorbed and this process is mediated by 1,25 dihydroxyvitamin D(1,25$[OH]_2$-D) (4, 7).

Calcium plays a significant role in maintaining and regulating physiological processes such as bone mineralization, muscle contraction and nerve conduction.

Milk is a major source of calcium with 250ml of milk providing about 300mg of calcium. Other sources include cheese, yoghurt, green leafy vegetables, legumes, nuts and calcium fortified cereals and juice (4).

The Institute of Medicine published updated recommended dietary allowances (RDA) for healthy normal children and these were endorsed by the American Academy of Paediatrics, see the table below (8):

Age	Calcium (mg/day)	Vitamin D (IU/day)
0-6 months*	200	400
6-12 months*	260	400
1-3 years	700	600
4-8 years	1000	600
9-18 years	1300	600

*Reflects Adequate Intake (AI) reference value not RDA. RDA in this group has not been established.

In deficiency states, the requirements will be higher until the patient's calcium and vitamin D status is replete.

A meta-analysis of randomized controlled trials investigating the effectiveness of calcium supplementation in preventing fractures found that although there was an increase in bone mineral content (BMC) by 1.7 % there was no effect of calcium supplementation on reduction of fracture risk (9).

2. Vitamin D and its metabolism

Vitamin D plays a pivotal role in the absorption of calcium, mineral homeostasis and ultimately the maintenance of skeletal integrity.
The effects of $1,25[OH]_2$-D are to maintain serum calcium and phosphate levels within normal physiological levels. $1,25[OH]_2$-D affects calcium levels by controlling the absorption of calcium from the intestines, increasing resorption from the kidneys and by its direct effects on bone and parathyroid hormone (PTH) secretion.
Very few foods naturally contain vitamin D_3, it is mainly found in oily fish (tuna, salmon) and small amounts are present in cheese, egg yolk and vitamin D fortified foods. Mushrooms provide vitamin D_2. Dietary derived vitamin D is fat soluble and absorbed from the gut and transported via chylomicrons and the lymphatic system to the liver for hydroxylation (4).

The major source of vitamin D is exposure to ultraviolet B rays from the sun. 7-dehydrocholesterol in the skin is converted to pre-vitamin D_3, which is in turn converted to vitamin D_3. Vitamin D_3 diffuses into circulation and is transported protein bound to the liver where it is hydroxylated to 25-hydroxyvitamin D (25[OH]-D), which is then transported to the kidney where it is further hydroxylated at the 1^{st} carbon by an enzyme 1-α-hydroxylase to a more potent $1,25[OH]_2$-D. In the kidney 25[OH]-D is converted either

into the potent 1,25[OH]$_2$-D if serum calcium is low or the inactive 24,25[OH]$_2$-D when serum calcium is normal.

25[OH]-D is a good marker of vitamin D stores as it has a half-life of 3-4 weeks. 1,25[OH]$_2$-D cannot be used as a marker of vitamin D stores as it is the active form and has a half-life of 4 hours (4).

The American Academy of Pediatrics and Pediatric Endocrine Society classify ≥50nmol/L of 25[OH]-D as vitamin D sufficiency. There are recommendations that 50nmol/L is the lower limit of sufficiency. When treating vitamin D insufficiency or deficiency then serum levels above 50nmol/L is the level to aim for (10, 11).

In a study by Ceroni et al, low levels of 25[OH]-D were found in adolescents irrespective of presence or absence of limb fracture. Of the 100 subjects who sustained fractures 12% were found to be deficient and 36% of the fracture group found to have insufficient levels. Deficiency was defined by 25-[OH]-D levels of less than 50nmol/L and insufficient defined by 25-[OH]-D levels between 50nmol/L-78nmol/L. In the non-fracturing control group 6% and 34% were found to have deficient and insufficient levels respectively (5). The prevalence of vitamin D deficiency in the fracture population is variable, ranging from 12% to 47% in various studies (5, 10-13). Two prospective studies found that a low 25[OH]-D is not a statistically significant risk factor for fracture risk (5, 12).

The confounding issue regarding the failure to associate low 25[OH]-D with fracture risk could be the fact that some of the literature reviewed used higher levels to classify for insufficiency and replete as equal or > 50nmol/L-78nmol/L and >78nmol/L respectively (5, 13).

The cut-off levels for replete, insufficiency and deficiency for vitamin D status is the subject of an ongoing debate (10). For the purpose of this study 50nmol/L or more will be used to define sufficiency, <30nmol/L to define deficiency and 30-49 will define insufficiency in line with recommendations by Institute of Medicine(10).

Ethnicity and Genetics

Ultraviolet B (UVB) radiation from the sun within ranges of 290 to 315nm is the major source of vitamin D. Sun exposure to arms and legs for a period of 5-15 minutes two to three times a week will produce about 3000 IU of vitamin D. The best time for exposure is between 10 am to 3 pm to ensure adequate synthesis of vitamin D (4).

In dark skinned individuals with greater amounts of melanin pigmentation, which absorbs UVB radiation, longer sun exposure is required for adequate conversion of 7-dehydrocholesterol to Vitamin D$_3$. Inadequate exposure to the sun will easily lead to below normal 25[OH]-D levels in dark skinned individuals. Covering the skin and sunscreen use will further impede vitamin D synthesis in these patients.

Despite the findings of a lower bone area (BA) and bone mineral content (BMC) in black subjects compared to white subjects in the Bt20 cohort, white subjects had a higher incidence of fractures compared to the black subjects. The reasons observed and hypothesized for this were greater physical activity in white children and possible protective genetic factors in the black population (14). None of the South African studies thus far have compared the vitamin D status and fracture risk in the Bt20 cohort.

A study by Ryan et al, reports that black subjects, in the African–American context, had lower vitamin D levels than their white counterparts, with no significant difference when comparing for fracture risk (13).

A study by Poopedi et al on the Bone Health sub-cohort on children aged 10 years from the South African Bt20 cohort also support the findings that white children had a higher 25(OH)D than black children with deficiency being found in 7% of black children and 1% white children. The study however did not assess the association of vitamin D and fracture risk (7)

Physical exercise

Exercise which increases mechanical loading during weight bearing activities, stimulates osteogenesis and promotes bone formation. Physical activity is associated with an increase in bone mineral density (BMD) in subjects and will therefore strengthen the skeletal system (4, 14).
The BMD increase is sport specific, depending on the bones that are experiencing the most impact or load (4). However, there are studies that show that increased physical activity and the mechanical forces applied to the skeletal system may have a contradictory increased risk to fractures in adolescents (14). This seems to be more related to the impact and high intensity exercise predisposing adolescents to a greater fracture risk.

Body habitus and hormonal effects

Obese children tend to have lower 25[OH]-D levels which is thought to be due to sequestration of fat soluble vitamin D into the adipose tissue where it can be stored, thus reducing circulating serum levels.

In one study, the results showed that 25[OH]-D levels decreased as weight increased (15). A study by Ryan et al showed that subjects with forearm fractures were more likely to be overweight, the mechanisms hypothesized for this include poor motor co-ordination and relative weakness of the bone for patients' weight(16).
Other studies do not support the findings that an increase in weight increases the fracture risk (13).

Hormones such as oestrogen, testosterone and growth hormone all play a part in the promotion of bone formation via various mechanisms. Anorexia nervosa is a good

example of the effects of malnutrition on bone health as it affects multiple hormonal axes. Hypogonadism in severe malnutrition leads to abnormal luteinizing hormone (LH) pulsatility and low gonadal steroids. Growth hormone (GH) increases in early puberty due to increase in oestrogen in girls and aromatization of testosterone in boys. Rising GH is associated with increase bone modelling and the increase in oestrogen prevents osteoclastic bone resorption. In anorexia, there is bone resistance to effects of GH (17). Relative hypercortisolaemia in anorexia impacts on bone health by decreasing bone mineral density. All the above mentioned factors render the malnourished individual at a higher risk for fractures.

2. Justification of Study

As mentioned earlier, the study by Thandrayen et al. reported on the fracture rates in the Bt20 cohort over a 15 year period and found that males were more likely to fracture then females (27.5% and 16,3% respectively) and that white children fractured more than black children (41.5% vs 19%) (3).

We plan to investigate whether there are any associations between vitamin D status and fracture risk in adolescents within the different ethnic groups in South Africa. This association between vitamin D status and fracture risk has not been investigated previously in this cohort.

3. Aims and Objectives

The aim of the study is to determine whether there is an association between the vitamin D status at 10 years of age and fracture risk (in the first 15 years of age) in adolescents within different ethnic groups.

Objectives:

1.
    a. To investigate 25[OH]-D levels at 10 years of age, in children with and without fracture and determine whether there is an association between 25[OH]-D levels and fracture risk at 10 and 15 years of age.
    b. Investigate BMI in relation to 25[OH]-D levels at 10 years of age.
    c. To assess physical activity score/ MET score at 10 years of age between fracturing and non-fracturing group at 10 and 15 years of age.
    d. To assess bone mass and body composition measurements whole body less head bone mineral area and content (WBLH BA and BMC); hip, radius and lumbar spine BA and BMC; fat and lean mass at 10 years of age between fracturing and non-fracturing group at 10 and 15 years of age.
2. The association between 25[OH]-D levels at 10 years of age will be assessed in relation to the BMI, physical activity scores, bone mass measurements, body composition measurements and these will be compared at both 10 and 15 years of age.

4. Methodology

4.1 Study design

This is a retrospective study utilizing data collected from children in the Bone Health sub-cohort of the Birth to Twenty cohort.

4.2 Sample population

Sample size and inclusion criteria:
The study population comprises children of black and white ethnicity living in urban Johannesburg and Soweto who were delivered in the public sector between April 23 and June 8 1990 and were enrolled in the Bone Health sub-cohort of the Birth to Twenty cohort study when they were 9 years of age. The total sample size comprises of 311 participants.

Exclusion criteria
Children with chronic diseases and chronic medication that would impact on the results by affecting the bone mineral content and density were excluded. Participants suffering from conditions such as rickets, rheumatoid arthritis, asthma and conditions affecting the patients' mobility were excluded. Children on chronic medications such as anti-epileptic and steroid medications were also excluded.

5. Data

Data collection

Data will be taken from the existing database of the Bone Health sub-cohort of the Bt20 cohort. Data in the database was collected by means of a retrospective completion of a fracture questionnaire administered at 15 (Appendix B) years of age.
The participants completed the questionnaire and parents/caregivers verified the information given. The questionnaire enquired about previous fractures and age fracture sustained, fracture site with the aid of diagram and the cause for fracture. For the purposes of this study, we'll look at whether subjects fractured or not and the number of fractures were sustained.

Variables to be analysed:

Primary

- 25-[OH]-D levels will be analysed and it will be classified as follows:
  Sufficient ≥50 nmol/L
  Insufficient 30-50 nmol/L
  Deficient <30 nmol/L

The levels will be compared at 10 years between the fracturing and non-fracturing groups.

Secondary

- BMI Z scores
  Will be calculated using the WHO Anthroplus software. Important variables to be entered will be the gender, weight, height and BMI calculated using the formula kg/m^2 and given as Z-scores for age and gender at 10 years and 15 years of age. Height was measured to the nearest millimetre using a stadiometer (Holtain, Crosswell, UK) with the patient barefoot. Weight was measured to the nearest 100g using a digital scale (Dismed, Halfway House, South Africa) with patient wearing light clothing and barefoot.

  Z-scores of ≤-2 will be used for thinness and ≤ -3 for severe thinness. Z-score of ≥+1 will denote overweight and ≥+2 denote obesity and ≥ +3 will denote severe obesity. Less than +1 and> -2 Z-score will be regarded as normal.

  BMI will be assessed between the different groups and correlated with the 25-[OH]-D level. An association between the BMI, 25-[OH]-D and fracture risk will be investigated.

- Physical activity scores or MET score level
  A questionnaire quantifying total physical activity (PA) for the past twelve months were administered at ages of 10 and 15 years of age via interview in the Bone health sub-cohort of the Bt20 longitudinal study. The score looks at the intensity, duration and frequency of all formal PA (sport at school/club level), informal PA (play activities) and sedentary. Physical activity is scored in minutes per week multiplied by metabolic equivalent of task (MET) where one MET is defined as energy expenditure for sitting quietly (1kcal/kg/hour) which for an average adult is approximately 3.5ml of oxygen/kg/minute. The total formal and informal PA scores were then converted into Z-scores and non-fracturing black females will be used as the control group.

  The data will be analysed to see if or how physical activity affects 25-[OH]-D levels and fracture risk.

- Bone mass and body composition measurements:
  Whole body less head bone area and bone mineral content (WBLH BA and BMC); hip, radius and lumbar spine BA and BMC, fat and lean mass were measured using dual energy x-ray absorptiometry (DXA) at 10 and 15 years of age.

- The number of fractures at 10 and 15years will be analysed.
- Comparison will be done between the ethnic groups (black vs white) but within the gender groups (male vs female) and between those with and without fractures.

Data analysis

Collected data will be entered into a Microsoft Excel spreadsheet and statistical analysis will be performed using Statistica Software (Statsoft USA, version 12).
Fisher exact and Chi-squared test will be used to compare categorical variables such as fractured or not fractured, within gender and ethnic groups. Continuous variables such as 25-[OH]-D levels will be analysed between groups using student-t test or Mann-Whitney U test depending on the distribution of the data. A p value of less than 0.05 will be used to denote statistical significance. Logistic regression analyses will be performed to determine what factors at 10 and 15 years of age were associated with fracture risk in the first 15 years of age in South African black and white adolescents.

6. Ethical consideration

All participants provided assent and informed written consent was obtained from the parent or caregiver when enrolled onto the Bt20 cohort study. Confidentiality will be maintained as no identity details of subjects will appear on the data spreadsheet.

The protocol will be submitted to the Human Research Ethic Committee of the University of the Witwatersrand to obtain ethical clearance for this study.

7. Funding

The Birth to Twenty Cohort from which the data from this study is obtained was funded by the Wellcome Trust (UK), Medical Research Council of South Africa, and the Human Sciences Research Council of South Africa.

There is no external or internal funding for the cost of this current study. The cost mainly includes that of stationery, photocopying and printing for which, I, the investigator will be held responsible for.

8. Limitations

Fracture data was collected by means of questionnaires completed by the subjects and their parents on the recall of fractures. The unavailability of radiographs to confirm that there was a fracture serves as a limitation of the study, however to assess the quality of data Dr Thandrayen verified fracture history on a sub-sample by telephonic interview as part of her thesis on fracture and bone mass on this study population. Telephonic verification was done 6-12 months after completing the initial questionnaire

9. Timeline

	October 2016 June 2017	July August 2017	September October 2017	November 2017 January2018
Literature review				
Protocol preparation				
Protocol assessment				
Ethics				
Data collection				
Data analysis				
Write dissertation				

References:

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Appendix A

Data Collection sheet
Bt20: Bone health

	ID		
Ethnicity **Black** **White**		**Fractured** Yes No	
Gender **Male** **Female**			
25[OH]D nmol/L	10 years		
Height cm	10 years	**15 years**	
***Weight(Kg)**	10 years	15 years	
Body composition **Lean mass(g)** **Fat mass(g)**	10 years	15 years	
DXA measurements **WBLH BA (cm_2)** **WBLH BMC (g)** **Radius BA (cm_2)** **Radius BMC (g)** **Hip BA (cm_2)** **Hip BMC (g)** **Lumbar spine BA (cm_2)** **Lumbar spine BMC (g)**	10 years	15 years	
***BMI Z score (kg/m^2)**	10 years	15 years	
Number Fracture	10 years	15 years	
***MET score (kcal/kg/minute)**	10 years	15 years	

*Rounded to one decimal place

Appendix 2 Ethics clearance certificate

Declaration and ethics clearance 2 .pdf (Command Line)

Appendix 3 South African Orthopaedic J Author guidelines

To submit a manuscript click here

Authors submitting articles for consideration for publication by the journal are required to familiarise themselves with the journal Ethics and Malpractice policy prior to submission. The policy is available on the journal website: https://www.saoj.org.za

Criteria for publication

- The article falls within the scope of the journal.
- Methods, statistics, and other analyses are performed to a high technical standard and are described in sufficient detail.
- Results reported have not been published elsewhere.
- Conclusions are presented appropriately fashion and are supported by the data.
- The article is presented in an intelligible fashion and is written in standard English (British usage).
- The research meets all applicable ethical standards.
- The article adheres to guidelines provided in the instructions for authors section.

Guidelines for authorship

- Each author should participate and is responsible for the content and design of the study, the preparation of the manuscript and its revisions, and final approval.
- In order to qualify for authorship, authors should satisfy all four the criteria for authorship as specified by the ICMJE:
    1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
    2. Drafting the work or revising it critically for important intellectual content; AND
    3. Final approval of the version to be published; AND
    4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
- Other ‘contributors’ or ‘collaborators’ can be acknowledged at the end of the manuscript together with their contribution. Those whose contributions do not justify authorship may be acknowledged individually or together as a group under a single heading (e.g., “Clinical Investigators” or “Participating Investigators”), and their contributions should be specified (e.g., “served as scientific advisors,” “critically reviewed the study proposal,” “collected data,” “provided and cared for study patients”, “participated in writing or technical editing of the manuscript”.
- The South African Orthopaedic Journal accepts a maximum of 8 authors per article. If there are more than eight authors, the first eight authors must be listed along with the group name at the end. The remaining authors and their affiliations must then be listed in an appendix.
- On submission of your article, the ORCID (Open Researcher and Contributor ID) identifier of at least the corresponding author will be required. ORCID provides a persistent digital identifier that distinguishes you from every other researcher and supports automated linkages between you and your professional activities, ensuring that your work is recognised. To register and find more information, please visit: http://orcid.org

Registration of clinical trials

- A clinical trial is defined as any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects of health outcomes. Interventions include drugs, surgical procedures, devices, behavioural treatments, dietary interventions, and process-of-care changes.
- Clinical trials should be registered in a public trials registry in accordance with International Committee of Medical Journal Editors
- Trials must be registered and approved by the relevant authorities before the onset of patient enrolment.
- The Medicines Control Council (MCC) reference number and the SA National Clinical Trial Register (SANCTR) registration number should be included at the end of the abstract of the article.
- Purely observational studies (those in which the assignment of the medical intervention is not at the discretion of the investigator) do not require registration.

Reporting guidelines

- All articles should be prepared in accordance with the guidelines relevant to the study design, as described in the Equator Network Guidelines (https://www.equator-network.org/reporting-guidelines/)
- Randomised trials should be accompanied by a flow diagram that illustrates the progress of patients through the trial, including recruitment, enrolment, randomisation, withdrawal and completion, and a detailed description of the randomisation procedure.

Reporting of statistics

In terms of the statistical reporting, the Equator Network advises on the use of the SAMPL guideline: https://www.equator-network.org/2013/02/11/sampl-guidelines-for-statistical-reporting/

The SAMPL guidelines provide two guiding principles

1. *"Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results."* When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as *P* values, which fail to convey important information about effect size.
2. *Provide enough detail that the results can be incorporated into other analyses.* This requires reporting the descriptive statistics from which other statistics are derived, such as the numerators and denominators of percentages, especially in risk, odds, and hazards ratios. Likewise, P-values are not sufficient for re-analysis. Needed instead are descriptive statistics for the variables being compared, including sample size of the groups involved, the estimate (or effect size) associated with the P-value, and a measure of precision for the estimate, usually a 95% confidence interval.

Some specific guidelines applicable to the SAOJ:

- Consistency is one of the most important factors in presenting a well-formatted, professional manuscript.
- The nature of the measurements and variables reported on will often dictate the amount of precision required. Report numbers - especially measurements? with an appropriate degree of precision. For ease of comprehension and simplicity, round to a reasonable extent.
- The recommendation is to report the number of decimals that have both clinical and statistical meaning and consistently reporting all other variables in the same manner.

- Note: Generally, for descriptive purposes, percentages are reported as whole numbers except when dealing with really large sample sizes
- At least for the primary outcomes, report a measure of precision (a confidence interval).
- Although not preferred to confidence intervals, if desired, *p* values should be reported as equalities to three decimal places (e.g., $p = 0.031$ and not as inequalities: e.g., $p < 0.05$). Do NOT report NS; give the actual *P-value*. The smallest *P*-value that needs to be reported is $P < 0.001$.
- Report numerators and denominators for all percentages
- Summarize data that are approximately normally distributed with means and standard deviations (SD). Use the format: mean (SD) not mean ?
- Summarize data that are not normally distributed with medians and interpercentile ranges, ranges, or both.
- Do NOT use the standard error of the mean (SE) to indicate the variability of a data set. Use standard deviations, inter-percentile ranges, or ranges instead.

Formatting examples:

- p = 0.028 or p < 0.001
- (43% vs 21%; p = 0.002)
- (odds ratio (OR) 0.38; 95% confidence interval (CI) 0.71 to 1.82; p = 0.822) or after first use (OR 1.62; 95% CI 1.41 to 1.86; p < 0.001)
- *Descriptive stats normal distribution:* mean age 36 years (SD 4 years) or 36 years (SD 4; range 40 to 97 years)
- *Descriptive stats non-normal distribution:* median age 36 years (IQR 44 to 88 years) or 36 years (IQR 44 to 88 years; range 40 to 97 years)
- *Descriptive stats percentage:* (149 of 202; 74%)

Formatting of submissions

Text formatting

- Use Helvetica or Arial font, size 11.
- Use double line spacing throughout the document.
- Number the pages of the blinded manuscript consecutively.
- Use italics for emphasis.
- When referring to an article with multiple authors, please use the following format: Rabinowitz et al. published their retrospective review.
- Do not use field functions.
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- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Headings

- Use no more than three levels of displayed headings.

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- Define abbreviations and acronyms at first mention and use consistently thereafter.

Units

- Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

Figures

- Figures should be numbered consecutively with illustration Arabic numbers 1, 2, 3, etc.
- The figure should be listed in the text as follows: … wound irrigation and splinting (*Figure 1*).
- Figures should be clear and easily understandable with a full descriptive legend stating any areas of interest and explaining any markings, letterings or notations. All figures and figure legends should be understandable as a stand-alone item, without having to read the main body of the text.
- For radiographs, please ensure you state the view used and the time point at which it was taken, as well as the demographic details of the patient if applicable.
- Please submit the original JPEG (300 dpi) or TIFF of all photographs, as well as the figure saved as a Word document. The Word version of the figure should be complete with the legend and any necessary markings such as letters or arrows.
- Figures such as graphs and algorithms should be in Word or PowerPoint in order to be editable.
- Figures should not be imbedded in the text file but should be submitted as separate individual files. Each figure should be a separate file, entitled Figure 1, Figure 2, etc.
- Remove all markings, such as patient identification, from radiographs before photographing. Clinical photos must be adequately anonymised.
- A statement of patient consent for clinical photographs must be provided on the title page.
- In images depicting X-rays of children there should exhibit adequate shielding of radiation.
- All line or original drawings must be done by a professional medical illustrator.
- We accept a maximum of six figures. You may apply to the Editor-in-Chief for permission to include more figures if considered critical to the clarity and completeness of the submission.
- Do not submit any figures, photos, tables, or other works that have been previously copyrighted or contain proprietary data unless you have obtained and can supply written permission from the copyright holder to use that content.

Tables

- Tables should carry uppercase Roman numerals, I, II, III, etc.
- Tables should always be cited in the text in consecutive numerical order.
- The table should be identified in the text as follows: Details of results are listed in *Table I*. Or, alternatively,? high-energy trauma that is often associated with these fractures (*Table II*).
- Tables should be used to present information in a clear and concise manner. All tables should be understandable without the main text.
- For each table, please supply a table heading explaining the components of the table.
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- Footnotes to tables should be indicated by superscript lower-case letters and included beneath the table body.
- Please submit tables as editable text and not as images. They should be created using the Table tool in Word.
- Do not embed tables in the text file but submit them as separate individual files. Each table should be a separate file, entitled Table I, Table II, etc.
- We accept a maximum of eight tables.
- Do not duplicate information given already in the text.

- Do not submit any figures, photos, tables or other works that have been previously copyrighted or contain proprietary data unless you have obtained and can supply written permission from the copyright holder to use that content.

References

- References should be numbered consecutively in the order that they are first mentioned in the text and listed at the end in numerical order of appearance.
- Identify references in the text by Arabic numerals in superscript after punctuation.
- References should not be a listing of a computerised literature search but should have been read by the authors and have pertinence to the manuscript.
- Accuracy of references is the authors' responsibility, and the author is to verify the references against the original documents.
- Manuscripts in preparation, unpublished data (including articles submitted but not in the press) and personal communications may not be included in the reference listing. They may be listed in the text in parentheses only if absolutely necessary to the contents and meaning of the article.
- The titles of journals should be abbreviated according to the style used in Index Medicus, obtainable through the website http://www.nlm.nih.govshould
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Journal article:

Sidhu GS, Ghag A, Prokuski V, Vaccaro AR, Radcliff KE. Civilian gunshot injuries of the spinal cord: a systematic review of the current literature. *Clin Orthop Relat Res* 2013;**471**:3945-55.

Ideally, the names of all authors should be provided, but the usage of *et al*. in long author lists (more than six authors) will also be accepted: Fong K, Truong V, Foote CJ, *et al*. Predictors of nonunion and reoperation in patients with fractures of the tibia: an observational study. *BMC Musculoskelet Disord* 2013;**14**:103.

Online journal article:

Caetano-Lopes J, Lopes A, Rodrigues A, *et al*. Upregulation of inflammatory genes and downregulation of sclerostin gene expression are key elements in the early phase of fragility fracture healing. *PLoS One* 2011;**6**:e16947.

Web reference (with authors):

Cierny G, DiPasquale D. Adult osteomyelitis protocol.
http://www.osteomyelitis.com/pdf/treatment_protocol.pdf.

(date last accessed 05 March 2013).

Web reference (no authors listed):

No authors listed. International commission on radiological protection. http://www.icrp.org (date last accessed 20 September 2009).

Chapter in a book:

Young W. Neurophysiology of spinal cord injury. In: Errico TJ, Bauer RD, Waugh T (eds). *Spinal Trauma*. 3rd ed. Philadelphia: JB Lippincott; 1991: 377-94.

Dissertation:

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Abstract:

Peterson L. Osteochondritis of the knee treated with autologous chondrocyte transplantation [abstract]. ISAKOS Congress, 2001.

Structure and content of submission

- We accept a maximum of 3 500 words, including the abstract and body of the text (excluding references).
- Exceptions to this rule may be made for systematic reviews and meta-analysis at the discretion of the Editor-in-Chief.
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- The title should be concise and informative.

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Authors are to insert a section at the end of the title page entitled declarations (please provide the author's name, signature and date). The following statements are required under the declarations section:

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The authors confirm that all authors have made substantial contributions to all of the following:

- The conception and design of the study, or acquisition of data, or analysis and interpretation of data.
- The drafting of the article or its critical revision for important intellectual content.
- Final approval of the version to be submitted.

Sound scientific research practice

The authors further confirm that:

- The manuscript, including related data, figures and tables, has not been previously published and is not under consideration elsewhere.
- No data have been fabricated or manipulated (including images) to support conclusions.
- This submission does not represent part of a single study that has been split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (e.g. 'salami-publishing').

Plagiarism

The authors confirm that the work submitted is original and does not transgress the plagiarism policy of the journal.

- No data, text or theories by others are presented as if they were the authors' own.
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A conflicting interest exists when professional judgment concerning a primary interest (such as the patient's welfare or the validity of research) may be influenced by a secondary interest (such as financial gain or personal rivalry). It represents a situation in which financial or other personal considerations from authors, reviewers or editors have the potential to compromise or bias professional judgment and objectivity. It may arise for the authors when they have a financial interest that may influence their interpretation of their results or those of others. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, grants or other funding. All potential conflicts of interest need to be declared. The conflict of interest statement should list each author separately by name, e.g.,

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If no conflicts of interest exist, state this as follows:

'The authors declare they have no conflicts of interest that are directly or indirectly related to the research.'

Funding sources

All sources of funding should be declared. Also, define the involvement of study sponsors in the study design, collection, analysis and interpretation of data; the writing of the manuscript; and the decision to submit the manuscript for publication.

List all funding sources as follows:

'This work was supported by the xxxx (grant numbers xxxx, yyyy).'

When funding is from a block grant or other resources available to a university, college or other research institution, submit the name of the institute or organisation that provided the funding.

If no funding was received, state as follows:

'No funding was received for this study.'

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'The author/s declare that this submission is in accordance with the principles laid down by the Responsible Research Publication Position Statements as developed at the 2nd World Conference on Research Integrity in Singapore, 2010.'

Available from: http://publicationethics.org/resources/international-standards-for-editors-and-authors

Institutional Review Board (IRB) ethical approval must have been given if the study involves human subjects or animals. Please provide the approval number. IRB documentation should be available upon request.

‘Prior to the commencement of the study ethical approval was obtained from the following ethical review board: *Provide name and reference number’*

- For studies with human subjects include the following:

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‘Informed written consent was or was not obtained from all patients for being included in the study.’

‘Consent was obtained from patients for the use of clinical photographs and these images were adequately anonymised.'

- For studies with animals, include the following sentence:

‘All institutional and national guidelines for the care and use of laboratory animals were followed.’

- For articles that do not contain studies with human or animal subjects:

‘This article does not contain any studies with human or animal subjects.’

- If doubt exists whether the research was conducted in accordance with the Helsinki Declaration, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study. If any identifying information about patients is included in the article, the following sentence should also be included: Additional informed consent was obtained from all patients for which identifying information is included in this article. The Helsinki Declaration 2008 can be found at http://www.wma.net/en/30publications/10policies/b3/

Please provide the names and email addresses of two reviewers.

Title Page Example

Title of Submission

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Declarations:

Authorship

The authors confirm that all authors have made substantial contributions to all of the following:

- The conception and design of the study, or acquisition of data, or analysis and interpretation of data.
- The drafting of the article or its critical revision for important intellectual content.
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Sound scientific research practice

The authors further confirm that:

- The manuscript, including related data, figures and tables, has not been previously published and is not under consideration elsewhere.
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Conflict of interest statement

John Smith declares that he has no conflict of interest. Paula Taylor has received research grants from Drug Company A.

Funding sources

No funding was received for the purposes of performing this study.

Compliance with ethical guidelines

The author/s declare that this submission is in accordance with the principles laid down by the Responsible Research Publication Position Statements as developed at the 2nd World Conference on Research Integrity in Singapore, 2010.

Prior to the commencement of the study ethical approval was obtained from the following ethical review board: *Provide name and reference number.*

All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Informed written consent was or was not obtained from all patients for being included in the study.

Consent were obtained from patients for the use of clinical photographs/ and these images were adequately anonymised.

Author Name	**Signature**	**Date**
J Smith		15/8/2017
		16/8/2017

Appendix 4 Plagiarism declaration

Declaration and ethics clearance 2 .pdf (Command Line)

Appendix 5 Turnitin Report

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