

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

**USING QUANTITATIVE ULTRASOUND FOR EPIDEMIOLOGICAL RESEARCH:
ASSOCIATIONS WITH RISK FACTORS FOR LOW BONE MINERAL DENSITY
IN SOUTH AFRICAN PREMENOPAUSAL WOMEN**

**By Deborah Constant
CNSDEB001**

**Submitted to the University of Cape Town in part fulfillment of the requirements for
the degree Masters in Public Health**

**Faculty of Health Sciences
UNIVERSITY OF CAPE TOWN**

DECEMBER 2008

**Supervisor:
Prof. Margaret Hoffman
Women's Health Research Unit
School of Public Health & Family Medicine
University of Cape Town**

DECLARATION

I, Deborah Constant, hereby declare that the work on this dissertation thesis is based on my original research and has not, in whole or in part, been submitted towards another degree, at this university or elsewhere

The University is empowered to reproduce either the whole or any portion of the contents for purposes of research.

.....
SIGNATURE

.....
DATE

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Prof Margaret Hoffman for her guidance and astute commentary during the course of this project as well as during the analysis and write up of this dissertation. Her honesty, encouragement and ability to nurture and develop a newcomer to the practice of public health research and her attention to detail has been an example to me of the best possible qualities a student could hope for in a supervisor.

I would like to thank Prof Lynn Rosenberg for her clear direction, and Dr Yuqing Zhang for his guidance in statistical methods.

Finally I would like to extend my thanks to my husband, my brother, and my mother for their encouragement and their interest in my progress with this effort.

University of Cape Town

ABSTRACT

Introduction. Quantitative Ultrasound (QUS) measurement at the calcaneum is a convenient, cost effective and noninvasive method of determining bone strength well suited to community-based research in countries with limited resources. Although only moderately correlated with dual X-ray absorptiometry (DEXA) measurement of bone mineral density (BMD) at the hip and spine, quantitative ultrasound has shown to be a reliable predictor of osteoporotic fracture. This study aims to evaluate the use of QUS in epidemiological studies in South African settings. To this end, this report determines whether characteristics associated with QUS measures of bone strength in a large sample of premenopausal South African women are similar to those known to be associated with the BMD as measured by DEXA, and compares these associations with those in other populations.

Methods. This cross-sectional study included 3493 black and mixed race women aged 18 - 44 living in Cape Town. Trained study nurses administered structured interviews on reproductive history and lifestyle factors. In addition they took height, weight and calcaneal QUS measurements using the Sahara device. Adjusted means of QUS measures according to categories of risk factors were obtained using multivariable regression analysis.

Results. In both groups associations between QUS measures and age, Body Mass Index (BMI), age at menarche, parity and primary school physical activity were similar to those known to be associated with BMD as measured by DEXA. There were no clear associations between QUS and educational level, alcohol use, cigarette smoking, and current calcium intake.

Conclusion. The data give support to the use of QUS as an epidemiological tool in large studies of bone strength in premenopausal women.

TABLE OF CONTENTS

| | |
|--|-----------|
| 1. INTRODUCTION..... | 1 |
| 2. BACKGROUND..... | 2 |
| 2.1. Bone health and peak bone mass..... | 2 |
| 2.2. Measurement technologies for bone strength | 3 |
| 2.3. Quantitative ultrasound mechanics..... | 4 |
| 2.4. QUS precision and accuracy..... | 4 |
| 2.5. Uses for QUS: Predictive, diagnostic, monitoring..... | 9 |
| 2.6. The South African context..... | 12 |
| 3. AIM AND OBJECTIVES..... | 13 |
| 4. METHODS..... | 13 |
| 4.1. Overview..... | 14 |
| 4.2. Study subjects..... | 14 |
| 4.3. Interview..... | 15 |
| 4.4. Measurement..... | 18 |
| 4.5. Quality Control..... | 18 |
| 4.6. Comparison of QUS machines..... | 19 |
| 4.7. Agreement between QUS and DEXA: Small Study..... | 19 |
| 4.8. Statistical analysis..... | 20 |
| 5. RESULTS..... | 22 |
| 5.1. Description of subjects..... | 22 |
| 5.2. Exploratory analysis..... | 25 |
| 5.3. Final multivariable analysis..... | 28 |
| 5.4. Quality Control..... | 34 |
| 5.5. Comparisons between QUS machines..... | 37 |
| 5.6. Agreement between QUS and DEXA: Small study | 37 |
| 6. DISCUSSION..... | 41 |
| 6.1. Risk factor Associations..... | 41 |
| 6.2. Strength of association with risk factors..... | 49 |
| 6.3. Normative data for black and mixed race women..... | 49 |
| 6.4. QUS and DEXA measures: The question of validity..... | 51 |
| 6.5. Short term and long term precision..... | 52 |
| 6.6. Strengths and Limitations..... | 53 |
| 7. CONCLUSION..... | 54 |
| 8. REFERENCES..... | 55 |
| 9. APPENDICES..... | 61 |

LIST OF TABLES

| | |
|---|----|
| Table 1: Impact factors allocated to sports and leisure activities..... | 15 |
| Table 2: Demographic, socioeconomic and reproductive characteristics of study participants..... | 23 |
| Table 3: Lifestyle characteristics of study participants | 24 |
| Table 4: Exploratory analysis for QUS measures in Black women..... | 26 |
| Table 5: Exploratory analysis for QUS measures in mixed race women | 27 |
| Table 6: Associations of risk factors with BUA, SOS and QUI among Black women | 29 |
| Table 7: Associations of risk factors with BUA, SOS and QUI among Mixed race women | 31 |
| Table 8: Adjusted means for QUS measurements according to smoking in mixed race women | 33 |
| Table 9: Coefficients of variation for BUA , SOS and QUI for training trials of operators | 34 |
| Table 10: BUA and SOS differences for the two Sahara machines | 37 |
| Table 11: Correlation coefficients for QUS and DEXA | 37 |
| Table 12: Bland Altman statistics for agreement between QUS devices and DEXA | 38 |
| Table 13: Adj Mean BUA, SOS and QUI in height categories, by BMI strata | 61 |
| Table 14: Adj Mean BUA, SOS, QUI in Physical activity categories, by BMI strata | 62 |
| Table 15: Adj Mean BUA, SOS and QUI in Age at menarche categories, by BMI strata .. | 63 |

LIST OF FIGURES

| | |
|--|----|
| Figure 1: Mean Monthly phantom QAB..... | 36 |
| Figure 2: Mean Monthly phantom QAB | 36 |
| Figure 3: Bland Altman plot of BMD measured by MP QUS and by DEXA..... | 40 |
| Figure 4: Bland Altman plot of BMD measured by GU QUS and by DEXA..... | 40 |

ABBREVIATIONS

| | |
|---------|---|
| BMD | Bone Mineral Density |
| BMI | Body Mass Index |
| BUA | Broadband Ultrasound Attenuation |
| Ca | Calcium |
| CI | Confidence Interval |
| CV | Coefficient of Variation |
| DEXA | Dual X-ray Absorptiometry |
| ESOPO | Epidemiological study On the Prevalence of Osteoporosis |
| Fem | Femoral |
| GU | Gugulethu |
| IC | Injectable Contraceptive |
| IRB | Internal Review Board |
| ISCD | International Society for Clinical Densitometry |
| MANOVA | Multivariable Analysis Of Variance |
| MP | Mitchells Plain |
| MRC/UCT | Medical Research Council/University of Cape Town |
| NIH | National Institute of Health |
| OC | Oral Contraceptive |
| OPUS | Osteoporosis and Ultrasound Study |
| QAB | Quality Assurance Broadband attenuation |
| QAS | Quality Assurance Speed of sound |
| QC | Quality Control |
| QUI | Quantitative Ultrasound Index |
| QUS | Quantitative Ultrasound |
| RCT | Randomized Controlled trails |
| SD | Standard Deviation |
| SOS | Speed Of Sound |
| TIH | Total Impact Hours |
| WHO | World Health Organization |

1. INTRODUCTION

Osteoporosis is a skeletal disorder characterized by low bone mass and micro-architectural deterioration with subsequent bone fragility which commonly results in fractures of the spine, hip or wrist. (Hans and Krieg, 2008). Approximately 200 million people are affected world wide (Nayak *et al.*, 2006) and thus osteoporosis has been identified by the World Health Organization (WHO) as a priority health issue (WHO, 2003). Currently, the WHO diagnosis of osteoporosis rests on the evaluation of bone mineral density (BMD) by Dual X-ray absorptiometry at the hip and the lumbar spine. Dual X-ray absorptiometry (DEXA) is the most accurate of commonly available instruments for measuring BMD in clinical settings such as hospitals in urban centers.

An alternative method for measuring bone quality is Quantitative Broadband Ultrasound (QUS). QUS standard equipment measures bone quality at the calcaneum but not at the lumbar spine or hip – the latter two anatomical sites most commonly involved in debilitating osteoporotic fracture. However QUS is significantly cheaper than DEXA and noninvasive, and the machine is readily portable making it a practical tool for large epidemiological investigations, particularly in community settings. In resource-limited countries like South Africa, it would be highly desirable to be able to use the more portable and cheaper ultrasound method for conducting large epidemiological studies of bone quality.

2. BACKGROUND

2.1 Bone health and peak bone mass

Growth in bone size and strength occurs during childhood and early adolescence, but bone accumulation is not completed until the third decade of life, long after linear growth has ceased due to closure of the long bone epiphyses. The age of peak bone mass is however considered to be variable and is affected by environmental factors (Micklesfield *et al.*, 2005). The bone mass attained early in life is perhaps the most important determinant of life-long skeletal health. Thus individuals with the highest peak bone mass after adolescence have the greatest protective advantage against the decrease in BMD associated with increasing age, illness, and diminished sex-steroid production. Maximizing bone mass early in life presents a critical opportunity to reduce the impact of bone loss related to aging (NIH Consensus Development Panel, 2001).

Genetic factors exert a predominant influence on peak bone mass, but physiological, environmental, and modifiable lifestyle factors can also play a significant role. Among these are adequate nutrition and body weight, exposure to sex hormones at puberty, and physical activity. Adult bone mass may also be related to fracture occurrence in childhood and adolescence.

Research indicates that reliable predictors of low bone mass are age, female gender, oestrogen deficiency, low weight or BMI, white race, family history of osteoporosis, history of prior fracture, smoking and late menarche. Inconsistent predictors are: use of carbonated drinks or caffeine beverages and childhood physical activity (*ibid*). Impaired absorption or low dietary intake of Vitamin D and calcium, and excessive use of alcohol and tobacco are risk factors for low bone mass, however the effect of moderate intake of these substances is

unclear (Moyad, 2003). In contrast, grip strength and current high impact physical activity are associated with increased bone mass (NIH Consensus Development Panel, 2001). Most of these findings are from studies of post-menopausal women, and the relative importance of these factors in premenopausal women is not as well understood (Tudor-Locke and McColl, 2000).

2.2 Measurement technologies for bone strength

The definition of osteoporosis as fragility of bone leading to fracture does not include diagnostic criteria. The operational definition developed by the WHO is in terms of bone mineral density (BMD) assessment and dual-energy x-ray absorptiometry (DEXA) is considered to be the most widely validated technology for this assessment (WHO, 2004). Osteoporosis is diagnosed in terms of standard deviations away from a healthy adult reference population (30 yrs) and is defined as 2.5 or more standard deviations (T- scores) below this mean (WHO, 2004). The primary focus on hip BMD, specifically at the femoral neck, is because this is the most common fracture site and the greatest morbidity associated with osteoporotic fracture is from fracture of the neck of femur.

DEXA is commonly available for measuring BMD in clinical settings such as hospitals, however it involves the use of radiation, is expensive and is usually only situated in urban centers. An alternative method for measuring bone quality is Quantitative Broadband Ultrasound (QUS). The correlation between heel QUS measures and heel BMD as measured by DEXA is fairly high (0.82 – 0.85: Sahara Users Guide; Hologic, 1998).

It is well established that the QUS parameters Broadband Ultrasound Attenuation (BUA) and Speed of Sound (SOS) are associated with overall bone strength – reports demonstrate correlations with bone strength of 70 – 80% (Krieg M-A *et al.*, 2008). Indeed bone strength

is related not only to bone density but also to macro - and micro-architecture and bone turnover (ibid) and QUS is thought to measure these bone qualities in addition to BMD (Gluer 2008). Specific aspects of micro-architecture that QUS is thought to measure include the number, thickness and orientation of trabeculae, and the elasticity of bone (Kroke *et al.*, 2000, Gregg *et al.*, 1999, Toyras *et al.*, 1999).

2.3 Quantitative ultrasound

The Sahara Ultrasound device measures two parameters: Speed of sound (SOS) in m/s, and Broadband ultrasonic attenuation (BUA, dB/MHZ). SOS is the distance between the two transducers divided by the time it takes for the signal to pass from one transducer through the heel to the opposite transducer. BUA is the slope the regression line for the relationship between the ultrasound attenuation and the sound frequency over the range 0.1 to 1 MHz. (McClellan *et al.*, 2000). Higher values of BUA and SOS are associated with greater bone mass. The quantitative ultrasound index (QUI) or stiffness index is a linear combination of both BUA and SOS ($QUI = (BUA + SOS) \times 0.41 - 571$). Heel QUI is highly correlated with heel BMD as measured by DEXA (Hologic, 1998, Prins *et al.*, 1998, Grampp *et al.*, 1997). Different types of QUS (dry and water based) report different parameters which has made it difficult to validate QUS technology and the official position of the International Society for Clinical Densitometry (ISCD) is that BMD from different types of QUS devices cannot be compared. The ISCD recommend using BUA and SOS and the QUI, the so-called stiffness index, if necessary (Krieg M-A *et al.*, 2008).

2.4 QUS precision and accuracy

Good quality measurements need to be both accurate and precise. Accuracy is the degree of closeness of a measured quantity to its actual value. Thus accuracy reflects the “true-ness” of

a measurement. Translated into clinical terms, QUS must be sufficiently accurate to correctly discriminate between healthy and osteoporotic subjects. Precision, also called repeatability, is defined as the degree to which repeated measurements show the same or similar results. Poor precision adds random error to results and detracts from the ability of the measurement to be used for monitoring purposes. For the Sahara QUS device, results from 6 studies on short term precision measured by the coefficients of variation for BUA, SOS and QUI were as follows. BUA: Mean = 4.1% (Range 2.7 – 5.0), SOS: Mean = 0.3% (Range 0.2 - 0.4) and QUI: Mean = 2.6 % (Range 1.6 – 3.5) (Krieg M-A *et al.*, 2008).

To maximize precision of QUS measurements when carrying out bone status assessment the quality control (QC) practices are a critical aspect because changes due to osteoporosis or its treatment are very small. As a first point of departure, the same subject should always be measured on the same machine. The complexity of the QC process will depend on whether the measurement is for a single study site or for a multi-center trial (Langton and Njeh, 2008).

Factors affecting precision

The Sahara QUS system is affected by body positioning, temperature and soft tissue properties as well as aging of the system components, specifically the phantom used in daily QC checks and the transducer pads.

Positioning of the foot is probably the major contributor to clinical imprecision. (Langton and Njeh, 2008) To obtain a precise result, anatomically consistent regions of bone must be measured. For sequential measurements used in individual monitoring, precision will be affected if the subject's heel is not correctly positioned in the heel positioner supplied by the manufacturer.

Precision of QUS can be affected by the temperature of both the environment and of the subject's limb. Peripheral temperature of skin at the calcaneum can be low, substantially lower than core body temperature especially in the colder months. Studies have shown that SOS varies significantly with temperature (Ikeda and Iki 2004, Pocock *et al.*, 2000). Over a range from 15 – 40 degrees Celsius, a strong negative correlation was demonstrated between temperature and SOS (Pocock *et al.*, 2000). Specifically, a one degree Celsius increase in temp was shown to be associated with a 3.6 m/s drop in SOS (Krieg M-A *et al.*, 2008). BUA was more stable, but nonetheless did show a small positive correlation with increased temperature (Pocock *et al.*, 2000). These opposite associations for BUA and SOS with temperature make it difficult to interpret the mechanism behind the fluctuations. From a practical standpoint, it is recommended that the temperature of the examination room should be between 20 and 27 degrees C (Sahara Hologic Guide, 1998) and that the subjects foot should be warmed if necessary prior to taking the QUS measurement (Krieg M-A *et al.*, 2008).

Transmission of SOS is similar in soft tissue and in trabecular bone. However inaccuracies of SOS and BUA caused by variation in composition and thickness of soft tissues can be significant in longitudinal studies. Thus if the amount or consistency of the soft tissue around the heel varies within an individual between measurements this can influence measurement precision. A 6mm increase in heel soft tissue thickness due to oedema was shown to result in a drop of 24 m/s in SOS (*ibid*) and a similar reduction in BUA was demonstrated in the presence of oedema (Chappard *et al.*, 2000).

Monitoring QUS precision

The daily use of the manufacturer – specific phantoms is the principal method of detecting malfunction in the Sahara device resulting from aging of the transducer pads, inadequacy of the coupling path or electrical failure at any point in the circuitry.

The properties of the phantoms do change with age and their reliability is temperature dependant. A conservative acceptable range for the daily phantom QC is provided by the manufacturers to allow operators to check the performance of the system.

The daily QC process generates two measurements: Quality Assurance Broadband Attenuation (QAB) and Quality Assurance Speed of Sound (QAS). These reflect the phantom properties as well as the machine operation and are influenced by the temperature of the room. QAB and QAS equal the measured phantom BUA and SOS divided by the actual phantom BUA and SOS that was entered at the point of manufacture. Thus, in terms of instrument performance, theoretically QAB and QAS measurements would equal 1.00 if instrument precision was 100%. However the ultrasonic properties of the phantom due to temperature are more variable than the short term precision of the Sahara (Hologic User Guide, 1998). Because of this, daily fluctuations for these QAB and QAS would occur even if the Sahara system functioned with zero variation. According to the manufacturer, if the machine passes its QC test, it is performing within its specifications. In-built precautions are such that readings beyond the limits of 1.13 or 0.87 for QAB and 1.013 or 0.987 for QAS cause the machine to default to “Repeat QC in one hour” to allow for stabilization of the phantom temperature. Subsequent failure to pass the QC indicates a malfunction of the machine, or damage to the phantom. In addition, QC readings that remain at the outer limits

over time indicate that either the machine function or the condition of the phantom is sub-optimal, with the possible loss of precision for QUS output (Hologic User Guide, 1998).

Measuring individual versus measuring large groups

Effective monitoring of bone status over time in an individual depends on the precision of the instrument as well as the magnitude of the expected change – which will in turn depend on the bone status of the individual concerned.

Thus, for 2 single measurements over time in an individual, the variation between the measurements will be the sum of the true variation within the individual and the variation due to measurement error:

$$V_{\text{total}} = V_{\text{error}} + V_{\text{within individual}}$$

With single measurements it is not possible to distinguish between the true difference and random error. If the random error of the instrument is known to be larger than the magnitude of the true expected change, the instrument cannot be used for this purpose. According to the manufacturer (Hologic, Users Guide 199) the Sahara precision error for BMD is $0.014\text{g}\cdot\text{cm}^2$. This is equivalent to 2.4 times the annual rate of loss in the US reference population (given as $0.0057\text{g}/\text{cm}^2/\text{yr}$). Using this precision error, the minimum significant change which can be determined with 95% confidence is $0.0388\text{g}/\text{cm}^2$ which corresponds to approximately 7% of the mean BMD of a 55 yr old Caucasian female. For DEXA of the spine or hip, the precision error is approximately half this rate.

Random error in QUS is due to operator error, normal biological variation as well as machine imprecision. To minimize this error, operator training can be optimized specifically with respect to positioning of the subject. Room temperature and temperature of the subject's foot can be controlled, and soft tissue influences can be taken into account. However machine

precision is dependent on further technological development and the current state of the technology precludes the use of QUS for the purpose of monitoring individuals.

In contrast, when comparing measurements of groups:

$$V_{\text{total}} = V_{\text{error}} + V_{\text{between individuals.}}$$

Since the V_{error} is random and therefore normally distributed, this quantity should not bias the group mean, thus group means can be compared. However the V_{total} will be larger when the measurement instrument is imprecise resulting in broader confidence intervals than would be the case for a more precise technique. Statistically significant comparisons for QUS therefore rely on a large sample and, as with individuals, the magnitude of the expected change.

2.5 Uses for QUS: Predictive, diagnostic, monitoring.

Prediction of osteoporotic fracture

The evidence has been controversial concerning the usefulness of QUS as a screening tool for subsequent risk of fracture. However numbers of large prospective and retrospective studies have confirmed that the predictive power of calcaneal ultrasound measurement for osteoporotic fracture at the hip and lumbar spine is as good as that of BMD measured by DEXA at these sites (Camozzi V *et al.*, 2007, Gluer, 2006, Huopio *et al.*, 2004, López-Rodríguez F *et al.*, 2003, Langton and Langton, 2000, Frost *et al.*, 1999, Bauer DC *et al.*, 1997, Hans D *et al.*, 1996). Other studies have cautioned that the accuracy of prediction of fracture from calcaneal QUS is not very high, and also that QUS should not be used in screening for individuals at risk of osteoporosis (Gemalmaz *et al.*, 2007, El-Desouki MI *et al.*, 2005, Cetin A *et al.*, 2001, Cummings SR *et al.*, 1993)

The most recent official position of the ISCD is endorsed by the American association of Clinical Endocrinologists, the American society for Bone and mineral research and The Endocrine Society. This position states that QUS measured at the heel is able to predict fragility fracture in post menopausal women and older men as well as, and independently of DEXA. The ISCD also confirms that discordant DXA and QUS results are not necessarily due to deficiencies of the device. In particular, the Sahara has been verified in substantive prospective studies for fracture prediction (Krieg M-A *et al.*, 2008)

Diagnosis of osteoporosis according to the WHO definition

A number of studies have shown QUS measurements to be only moderately correlated with BMD at the hip and lumbar spine as measured by DEXA (Lappa V *et al.*, 2007, Alexandersen P *et al.*, 2005, Babaroutsi *et al.*, 2005, Frost *et. al.*, 2001, 2000, Gluer *et al.*, 1999 Gregg *et al.*, 1997). The current consensus is that the WHO criteria cannot be applied to devices other than DEXA (Krieg M-A *et al.*, 2008). Thus T- scores from QUS cannot be used according to WHO diagnostic classification for osteoporosis because they are not equivalent to T- scores from DEXA. Indeed, it is the interpretation of QUS results that is the most controversial area and therefore the area of greatest opportunity for research.

The sensitivity and the specificity of QUS in detecting WHO defined to osteoporosis at different T-score thresholds are inadequate.

$$\text{Sensitivity} = \frac{\text{Number of True positives}}{\text{Number of True positives} + \text{Number of False negatives}}$$

A QUS sensitivity of 100% would recognize all WHO DEXA-defined osteoporotic subjects as such. Thus if QUS demonstrated high sensitivity, a negative result could be used to rule out osteoporosis.

$$\text{Specificity} = \frac{\text{Number of True negatives}}{\text{Number of True negatives} + \text{Number of False positives}}$$

A specificity of 100% means that all healthy people are recognized as healthy. For specificity, a positive result is used to rule in the disease. However any test with very high specificity may have very low precision if there are far more true negatives than true positives.

The specificity and sensitivity of QUS to detect osteoporosis are dependent on T-score threshold values. In an extensive meta-analysis (Nayak S *et al.*, 2006), it was shown that although calcaneal quantitative ultrasound can rule out DXA-determined osteoporosis with reasonable certainty in populations with low probabilities of DXA-determined osteoporosis, it has a high false positive rate. However it is less useful for screening populations with high probabilities of DXA-determined osteoporosis and cannot rule in or rule out DXA-determined osteoporosis. .

A possible solution is to establish QUS thresholds for high and low risk of osteoporosis. For individuals with QUS measures in between these two extremes, confirmation with DEXA would be required. This strategy was tested using data from the EPIDODS study on French women (aged over 74) in which both QUS and DEXA measurements were taken (Krieg M-A *et al.*, 2008). For QUI cutoffs of 59 Units and 83 Units using the Sahara system, and validated against DEXA defined osteoporosis, QUS sensitivity was 89% (11% false positives) and specificity was 87% (13% false negatives) (Krieg M-A *et al.*, 2008)

Monitoring response to treatment.

The ability of technology to monitor individual changes in response to interventions requires excellent precision and is also determined by the range of response to the intervention.

Changes in BMD following anti-resorptive therapy are known to be small and further

randomized controlled trials (RCTs) are needed to determine whether QUS parameters are sufficiently sensitive to detect these changes (Hans and Krieg, 2008). While QUS, especially SOS, is precise in the short term, only DXA is recommended by the ISCD for monitoring long term to ascertain significant change (Krieg M-A *et al.*, 2008). Insufficient numbers of RCTs, studies with low patient numbers and equivocal findings are deficiencies in the current state of research in this area. To progress, further quality control and quality assurance methods to check device stability also need to be established (Hans *et al.*, 2002).

2.6. The South African context

Currently, there is limited and controversial epidemiologic data on BMD and fracture incidence in the different ethnic populations in South Africa (Conradie *et al.*, 2005, Hough 2003, Solomon 1979). Cross sectional data found femoral neck BMD to be higher in black than in white South African women, however BMD at the lumbar spine was equivalent (Daniels ED *et al.*, 1995). Only one previous study has explored bone health among the mixed race community (Micklesfield *et al.*, 2007), and this was limited to children. The majority of South Africa's poorer black and mixed race population attend health centers for primary and secondary level health care where there is no access to DEXA. Also, research efforts into public health issues are generally carried out at these community-based health care facilities. In such resource-limited settings, it would be highly desirable to use the more portable and cheaper ultrasound method for conducting epidemiological studies of bone quality rather than rely on referral to a tertiary care facility in an urban center with all the associated problems of access and affordability.

3.1 AIM

The aim of this study was to determine whether Quantitative Ultrasound is sufficiently accurate and precise to be used in epidemiological studies of bone strength in black and mixed race premenopausal South African women.

3.2 OBJECTIVES

1. To describe BUA, SOS and QUI in black and mixed race premenopausal South African women.
2. To determine whether factors associated with QUS measures of bone quality are similar to those known to be associated with BMD as measured by DEXA.
3. To compare QUS associations with those reported in the literature for other populations of premenopausal women.
4. To monitor the QC output from the two QUS machines used in the study to ensure adequate precision throughout the duration of the study.
5. To validate our results in a separate small sample by comparing output from both QUS devices used in the study to output from DEXA.

4. METHODS

4.1 Overview of the Study

The data were derived from of a cross sectional study aimed at determining the effects of injectable progestin contraceptive use on bone mass in premenopausal women (Rosenberg *et al.*, 2007). The study was conducted between September 2002 and September 2005 at community health facilities in two areas – Gugulethu and Mitchells Plain, both situated close to Cape Town in the Western Cape Province, South Africa. Black women attended the Gugulethu facility and women of mixed race attended Mitchells Plain. These populations were selected to meet the aim of the original study. White women not included as very few have been exposed to injectable progestin contraceptives. Sampling was on a convenience basis and each of the two sites was allocated a Sahara Ultrasound machine (Hologic) for the duration of the study in order to measure bone mass.

A structured questionnaire was administered by trained nurse interviewers in the subject's preferred language (English, Afrikaans or Xhosa), and each subject's height, weight and calcaneal ultrasound measurements were taken. The study protocol was approved by the IRBs of Boston University and the University of Cape Town.

4.2 Study subjects

Women eligible for the study were aged 18-44, premenopausal, had not been pregnant or had not breastfed in the previous year, and did not have an illness or use medication that would influence their bone health. Medical conditions for exclusion from the study were illnesses requiring bed-rest for more than 6 weeks in the past 12 months, thyroid, parathyroid and pituitary disease, cancer, sarcoidosis, rheumatoid arthritis, chronic liver and kidney disease, rickets, Pagets disease and osteoporosis. Women taking anticonvulsants, systemic

corticosteroids, drugs for hypo, hyper-thyroidism or parathyroidism, thiazide diuretics or calcium supplements for more than six months in the last five years were also excluded.

Written informed consent was obtained from all subjects.

Of the 4362 women approached, 3957 were willing to participate, of whom 413 were excluded for failing to meet study criteria or because the Sahara measurement failed, leaving 3544 women. We excluded 51 from the analysis because of ultrasound measures below or above the 0.5 percentile of measures. This equated to broadband ultrasonic attenuation (BUA) ≤ 45 and ≥ 117 or speed of sound (SOS) ≤ 1500 and ≥ 1645.5 , leaving 3493 women, of whom 1598 were black and 1895 were of mixed race.

4.3 Interview

The study nurses administered standard questionnaires to collect information on demographic factors, reproductive and contraceptive history, and lifestyle factors.

Information was obtained on age, ethnicity, highest standard passed at school, training after school and employment status. Details were elicited for marital status, age at menarche, age at each pregnancy and contraceptive use before and in intervals between pregnancies. These latter details were required specifically for the original study on use of injectable contraceptives and bone health.

Alcohol and smoking

Information was obtained for never, past or current use, as well as duration of alcohol intake in years and quantity of week and weekend drinking. For current and past cigarette smokers, women were asked about the number of cigarettes smoked per day and years of smoking as well as years since last smoked for past smokers.

Current calcium intake

Questions were based on a previous study that evaluated intake of calcium rich food in a similar population in terms of daily intake of dairy servings (1 dairy serving = 1 glass of milk) and which had been validated against three day records (Micklesfield, unpublished data). Women were asked about the quantity and frequency of milk drinks, milk in coffee tea and cereal, as well as frequency of eating cheese, pizza, macaroni and cheese and sardines.

Physical activity

The physical activity section of the questionnaire was adapted to the South African context (Micklesfield *et al.*, 2003, Kriske *et al.*, 1988). A previous study on black and mixed race women aged 22 to 59 (Micklesfield *et al.*, 2003) showed that adjusted BMD of the femoral neck, as measured by DEXA, was most strongly associated with physical activity that was undertaken during adolescence (14 – 21 years). In Micklesfield's study, activities were categorized into household, occupational, leisure time and transport, and results showed that walking for transport and walking with loads, as well as sporting activities were the best predictors of total proximal femur BMD. These results informed the design of our own questionnaire as to which physical activities to focus on, and how to measure them.

For our study historical information on physical activity was obtained for three epochs – primary school, high school and post-school. Information was obtained on walking for transport, for daily tasks (e.g. carrying water) and at work, as well as on leisure-time activity and sports participation.

For each of these activities, if the duration was above 30 minutes, we recorded information on the number of hours and minutes, number of days per week, number of months per year and numbers of years

To calculate the total amount of hours for each activity in each epoch the following algorithm was used.

$$\text{Total Hours} = (\text{Total minutes /day}) /60 * (\text{Total days/week}) * 4.3 * (\text{Total months /year}) * (\text{Total years})$$

To take account of load bearing during activities, the impact of loading from each physical activity was ranked on a 0 to 3 scale with “0” indicating non-weight-bearing and “3” indicating high impact. (Groothausen *et al.*, 1997). Impact factors for walking activities involving herding cattle, walking to public transport, for pleasure, to the shops scored 1. Walking to fetch water and walking to collect wood – in both cases carrying load halfway scored 1.5. Walking carrying a child (both ways) scored 2.

Leisure and sporting activity impact scores are shown below in Table 1.

Table 1: Impact factors allocated to sports and leisure activities

| Activity | Impact factor |
|---|---------------|
| Swimming, Cycling | 0 |
| Walking | 1 |
| Jogging, Tennis, Dancing Athletics, Hockey, Softball/soccer | 2 |
| Volley-ball, netball, Aerobic dancing | 3 |

Total impact hours (TIH) were calculated for each activity in each of the three epochs by multiplying total hours by the impact factor. Thus activities with an impact factor of 0 did not count toward the TIH. As the range of TIH for was very large, we limited our analysis to quartiles for each epoch.

4.4 Measurement

Weight and height measurements were taken using a SECA (Hamburg, Germany) scale and SECA height measure with participants wearing only light clothing and no shoes. Body Mass Index (BMI) was calculated as weight divided by height squared in kg/m^2 . Bone mass was assessed using a gel-coupled (dry) quantitative ultrasound system, the Sahara (Hologic: SN 03281, SN 03278).

4.5 Quality Control

Operator Proficiency

The study nurses were trained in using the QUS machines until they achieved proficiency such that the coefficient of variation (CV) for repeated measures of the same subject was within the machine specification as laid down by Hologic.

Instrument Quality Control

During the study proper, on a daily basis a phantom was used to check the quality control (QC) of each machine. The machine output for this procedure is in terms of QAB and QAS. These are dimensionless quantities calculated by dividing the phantom BUA and SOS measured in the calibration procedure by the values that were specified for that phantom by the manufacturer. Thus $QAB = \text{Phantom BUA (QC measurement)} / \text{Phantom BUA (manufacturer specification)}$. Likewise $QAS = \text{Phantom SOS (QC)} / \text{Phantom SOS (specification)}$. QAB and QUS readings were required to fall within the limits of 0.86–1.14 and 0.986–1.014 respectively, with optimum readings of 1.00 for both. If the machine did not pass the QC, it was recalibrated. If it continued to fail QC, the transducers pads were replaced. The results for this daily QC check were evaluated throughout the study and are reported here as monthly mean QAB and QAS. If over a number of weeks, the mean QC

values for a machine drifted outside Sahara specification for QAB and QAS, but still passed its QC test, the machine was recalibrated.

4.6 Comparison of QUS machines

To compare the output from the two QUS machines, measurements on a number of subjects were done on each of the two machines by 1 operator at the same visit, the measurement on the second machine being done immediately after the first. These trials were done on three separate occasions over the duration of the study - on October 2003 (N=50), October 2004 (N=48) and October 2005 (N=48). The results were pooled to allow for comparison of mean BUA and mean SOS between the two machines over a range of response similar to that which we expected in the study proper.

4.7 Agreement between QUS and DEXA : Small Study

To assess correlation of the QUS measures with DEXA measures, a sample of 14 young women who had been recruited for another study were measured on both QUS machines and had a DEXA scan (DXA Hologic, Model: Discovery W S/N 80196) . These measurements were taken at the MRC/UCT Research Unit for Exercise Science and Sports Medicine at the same visit. Different operators performed the DEXA and the QUS measurements respectively. For DEXA, BMD results for femoral neck, femoral trochanter, total hip (the combination of femoral neck, femoral trochanter and intertrochanteric areas), and total lumbar spine (L1-4) are reported here. These are compared to BUA, SOS and estimated BMD from both Ultrasound machines separately.

4.8 Statistical Analysis

Categories for Risk factors

True categorical data (ethnicity, nulli-parity and never, past or current smoking or alcohol use) were left in their natural categories for analysis. The distributions of the continuous risk factor variables were examined for the study sample as a whole, and categories were formed for each factor. Decisions for category boundaries were made based on both the distributional characteristics of the variable as well as its biological nature. Physical activity in the different epochs and calcium intake were analyzed by quartile.

Distributions of the risk factors were calculated separately for black and mixed race women. Medians were compared for the two study groups using Kruskal-Wallis tests, and distributions across categories were compared using Chi-squared tests.

Multivariable Analysis

To systematically explore the effects of risk factors on BUA, SOS and QUI, we first calculated the crude means and standard deviations for these QUS measures for all categories of all risk factors for which we had data. Using these results, and our a priori knowledge from the literature of expected associations of QUS measures with age and with BMI, we calculated age-adjusted means and adjusted means for age + each risk factor for three strata of BMI for black and mixed race women separately. This was done for BUA, SOS and QUI. These results helped to inform our final multivariable model in which we decided to include age, height, BMI, education, age at menarche, nulli-parity, smoking and alcohol use, calcium intake and early physical activity. Adding contraceptive use did not affect the results. We obtained adjusted means of BUA, SOS and QUI using linear regression models and then tested the linear dose response relationship by entering the median value for

the exposure category of interest into a term in the regression model. To further investigate residual confounding by BMI of height, physical activity and age at menarche, the multivariable regression model was again tested according to three strata of BMI. Smoking was further analyzed among mixed race women but this was not possible in black women due to low numbers of smokers. Adjusted means were calculated for ever versus never smokers, for numbers of cigarettes smoked per day and for smoking years * numbers of cigarettes smoked per day.

Quality Control

Means, standard deviations and coefficients of variation were calculated for the operator and instrument quality control trials performed prior to commencement of the study proper.

For instrument QC over the duration of the study, monthly means for QAB and QAS were calculated and plotted on graphs to allow for the detection of any seasonal trend or consistent deviation beyond the recommended QC limits.

Comparison of QUS machines

T-tests of mean differences for BUA and SOS between the two machines were used to determine whether these differences were significant. In addition, BMD values from the two QUS machines generated in the separate small study (N=14) were compared using the Bland-Altman method to test for agreement.

Comparison of QUS and DEXA

Comparisons of QUS output and DEXA measurements were made using Pearson correlation coefficients as well as Bland-Altman analyses for determining agreement between different measurement methods. All analyses were performed using STATA software version 9.0 (StataCorp LP, Texas)

5. RESULTS

5.1. Description of subjects

The demographic, socioeconomic and reproductive characteristics of the study sample by ethnicity are shown in Table 2. Black women were slightly younger, were taller and had higher BMIs. For black women median age, height and BMI were 27 yrs, 159 cm and 27 kg/m² and for mixed race women these were 28 yrs, 154 cm and 25 kg/m². Black women had completed more years of education than women of mixed race - median education levels were Grade 11 and Grade 9 respectively. In addition, black women experienced menarche later than mixed race women (Median age at menarche = 15 years and 13 years), and had fewer live births (Median number of live births = 1 for black women and 2 for mixed race women). Using Kruskal-Wallis tests, age differences were significant at $p < 0.05$ while all other differences reached significance levels of $p < 0.001$.

For lifestyle characteristics (Table 3), black women drank less alcohol and smoked less. 90 % of black women had never smoked whereas 67% of mixed race women were smokers, either current or past. Among those who had ever smoked, the median number of cigarettes smoked per day and the median number of years smoking were 3 cigarettes per day and 2 years for black women; and 6 cigarettes per day and 4 years for mixed race women. Black women had a lower calcium intake than mixed race women (median daily servings = 8 and 13 respectively). For all of the physical activity epochs, total impact hours for physical activity were better analyzed in quartiles as the distributions were notably wide and skew.

Comparing distributions across quartiles, black women engaged in more physical activity in primary school, high school as well as after leaving school than mixed race women. All

lifestyle differences were significant at $p < 0.001$, using Kruskal-Wallis and Chi-squared tests.

Table 2: Demographic, socioeconomic and reproductive characteristics of study participants

| Characteristic | Black Subjects N=1598 N (%) | Mixed race Subjects N=1895 N (%) |
|---------------------------------|-----------------------------------|--|
| Age (years) * | | |
| 18 – 19 | 141 (8.8) | 177 (9.3) |
| 20 – 24 | 422 (26.4) | 512 (27.0) |
| 25 – 29 | 373 (23.3) | 363 (19.2) |
| 30 – 34 | 299 (18.7) | 311 (16.4) |
| 35 – 39 | 227 (14.2) | 284 (15.0) |
| 40 – 44 | 136 (8.5) | 248 (13.1) |
| Mean (Med) SD | 28.4 (27) 7.68 | 29.1 (28) 7.68 |
| BMI (kg/m²)** | | |
| < 23 | 235 (14.7) | 638 (33.7) |
| 23 - 24 | 221 (13.8) | 263 (13.9) |
| 25 - 29 | 533 (33.4) | 507 (26.7) |
| 30 - 34 | 386 (24.2) | 306 (16.2) |
| 35+ | 223 (13.9) | 181 (9.5) |
| Mean (Med) SD | 28.4 (27) 5.73 | 26.0 (25) 6.01 |
| Height (cm) ** | | |
| < 153 | 211 (13.2) | 762 (40.2) |
| 153 – 157 | 386 (24.2) | 491 (25.9) |
| 158 – 161 | 466 (29.1) | 408 (21.5) |
| 162+ | 535 (33.5) | 234 (12.4) |
| Mean (Med) SD | 159.0 (159) 5.62 | 154.7 (154) 6.41 |
| Education** | | |
| Grade 9 or less | 453 (28.4) | 1000 (52.8) |
| Grade 10 – 11 | 681 (42.6) | 580 (30.6) |
| Grade 12 | 464 (29.0) | 315 (16.6) |
| Median (Range) | 11 (0 ; 12) | 9 (0 ; 12) |
| Age at Menarche** | | |
| < 13 | 120 (7.5) | 591 (31.2) |
| 13 – 14 | 576 (36.1) | 771 (40.7) |
| 15 | 340 (21.3) | 231 (12.2) |
| 16+ | 427 (26.7) | 301 (15.9) |
| Missing | 135 (8.5) | 1 (0.1) |
| Mean (Med) SD | 14.7 (15) 1.75 | 13.5 (13) 1.90 |
| Parity** | | |
| None | 534 (33.4) | 286 (15.1) |
| 1+ | 1064(66.6) | 1609 (84.9) |
| Med live births (Range) | 1 (0 ; 6) | 2 (0 ; 7) |

* $p < 0.05$

** $p < 0.001$

Table 3: Lifestyle characteristics of study participants

| Characteristic | Black Subjects N=1598 N (%) | Mixed race Subjects N=1895 N (%) |
|---|--|---|
| Smoking Status** | | |
| Ever smoked** | 159 (9.9) | 1283 (67.7) |
| Never smoked | 1439 (90.1) | 612 (32.3) |
| Non-smoker** | 1439 (90.1) | 612 (32.3) |
| Ex-smoker | 59 (3.7) | 138 (7.3) |
| Current smoker | 100 (6.3) | 1145 (60.4) |
| Median (Range) cigarettes smoked (Ever smokers only)** | 3 (1 ; 20) | 6 (1 ; 60) |
| Median (Range) years of smoking (Ever smokers only)** | 2 (1 ; 5) | 4 (1 ; 5) |
| Alcohol Use** | | |
| Non-drinker | 1167 (73.0) | 998 (52.7) |
| Ex-drinker | 112 (7.0) | 210 (11.1) |
| Current drinker | 319 (20.0) | 687 (36.3) |
| Quartile of Calcium Intake** | | |
| 1 (0 – 5.25) | 540 (33.8) | 359 (18.9) |
| 2 (5.3 – 10.75) | 482 (30.1) | 401 (21.2) |
| 3 (10.8 – 15.25) | 287 (18.0) | 615 (32.5) |
| 4 (15.3 – 50.25) | 289 (18.1) | 520 (27.4) |
| Mean (Med) SD | 9.5 (8) 7.34 | 12.3 (13) 6.80 |
| Quartile of Primary School Physical Activity (TIH†)** | | |
| 1 (0 - 310) | 236 (14.8) | 638 (33.7) |
| 2 (311 – 1118) | 272 (17.0) | 600 (31.7) |
| 3 (1119 – 2582) | 389 (24.3) | 485 (25.6) |
| 4 (2583 – 31579) | 700 (43.8) | 172 (9.1) |
| Missing | 1 (0.1) | |
| Mean (Med) SD | 3364 (2124) 3648 | 1060 (722) 1284 |
| Quartile of High School Physical Activity (TIH†)** | | |
| 1 (0) | 631 (39.5) | 811 (42.8) |
| 2 (1 - 258) | 61 (3.8) | 254 (13.4) |
| 3 (259 - 986) | 353 (22.1) | 510 (26.9) |
| 4 (987 - 11834) | 553 (34.6) | 320 (16.9) |
| Mean (Med) SD | 929 (473) 1346 | 491 (917) 821 |
| Quartile of After School Physical Activity (TIH†)** | | |
| 1 (0) | 786 (49.2) | 664 (35.0) |
| 2 (1 - 413) | 63 (3.9) | 247 (13.0) |
| 3 (414 - 4128) | 262 (16.4) | 604 (31.9) |
| 4 (4129 - 52942) | 486 (30.4) | 380 (20.1) |
| Missing | 1 (0.1) | |
| Mean (Med) SD | 8408 (5582) 8680 | 4102 (2413) 5131 |

† Total Impact Hours

* p<0.05

** p<0.001

5.2 Exploratory analysis (Tables 4 and 5)

In black women there were appreciable differences in BUA, SOS and QUI across the BMI strata, and adding age to the model consistently altered the mean QUS values. In general, within BMI strata the mean age-adjusted QUS parameters were altered only by a small amount when adding additional variables to the age-adjusted mean. For BUA, adding age at menarche altered the age-adjusted mean in all BMI strata. Height and total physical activity also had a small effect. Age-adjusted mean SOS was somewhat influenced by age at menarche and to a lesser extent by height, number of live births, education, injectable and oral contraceptive use. Factors affecting QUI were similar to those for BUA and SOS combined. Other variables did not alter the age-adjusted mean.

For mixed race women there were also appreciable differences across the BMI strata, and again age was associated with BUA, SOS and QUI. For BUA, adding age at menarche, height, number of live births, Oral Contraceptive (OC) use, calcium intake and total physical activity resulted in small changes. This was the same for SOS and QUI except that physical activity in primary school rather than total physical activity predominated, and live births predominated over age at menarche. Opposite effects were present for height in the lowest and highest BMI strata.

Following this exploratory analysis, and guided by what is published in the literature on factors associated with DEXA BMD, it was decided to include the following variables in our multivariable model: Age, BMI, height, education, age at menarche, number of live births, alcohol use, cigarette smoking, Calcium (Ca) intake and physical activity level at primary school.

The effects of injectable contraceptive (IC) use on bone mass are reported elsewhere (Rosenberg *et al.*, 2006).

Table 4 Exploratory analysis for QUS measures in Black women (N=1598)

| | BMI<25 N= 456 | BMI 25-29.9 N=534 | BMI 30+ N=608 |
|---|-----------------------------------|------------------------------------|--------------------------------|
| Mean BUA | 76.9 | 80.7 | 85.0 |
| Mean BUA adj for age | 77.2 | 80.8 | 84.7 |
| Mean BUA adj for age & height | 77.1 | 80.8 | 84.7 |
| Mean BUA adj for age & age at menarche (excl missing) | 77.0 | 80.9 | 84.5 |
| Mean BUA adj for age & live births | 77.2 | 80.8 | 84.7 |
| Mean BUA adj for age & education | 77.2 | 80.8 | 84.7 |
| Mean BUA adj for age & ICuse (never,past. current) | 77.2 | 80.8 | 84.7 |
| Mean BUA adj for age & OCuse (never,past. current) | 77.2 | 80.8 | 84.7 |
| Mean BUA adj for age & smoking (never,past. current) | 77.2 | 80.8 | 84.7 |
| Mean BUA adj for age & alcohol (never,past. current) | 77.2 | 80.8 | 84.7 |
| Mean BUA adj for age & Ca intake | 77.2 | 80.8 | 84.7 |
| Mean BUA adj for age & Total exercise | 77.2 | 80.9 | 84.7 |
| Mean BUA adj for age & Primary school exercise | 77.2 | 80.8 | 84.7 |
| | BMI<25 | BMI 25-29.9 | BMI 30+ |
| Mean SOS | 1561.1 | 1563.0 | 1565.3 |
| Mean SOS adj for age | 1561.3 | 1563.1 | 1565.1 |
| Mean SOS adj for age & height | 1561.7 | 1563.0 | 1564.9 |
| Mean SOS adj for age & age at menarche(excl missing) | 1560.9 | 1563.0 | 1564.9 |
| Mean SOS adj for age & live births | 1561.2 | 1563.1 | 1565.2 |
| Mean SOS adj for age & education | 1561.0 | 1563.1 | 1565.3 |
| Mean SOS adj for age & ICuse (never,past. current) | 1561.0. | 1563.2 | 1565.2 |
| Mean SOS adj for age & OCuse (never,past. current) | 1561.4 | 1563.0 | 1565.1 |
| Mean SOS adj for age & smoking (never,past. current) | 1561.3 | 1563.1 | 1565.1 |
| Mean SOS adj for age & alcohol (never,past. current) | 1561.3 | 1563.1 | 1565.1 |
| Mean SOS adj for age & Ca intake | 1561.3 | 1563.1 | 1565.1 |
| Mean SOS adj for age & Total exercise | 1561.3 | 1563.1 | 1565.1 |
| Mean SOS adj for age & Primary school exercise | 1561.3 | 1563.1 | 1565.1 |
| | BMI<25 | BMI 25-29.9 | BMI 30+ |
| Mean QUI | 100.6 | 102.9 | 105.7 |
| Mean QUI adj for age | 100.8 | 103.0 | 105.4 |
| Mean QUI adj for age & height | 100.9 | 103.0 | 105.3 |
| Mean QUI adj for age & age at menarche (excl missing) | 100.6 | 103.0 | 105.3 |
| Mean QUI adj for age & live births | 100.8 | 103.0 | 105.4 |
| Mean QUI adj for age & education | 100.8 | 103.0 | 105.4 |
| Mean QUI adj for age & ICuse (never,past. current) | 100.7 | 103.1 | 105.5 |
| Mean QUI adj for age & OCuse (never,past. current) | 100.8 | 103.0 | 105.4 |
| Mean QUI adj for age & smoking (never,past. current) | 100.8 | 103.0 | 105.4 |
| Mean QUI adj for age & alcohol (never,past. current) | 100.8 | 103.0 | 105.4 |
| Mean QUI adj for age & Ca intake | 100.8 | 103.0 | 105.4 |
| Mean QUI adj for age & Total exercise | 100.8 | 103.1 | 105.4 |
| Mean QUI adj for age & Primary school exercise | 100.8 | 103.0 | 105.4 |

Table 5 Exploratory analysis for QUS measures in mixed race women (N=1895)

| | BMI<25 | BMI 25-29.9 | BMI 30+ |
|--|------------------|--------------------|----------------|
| Mean BUA | 75.5 | 78.5 | 80.5 |
| Mean BUA adj for age | 75.7 | 78.4 | 80.2 |
| Mean BUA adj for age & height | 75.7 | 78.4 | 80.1 |
| Mean BUA adj for age & age at menarche | 75.8 | 78.4 | 80.1 |
| Mean BUA adj for age & live births | 75.6 | 78.5 | 80.3 |
| Mean BUA adj for age & education | 75.7 | 78.4 | 80.2 |
| Mean BUA adj for age & ICuse (never,past. current) | 75.7 | 78.4 | 80.2 |
| Mean BUA adj for age & OCuse (never,past. current) | 75.7 | 78.3 | 80.1 |
| Mean BUA adj for age & smoking (never,past. current) | 75.7 | 78.4 | 80.2 |
| Mean BUA adj for age & alcohol (never,past. current) | 75.7 | 78.4 | 80.2 |
| Mean BUA adj for age & Ca intake | 75.7 | 78.4 | 80.1 |
| Mean BUA adj for age & Total exercise | 75.7 | 78.4 | 80.1 |
| Mean BUA adj for age & Primary school exercise | 75.7 | 78.4 | 80.2 |
| | BMI<25 | BMI 25-29.9 | BMI 30+ |
| Mean SOS | 1561.6 | 1559.2 | 1560.5 |
| Mean SOS adj for age | 1561.3 | 1559.8 | 1560.8 |
| Mean SOS adj for age & height | 1561.6 | 1559.8 | 1560.2 |
| Mean SOS adj for age & age at menarche | 1561.3 | 1559.7 | 1560.7 |
| Mean SOS adj for age & live births | 1560.9 | 1560.0 | 1561.2 |
| Mean SOS adj for age & education | 1561.2 | 1559.8 | 1560.8 |
| Mean SOS adj for age & ICuse (never,past. current) | 1561.3. | 1559.7 | 1560.9 |
| Mean SOS adj for age & OCuse (never,past. current) | 1561.3. | 1559.7 | 1560.7 |
| Mean SA adj for age & smoking (never,past. current) | 1561.4 | 1559.8 | 1560.7 |
| Mean SOS adj for age & alcohol (never,past. current) | 1561.3 | 1559.8 | 1560.8 |
| Mean SOS adj for age & Ca intake | 1561.3 | 1559.9 | 1560.8 |
| Mean SOS adj for age & Total exercise | 1561.3 | 1559.8 | 1560.8 |
| Mean SOS adj for age & Primary school exercise | 1561.2 | 1559.8 | 1561.0 |
| | BMI<25 | BMI 25-29.9 | BMI 30+ |
| Mean QUI | 100.2 | 100.6 | 101.8 |
| Mean QUI adj for age | 100.2 | 100.7 | 101.8 |
| Mean QUI adj for age & height | 100.3 | 100.7 | 101.5 |
| Mean QUI adj for age & age at menarche | 100.2 | 100.6 | 101.7 |
| Mean QUI adj for age & live births | 100.0 | 100.8 | 102.0 |
| Mean QUI adj for age & education | 100.1 | 100.7 | 101.8 |
| Mean QUI adj for age & ICuse (never,past. current) | 100.1 | 100.6 | 101.8 |
| Mean QUI adj for age & OCuse (never,past. current) | 100.2 | 100.6 | 101.7 |
| Mean QUI adj for age & smoking(never,past. current) | 100.2 | 100.7 | 101.7 |
| Mean QUI adj for age & alcohol (never,past. current) | 100.2 | 100.7 | 101.8 |
| Mean QUI adj for age & Ca intake | 100.2 | 100.7 | 101.8 |
| Mean QUI adj for age & Total exercise | 100.2 | 100.7 | 101.8 |
| Mean QUI adj for age & Primary school exercise | 100.1 | 100.7 | 101.9 |

5.3 Final multivariable analysis.

In black subjects (Table 6), the fully adjusted means for all QUS measures – BUA, SOS and QUI increased significantly with increasing age and with increasing BMI. All three measures decreased significantly with increasing age at menarche. SOS and QUI decreased with increase in height and were also lower among parous women when compared with nulliparous women. BUA was not affected by these variables. There were small but nonsignificant positive associations of BUA, SOS and QUI with physical activity in primary school. There was no association between the three QUS measures and education level, alcohol use, cigarette smoking and current calcium intake.

In women of mixed race (Table 7), fully adjusted mean BUA and QUI increased significantly with increasing age but for SOS, there was an increase which was nonsignificant. BUA increased significantly with increasing BMI but SOS decreased slightly. As a result, QUI also increased with BMI but to a lesser extent. SOS and QUI decreased as height increased, with a similar but weaker trend for BUA. All three QUS measures were significantly lower in parous compared to nulliparous women. All measures also decreased with increasing age at menarche, but none of the trends was significant. In addition BUA, SOS and QUI were all significantly and positively associated with primary school physical activity. There was a weak association of decreased SOS with smoking. No associations were found with education level, alcohol intake or calcium intake.

Further stratified analysis of associations between QUS measures and height, QUS and age at menarche, and QUS and physical activity, according to three levels of BMI (<25 kg/m², 25 – 29 kg/m², 30+ kg/m²) did not reveal any differences as compared with the original non-stratified analysis (For Tables: See Appendix 1).

Table 6. Associations of risk factors with BUA, SOS and QUI among Black women

| Risk factor | BUA Mean (SD) | BUA Adj LSM* | P for trend | SOS Mean (SD) | SOS Adj LSM* | P for trend | QUI Mean (SD) | QUI Adj LSM* | P for trend |
|-------------------------------|--------------------------|-------------------------|------------------------|--------------------------|-------------------------|------------------------|--------------------------|-------------------------|------------------------|
| Age (years) | | | 0.05 | | | 0.039 | | | 0.027 |
| 18 – 19 | 77.1 (12.3) | 78.8 | | 1564 (25.1) | 1561 | | 102.0 (14.2) | 101.5 | |
| 20 – 24 | 80.5 (13.3) | 81.4 | | 1563 (24.8) | 1563 | | 103.1 (14.3) | 103.1 | |
| 25 – 29 | 81.4 (12.6) | 81.2 | | 1561 (25.2) | 1562 | | 102.5 (14.2) | 102.7 | |
| 30 – 34 | 80.6 (13.4) | 80.1 | | 1561 (27.2) | 1562 | | 102.1 (15.6) | 102.3 | |
| 35 – 39 | 83.4 (14.1) | 82.6 | | 1566 (25.4) | 1567 | | 105.3 (15.1) | 105.2 | |
| 40 – 44 | 85.2 (14.3) | 83.8 | | 1568 (28.1) | 1568 | | 106.9 (16.4) | 106.3 | |
| BMI (kg/m²) | | | <0.001 | | | 0.044 | | | <0.001 |
| < 23 | 76.2 (12.6) | 76.4 | | 1562 (25.3) | 1562 | | 100.5 (14.6) | 100.6 | |
| 23 - 24 | 77.6 (13.0) | 77.9 | | 1561 (25.6) | 1561 | | 100.7 (14.7) | 100.9 | |
| 25 - 29 | 80.7 (12.9) | 80.8 | | 1563 (25.7) | 1563 | | 102.9 (14.6) | 103.1 | |
| 30 - 34 | 84.7 (13.2) | 84.5 | | 1565 (25.0) | 1565 | | 105.3 (14.5) | 105.3 | |
| 35+ | 85.5 (13.1) | 85.1 | | 1566 (28.1) | 1565 | | 106.2 (15.6) | 105.6 | |
| Height (cm) | | | 0.204 | | | <0.001 | | | 0.037 |
| < 153 | 79.7 (13.9) | 79.5 | | 1566 (26.5) | 1566 | | 103.9 (15.1) | 103.8 | |
| 153 – 157 | 82.3 (14.0) | 81.9 | | 1567 (26.9) | 1567 | | 105.2 (15.7) | 105.0 | |
| 158 – 161 | 81.1 (12.8) | 81.0 | | 1562 (24.9) | 1563 | | 102.8 (14.3) | 102.8 | |
| 162+ | 81.2 (13.1) | 81.6 | | 1561 (25.2) | 1561 | | 102.1 (14.6) | 102.4 | |
| Education | | | 0.497 | | | 0.831 | | | 0.962 |
| Grade 9 or less | 81.5 (13.9) | 80.8 | | 1565 (26.6) | 1564 | | 103.9 (15.4) | 103.2 | |
| Grade 10 – 11 | 81.1 (13.3) | 81.3 | | 1563 (25.6) | 1563 | | 103.1 (14.8) | 103.3 | |
| Grade 12 | 81.2 (13.0) | 81.5 | | 1563 (25.4) | 1563 | | 103.0 (14.5) | 103.3 | |
| Age at Menarche | | | 0.060 | | | 0.009 | | | 0.010 |
| < 13 | 82.8 (13.9) | 83.4 | | 1567 (23.5) | 1566 | | 105.4 (14.3) | 105.5 | |
| 13 – 14 | 81.2 (12.9) | 81.3 | | 1564 (25.7) | 1564 | | 103.5 (14.7) | 103.6 | |
| 15 | 80.9 (13.3) | 80.8 | | 1564 (26.7) | 1564 | | 103.3 (15.0) | 103.3 | |
| 16+ | 80.8 (13.5) | 80.6 | | 1560 (25.5) | 1560 | | 101.9 (14.9) | 101.7 | |

| | | | | | | | | | |
|-----------------------------------|-------------|------|--------------------|-------------|------|--------------------|--------------|-------|--------------------|
| Live Births | | | 0.926 | | | 0.003 | | | 0.000 |
| None | 79.8 (13.0) | 81.2 | | 1565 (24.5) | 1567 | | 103.5 (14.2) | 104.8 | |
| 1+ | 82.0 (13.5) | 81.3 | | 1562 (26.4) | 1562 | | 103.2 (15.2) | 102.5 | |
| Primary School PA (TIH‡) | | | 0.183 | | | 0.842 | | | 0.534 |
| 1 (0 - 310) | 79.8 (13.5) | 79.8 | | 1562 (25.9) | 1562 | | 102.1 (14.8) | 102.0 | |
| 2 (311 – 1118) | 81.4 (13.1) | 81.6 | | 1565 (26.7) | 1565 | | 104.1 (15.0) | 104.3 | |
| 3 (1119 – 2582) | 80.9 (12.9) | 81.0 | | 1563 (25.1) | 1563 | | 103.0 (14.5) | 103.0 | |
| 4 (2583 – 31579) | 81.9 (13.7) | 81.8 | | 1563 (25.9) | 1563 | | 103.5 (15.0) | 103.5 | |
| Smoking Status | | | 0.818 [†] | | | 0.865 [†] | | | 0.847 [†] |
| Non-smoker | 81.3 (13.2) | 81.2 | | 1563 (25.7) | 1563 | | 103.2 (14.8) | 103.2 | |
| Ex-smoker | 80.8 (15.7) | 81.6 | | 1566 (27.0) | 1565 | | 104.0 (15.6) | 103.9 | |
| Current smoker | 81.3 (14.7) | 82.1 | | 1565 (26.6) | 1564 | | 103.8 (15.9) | 104.0 | |
| Alcohol Use | | | 0.323 [†] | | | 0.304 [†] | | | 0.419 [†] |
| Non-drinker | 81.5 (13.2) | 81.6 | | 1563 (26.0) | 1564 | | 103.4 (14.8) | 103.5 | |
| Ex-drinker | 80.3 (14.5) | 80.3 | | 1566 (25.4) | 1566 | | 104.1 (15.3) | 104.0 | |
| Current drinker | 80.5 (13.7) | 80.3 | | 1563 (25.4) | 1562 | | 102.8 (15.0) | 102.3 | |
| Quartile of Calcium Intake | | | 0.851 | | | 0.686 | | | 0.695 |
| 1 (0 – 5.25) | 81.3 (13.9) | 81.2 | | 1563 (25.9) | 1563 | | 103.1 (15.1) | 103.1 | |
| 2 (5.3 – 10.75) | 81.0 (12.8) | 81.2 | | 1563 (26.1) | 1563 | | 103.1 (14.7) | 103.1 | |
| 3 (10.8 – 15.25) | 81.2 (13.7) | 81.1 | | 1565 (26.0) | 1565 | | 104.1 (15.0) | 104.0 | |
| 4 (15.3 – 50.25) | 81.6 (13.2) | 81.4 | | 1563 (25.1) | 1563 | | 103.1 (14.6) | 103.2 | |

* Control for age, BMI, height, education, age at menarche, number of live births, alcohol use, cigarette smoking, physical activity level at primary school and calcium intake.

‡ Total Impact Hours †p value for diff between groups using MANOVA

Table 7. Associations of risk factors with BUA, SOS and QUI among Mixed race women

| Risk factor | BUA Mean (SD) | BUA Adj LSM | P for trend | SOS Mean (SD) | SOS Adj LSM | P for trend | QUI Mean (SD) | QUI Adj LSM | P for trend |
|------------------------|--------------------------|------------------------|------------------------|--------------------------|------------------------|------------------------|--------------------------|------------------------|--------------------|
| Age (years) | | | 0.001 | | | 0.131 | | | 0.024 |
| 18 – 19 | 75.6 (13.6) | 74.8 | | 1565 (26.9) | 1559 | | 101.4 (15.5) | 98.8 | |
| 20 – 24 | 75.9 (13.1) | 75.7 | | 1560 (28.0) | 1558 | | 99.9 (16.0) | 99.0 | |
| 25 – 29 | 77.9 (13.3) | 77.8 | | 1561 (26.1) | 1561 | | 100.9 (15.1) | 100.8 | |
| 30 – 34 | 78.1 (13.7) | 78.1 | | 1560 (27.1) | 1562 | | 100.8 (15.7) | 101.5 | |
| 35 – 39 | 79.7 (14.8) | 80.2 | | 1562 (28.9) | 1564 | | 102.0 (17.1) | 103.2 | |
| 40 – 44 | 78.6 (15.1) | 79.2 | | 1558 (28.4) | 1561 | | 99.9 (16.9) | 101.5 | |
| BMI (kg/m2) | | | <0.001 | | | 0.467 | | | 0.164 |
| < 23 | 75.4 (13.7) | 75.6 | | 1563 (28.3) | 1563 | | 100.6 (16.3) | 100.7 | |
| 23 – 24 | 75.8 (13.2) | 75.8 | | 1559 (29.6) | 1559 | | 99.2 (16.6) | 99.1 | |
| 25 - 29 | 78.4 (13.9) | 78.4 | | 1559 (26.4) | 1560 | | 100.5 (15.6) | 100.6 | |
| 30 - 34 | 80.2 (13.7) | 79.8 | | 1560 (26.8) | 1560 | | 101.5 (15.6) | 101.2 | |
| 35+ | 80.9 (14.1) | 80.8 | | 1561 (26.7) | 1561 | | 102.3 (15.7) | 102.3 | |
| Height (cm) | | | 0.260 | | | <0.001 | | | <0.001 |
| < 153 | 78.4 (14.2) | 78.1 | | 1565 (27.7) | 1565 | | 102.6 (16.1) | 102.6 | |
| 153 – 157 | 77.3 (13.6) | 77.3 | | 1560 (28.1) | 1560 | | 100.2 (16.2) | 100.3 | |
| 157.1 – 161.9 | 76.7 (13.3) | 76.9 | | 1556 (25.3) | 1556 | | 98.6 (14.8) | 98.6 | |
| 162+ | 76.8 (14.2) | 77.4 | | 1558 (28.6) | 1557 | | 99.1 (16.8) | 99.1 | |
| Education | | | 0.058 | | | 0.517 | | | 0.262 |
| Grade 9 or less | 77.2 (14.1) | 77.0 | | 1560 (28.1) | 1560 | | 100.1 (16.4) | 100.3 | |
| Grade 10 – 11 | 77.7 (13.2) | 77.9 | | 1562 (26.2) | 1561 | | 101.1 (15.1) | 101.1 | |
| Grade 12 | 78.4 (14.2) | 78.6 | | 1563 (28.3) | 1561 | | 101.8 (16.5) | 101.4 | |
| Age at Menarche | | | 0.116 | | | 0.378 | | | 0.239 |
| < 13 | 78.3 (13.6) | 78.3 | | 1562 (27.0) | 1562 | | 101.4 (15.6) | 101.4 | |
| 13 – 14 | 77.3 (14.4) | 77.4 | | 1560 (28.5) | 1560 | | 100.4 (16.6) | 100.4 | |
| 15 | 76.9 (13.7) | 76.9 | | 1561 (26.9) | 1561 | | 100.5 (15.7) | 100.5 | |
| 16+ | 77.0 (13.2) | 77.0 | | 1560 (27.1) | 1560 | | 100.1 (15.5) | 100.2 | |

| | | | | | | | | | |
|-----------------------------------|-------------|------|--------------------|-------------|------|--------------------|--------------|-------|--------------------|
| Live Births | | | 0.003 | | | 0.000 | | | 0.000 |
| None | 78.0 (13.9) | 80.1 | | 1568 (27.3) | 1568 | | 103.8 (15.9) | 104.7 | |
| 1+ | 77.5 (13.9) | 77.1 | | 1560 (27.5) | 1559 | | 100.1 (16.0) | 100.0 | |
| Primary School PA (TIH‡) | | | 0.066 | | | 0.003 | | | 0.006 |
| 1 (0 - 310) | 76.9 (13.9) | 76.9 | | 1560 (27.4) | 1559 | | 99.1 (15.9) | 99.7 | |
| 2 (311 – 1118) | 77.4 (14.2) | 77.2 | | 1560 (28.2) | 1560 | | 100.4 (16.5) | 100.2 | |
| 3 (1119 – 2582) | 78.1 (13.4) | 78.3 | | 1562 (27.5) | 1563 | | 101.4 (15.8) | 101.8 | |
| 4 (2583 – 31579) | 79.1 (13.9) | 78.8 | | 1564 (26.3) | 1565 | | 102.7 (15.3) | 103.0 | |
| Smoking Status | | | 0.393 [†] | | | 0.090 [†] | | | 0.147 [†] |
| Non-smoker | 78.3 (13.8) | 77.7 | | 1562 (27.9) | 1562 | | 101.6 (16.1) | 101.3 | |
| Ex-smoker | 76.6 (13.6) | 75.9 | | 1556 (27.1) | 1556 | | 98.4 (15.7) | 98.2 | |
| Current smoker | 77.3 (13.9) | 77.7 | | 1560 (27.5) | 1560 | | 100.5 (16.0) | 100.6 | |
| Alcohol Drinking Status | | | 0.948 [†] | | | 0.850 [†] | | | 0.878 [†] |
| Non-drinker | 77.6 (13.7) | 77.5 | | 1561 (27.2) | 1561 | | 100.8 (15.9) | 100.7 | |
| Past drinker | 78.0 (14.6) | 77.8 | | 1560 (28.8) | 1562 | | 100.7 (16.8) | 101.2 | |
| Current drinker | 77.3 (13.9) | 77.5 | | 1561 (27.7) | 1560 | | 100.6 (16.0) | 100.5 | |
| Quartile of Calcium Intake | | | 0.156 | | | 0.401 | | | 0.276 |
| 1 (0 – 5.25) | 76.8 (13.2) | 77.0 | | 1560 (27.3) | 1560 | | 99.9 (15.6) | 100.1 | |
| 2 (5.3 – 10.75) | 77.2 (13.9) | 77.2 | | 1561 (27.7) | 1561 | | 100.8 (16.1) | 100.7 | |
| 3 (10.8 – 15.25) | 77.5 (13.9) | 77.5 | | 1560 (27.7) | 1560 | | 100.3 (16.0) | 100.4 | |
| 4 (15.3 – 50.25) | 78.4 (14.3) | 78.2 | | 1562 (27.7) | 1562 | | 101.6 (16.2) | 101.4 | |

Control for age, BMI, height, education, age at menarche, number of live births, alcohol use, cigarette smoking, physical activity level at primary school and calcium intake.

‡ Total Impact Hours † p value for diff between groups using MANOVA

Smoking in mixed race women (Table 8)

There was a marginal decrease in the adjusted mean BUA for ever versus never smokers, but no association was evident for numbers of cigarettes smoked, or for (numbers of cigarettes smoked x years of smoking). For the latter factor, BUA actually increased in the highest category of smoking to above non-smoker levels.

Mean SOS was also lower in ever-smokers than in never-smokers, although again there was no trend for either number of cigarettes or for (number of cigarettes/day x years of smoking). For QUI, results followed the same pattern as for SOS for the never- versus ever-smokers, but the effect was of smaller magnitude.

Table 8. Adjusted means for QUS measurements according to smoking in mixed race women.

| Risk factor | N | BUA Adj LSM* | SOS Adj LSM* | QUI Adj LSM* |
|--|----------|-------------------------|-------------------------|-------------------------|
| Smoking status | | | | |
| Never smoked cigarettes | 612 | 77.7 | 1562 | 101.3 |
| Ever smoked cigarettes | 1283 | 77.5 | 1560 | 100.4 |
| Numbers of cigarettes | | | | |
| Never smoked cigarettes | 612 | 77.7 | 1562 | 101.3 |
| Smoke/d 1-5 cigs/day | 363 | 77.1 | 1559 | 99.8 |
| Smoke/d 5 – 20 cigs/day | 769 | 77.6 | 1561 | 100.7 |
| Smoke/d > 20 cigs/day | 151 | 77.7 | 1559 | 100.0 |
| Number of cigarettes/day x Years of smoking | | | | |
| Never smoked cigarettes | 612 | 77.7 | 1562 | 101.3 |
| Smoke/d 1 - 40 cig years | 898 | 77.0 | 1560 | 100.2 |
| Smoke/d > 40 cig years | 385 | 78.5 | 1560 | 100.8 |

*Control for age, BMI, height, education, age at menarche, number of live births, alcohol use, physical activity level at primary school and calcium intake.

5.4 Quality Control

Short term machine and operator precision

Operator training sessions took place prior to commencement of the study proper. Table 9 shows the coefficients of variation (CVs) for BUA, SOS and QUI for each operator using one machine and measuring one subject N times. The high CVs for all operators for the Mitchells Plain (MP) machine for the trials on 28 May 2002, as compared with their results for the Gugulethu (GU) machine, suggested that there was a problem with the machine and it was sent back to the manufacturer for assessment. The machine was subsequently returned to us with new transducer pads and the manufacturer's guarantee of full working order. Both operators assigned to this machine had two subsequent training sessions, while interviewer 3 on the GU machine had a single further session in order to achieve CVs within the Hologic specifications.

Table 9: Coefficients of variation for BUA , SOS and QUI for training trials of operators.

| | | | | BUA | SOS | QUI |
|------------------------------|--------------------|----------------|----------|----------------|----------------|----------------|
| | | | | CV (%) | CV (%) | CV (%) |
| Hologic specification | | | | 3.7 | 0.3 | 2.6 |
| Date | Interviewer | Machine | N | | | |
| 28/05/02 | 1 | GU | 10 | 3.4 | 0.1 | 1.6 |
| 28/05/02 | 1 | MP | 10 | 5.0 | 0.6 | 3.5 |
| 05/08/02 | 1 | MP | 50 | 6.3 | 0.4 | 2.9 |
| 12/08/02 | 1 | MP | 19 | 2.1 | 0.3 | 2.2 |
| 28/05/02 | 2 | GU | 10 | 1.7 | 0.2 | 1.6 |
| 28/05/02 | 2 | MP | 10 | 4.7 | 0.4 | 2.2 |
| 12/08/02 | 2 | MP | 30 | 6.1 | 0.5 | 3.3 |
| 04/10/02 | 2 | MP | 40 | 2.7 | 0.3 | 2.1 |
| 28/05/02 | 3 | GU | 10 | 1.8 | 0.1 | 1.0 |
| 28/05/02 | 3 | MP | 10 | 3.6 | 0.4 | 2.6 |
| 14/08/02 | 3 | GU | 21 | 2.1 | 0.3 | 2.1 |

Long terms precision: Machine reliability over duration of study

The long term stability of BUA and SOS was monitored by recording the QAB and QAS. According to Hologic, over several months, typical mean values fluctuate within the following range: Mean QAB = 0.93 - 1.07 and Mean QAS = 0.993 - 1.007 (Hologic, 1998). It is recommended that if QAS and QAB results are beyond these limits for more than two weeks, the unit should be re-calibrated.

Figures 1 and 2 show monthly means for QAB and QAS for the duration of the study. Months corresponding to winter months are indicated by the blue arrows below the x-axis. In general, both machines showed similar fluctuations for QAB over the course of the study. QAS readings were relatively stable for the first two years of the study where after there were wider fluctuations and readings for the MP machine drifted towards the lower end of acceptable range. Recalibration procedures were followed and transducer pads were changed in month 13 and 27 for the GU machine, and in month 9 and 19 for the MP machine. Services were performed in month 23 on the GU Machine and in months 9 and 22 on the MP machine. There was no obvious seasonal drift in QAB or QAS corresponding to the colder or warmer months.

Fig 1: Mean Monthly phantom QAB.

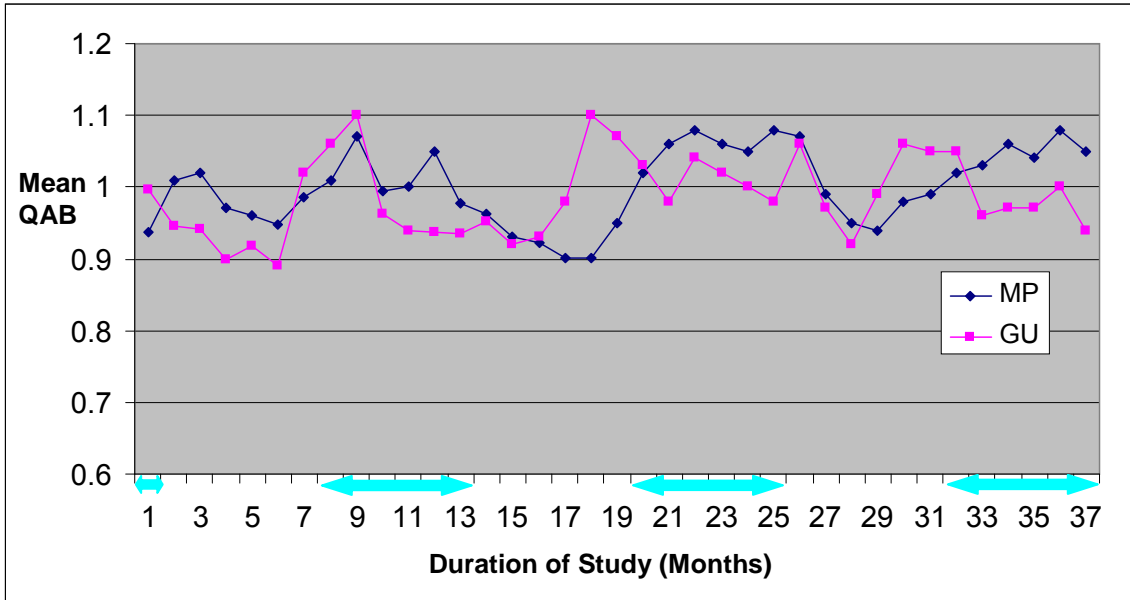
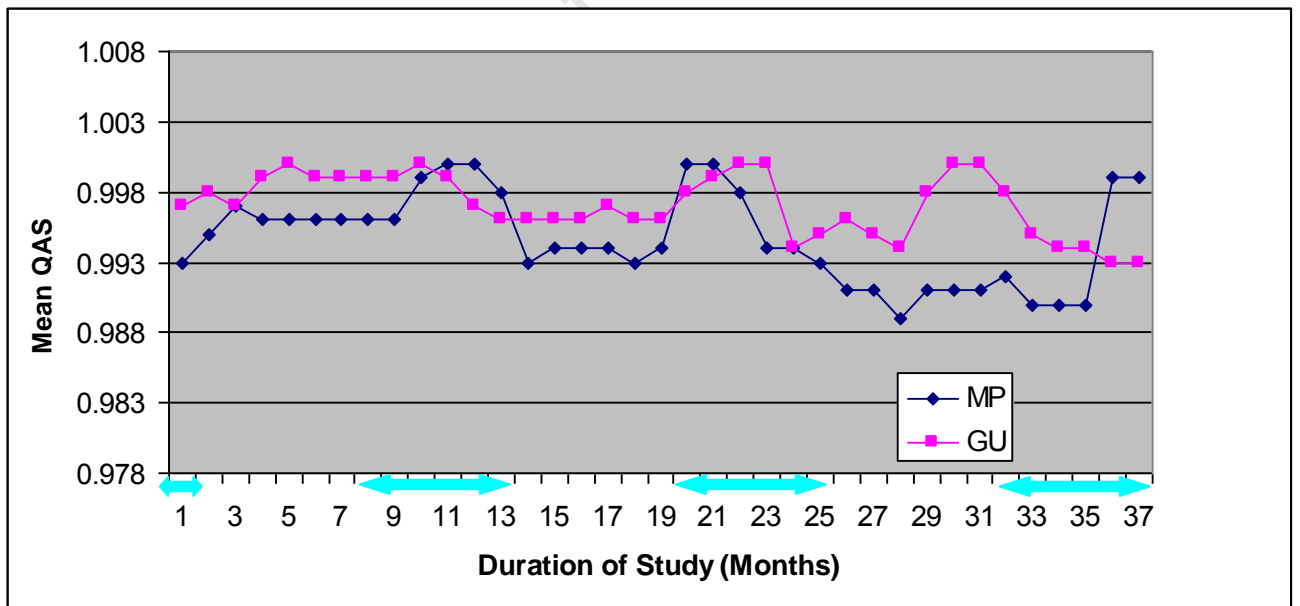


Fig 2: Mean Monthly phantom QAS.



5.5. Comparisons between QUS machines:

Table 10: BUA and SOS differences for the two Sahara machines.

| | N | Mean Difference | Std. Dev Difference | Min | Max |
|----------------------------|-----|--------------------|------------------------|-------|------|
| BUA(MP) – BUA(GU) | 146 | 0.4 | 5.35 | -23.3 | 11.9 |
| SOS (MP) – SOS (GU) | 146 | 3.3 | 9.67 | -30.9 | 35 |

Table 10 shows pooled results of measurements on 48 to 50 different subjects by a single operator performed at annual intervals - on October 2003 (N=50), October 2004 (N=48) and October 2005 (N=48). The MP machine read slightly higher for both BUA and for SOS than the GU machine. In the case of BUA, this difference was not significant ($t = 0.8233$, $p = 0.412$). For SOS however, the difference between the two machines was highly significant ($t = 4.139$, $p=0.000$)

5.6. Agreement between QUS and DEXA: Small study

Results for the small study (N=14) comparing DEXA BMD at different hip sites and at the lumbar spine with Sahara QUS results from both machines are shown in Table 11.

Table 11: Correlation coefficients for QUS and DEXA

| | GU BUA | GU SOS | GU QUI | MP BUA | MP SOS | MP QUI |
|------------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| DEXA Fem neck | 0.69 | 0.65 | 0.68 | 0.72 | 0.61 | 0.67 |
| DEXA:Fem Trochanter | 0.78 | 0.84 | 0.84 | 0.77 | 0.82 | 0.83 |
| DEXA: Fem Total hip | 0.74 | 0.73 | 0.75 | 0.74 | 0.70 | 0.74 |
| DEXA: L1-14 | 0.60 | 0.54 | 0.58 | 0.53 | 0.49 | 0.52 |

For the Gugulethu sonometer, all the correlation coefficients were significant at $p<0.05$).

For the Mitchell's Plain sonometer the correlation coefficients were also significant

($p<0.05$) except for at the lumbar spine where results were nearly significant ($p <0.075$).

Bland-Altman analysis of agreement

Although commonly used, Pearson's correlation coefficient can be misleading in measuring agreement (Bland and Altman, 1986). The correlation coefficient measures the strength of relation between two variables, and perfect correlation occurs ($r = 1$) if the points lie along any straight line. For perfect agreement between 2 variables, the points must lie along the line of equality. In addition, the correlation coefficient is affected by the range of the response but it is not influenced by the scale of the measurement. For these reasons, the Bland-Altman method offers a more accurate approach for analyzing agreement between two different methods measuring the same quantity (Bland and Altman, 1986).

We used the Bland-Altman approach for comparison of BMD between the two QUS machines and between each QUS machine and DEXA (see Table 12)

Table 12: Bland Altman statistics for agreement between QUS devices and DEXA. (N=14)

| | Mean Difference | 95% CI | Limits of agreement (Mean diff \pm 2SD) |
|--------------------------------------|------------------------|-----------------|---|
| BMD (GU) and BMD (MP) | -0.008 | -0.025 ; 0.009 | -0.066 ; 0.051 |
| BMD (MP) and DEXA at Fem neck | -0.281 | -0.338 ; -0.224 | -0.477 ; -0.085 |
| BMD (GU) and DEXA at Fem neck | -0.273 | -0.329 ; -0.218 | -0.466 ; -0.081 |

Comparing results for the two QUS machines shows again that the MP machine was reading slightly higher than the GU machine for mean BMD for these subjects. However, the 95% Confidence Interval (CI) overlaps zero. When QUS is compared with DEXA, both QUS machines showed similar lower means for calcaneal BMD than mean BMD as measured by DEXA at the femoral neck. For both QUS machines, these differences are significant (95% CIs do not overlap zero). The Limits of agreement are estimates from the data (Mean difference \pm 2SD) that can be applied to the population in general when

comparing the two methods. If these limits are beyond what is clinically acceptable, the two methods are not interchangeable. The limits of agreement of the two QUS BMD measurements with the DEXA BMD are similar for both machines. The range of these limits is 0.392g/cm^2 (Lower limit; Upper limit -0.477 ; -0.085) for the MP machine and 0.385g/cm^2 (Lower limit; Upper limit -0.466 ; -0.081) for the GU machine. The Standard deviation (SD) of the DEXA(fem neck) BMD for these 14 subjects was 0.117 g/cm^2 (not shown in Table 12). For both machines the range encompassed by the limits of agreement is more than 3 SDs of the DEXA measurement, suggesting that the two methods do not agree sufficiently to be used interchangeably.

Bland-Altman plots allow for graphical representation of any trend in variance and should be interpreted visually and not by means of analysis. Figures 3 and 4 plot the differences between QUS and DEXA for BMD against the average for the two methods for each machine respectively. These graphs show no relationship between the size of the measurement and the size of the variance, nor is there a trend in the variance that relates to the size of measurement.

Fig 3: Bland Altman plot of BMD measured by MP QUS and by DEXA

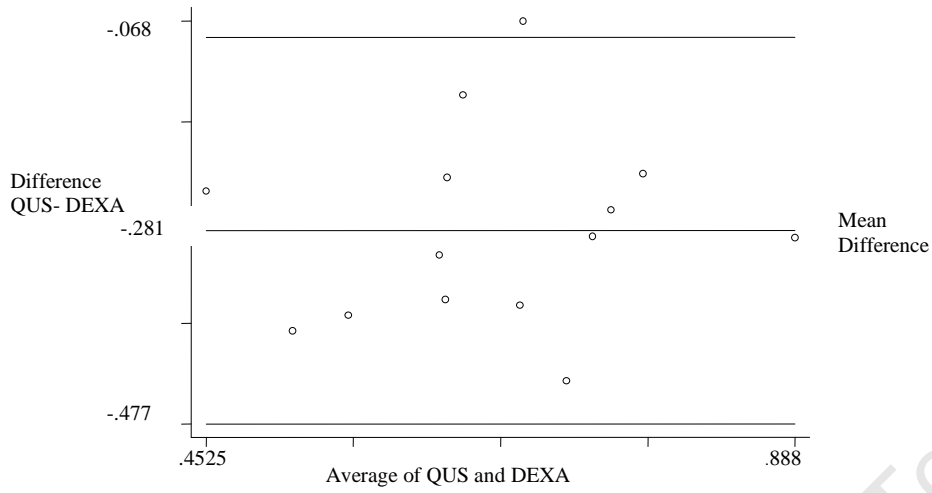
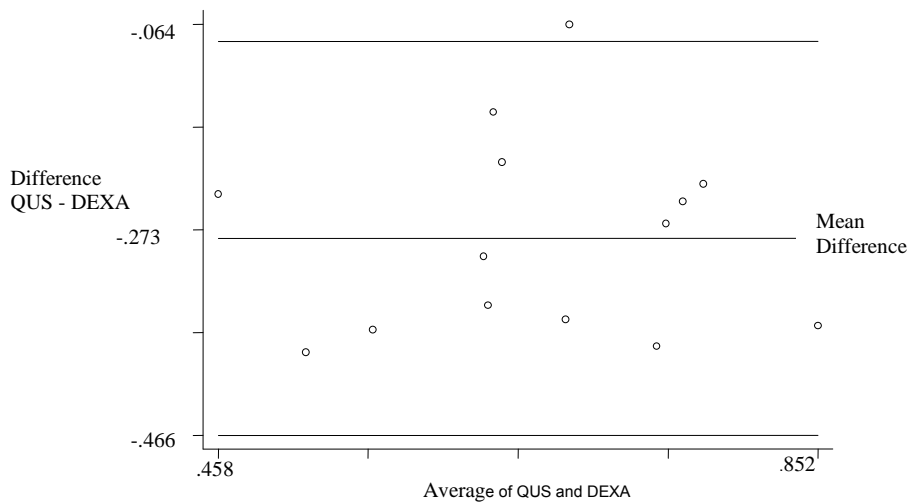


Fig 4: Bland Altman plot of BMD measured by GU QUS and by DEXA



6 DISCUSSION

6.1 Risk factor Associations

Our results showed that age, BMI, height, early physical activity as well as age at menarche and parity were associated with either BUA, SOS or QUI individually or with more than one of these ultrasound measures. Except for BMI in women of mixed race, the associations of these variables with QUI were the same as with SOS. There were differences between our two populations for associations of QUS measures with age at menarche and with primary school physical activity. Stratified analyses according to categories of BMI did not resolve these differences. Nor did this approach alter the inverse association of QUS measures with height. The effect of depot contraceptive progestins on bone health was evaluated in our original study and lower bone mass in current users was confirmed (Rosenberg *et al.*, 2007).

The associated factors found in our study are similar to those known to be consistently associated with BMD as measured by DEXA in premenopausal women according to a review by Tudor-Locke and McColl (2000). In this review associations with BMD included age, BMI, age at menarche, smoking, load bearing exercise and use of depot contraceptive progestins. Additional factors reported to be associated with BMD that our study did not assess are as follows: Use of calcium supplementation where dietary intake is low, Vitamin D levels, primary amenorrhea, and family history of osteoporosis.

Associations with individual risk factors:

The following section examines the QUS associations for the individual risk factors in our two study populations, and compares these results to those reported for BMD measured by

DEXA. Our QUS findings are then compared with QUS findings reported in the literature for premenopausal women from other populations.

Age

In our study QUS measures increased with increasing age in black women up to 44 years whereas in women of mixed race these measures increased up to 39 years and then decreased in the oldest age category. This decrease in our mixed race women of 40 years and older suggests the onset of bone loss in late premenopausal years. As this apparent drop-off of QUS values after 40 years was not present in our black women and since BMI is highest in the oldest category of black women, their continued increase in QUS with age may be due to a protective effect of BMI which was unresolved in our analysis.

The onset of bone loss just prior to menopause has been shown in DEXA studies where it is generally reported that peak BMD at the femoral neck had occurred by 20 years of age, where after it remained stable until the perimenopausal years when it began to drop. In comparison, total body BMD showed continuous small increments with age (Khan and Syed, 2004) possibly due to continued periosteal apposition at the vertebral bodies, with the onset of loss occurring in the late premenopausal or in the perimenopausal years. (Tudor-Locke and McColl 2000).

Minor population differences in the association of bone mass and age may stem from both genetic as well as environmental factors such as physical activity and nutrition (Micklesfield *et al.*, 2005). QUS studies on pre- and post-menopausal European (Adami *et al.*, 2004, Langton and Langton, 1997) and Asian women (Vu *et al.*, 2005) have shown an increase of both SOS and BUA up to fourth decade followed by a significant decrease after 45 years. Other studies of premenopausal women have not consistently reflected changes in

QUS with age (Gregg *et al* 1999). This may be due to the relative stability of bone mass prior to menopause as it is generally accepted that the rapid age-related loss of bone with accompanying osteopenia is most apparent in the immediate post-menopausal phase (Gregg *et al.*, 1997). Alternatively it may simply reflect the preponderance of cross sectional over the more appropriate prospective QUS studies.

Body Mass Index

Increased BUA, SOS and QUI with increasing BMI was found in our study in black women. BUA, to a lesser extent QUI, but not SOS increased in mixed race women. We therefore interpret these results cautiously as supporting a general positive association between BMI and QUS measures. BMI has demonstrated a consistent positive relationship with bone mineral measurements by DEXA (Tudor-Locke and McColl 2000).

Positive associations of BUA and SOS with BMI have been demonstrated in different premenopausal populations including European women (Babaroutsi *et al.*, 2005, Kroke *et al.*, 2000), Arabian women (Saadi *et al.*, 2003) and White and African- Americans (Gregg *et al.*, 1999 Gregg *et al.*, 1997). However, as with SOS in our mixed race women, not all studies have demonstrated this relationship. The multicentre European OPUS study (Stewart *et al.*, 2006) the ESOPO study on Italian women (Adami *et al.*, 2004) and studies on Turkish women (Gemalgez *et al.*, 2007) did not report the association of QUS measures with BMI as significant. In these studies weight rather than BMI was associated with some but not all QUS measures. These inconsistencies are hard to rationalize, but it is possible they stem from variation in body composition. Since the main association of body mass with QUS measures is due to lean body mass rather than fat mass (Kroke *et al.*, 2000, Gregg *et al.*, 1999) and as lean body mass and fat mass affect bone mineral

unequally, the association of weight or BMI with QUS measures could be expected to be somewhat inconsistent.

Height.

In both our study groups, height was negatively related to SOS and QUI but there was no association with BUA. As an increase in height is usually accompanied by a concomitant increase in weight and therefore increased mechanical loading on weight-bearing bones, positive associations with bone mass are to be expected. We surmised that as our taller women in both study groups had a significantly lower BMI than our shorter women (results not shown) this may account for the decrease in SOS. However, when analyzed in three separate BMI strata, the negative association of height with SOS and QUI measures remained for both study groups in both the lighter and the heavier BMI categories.

For BMD measured by DEXA, height has been demonstrated to be positively and significantly related to BMD at the femoral neck, but not at the lumbar spine (Mazess and Barden 1991).

A negative association with estimated BMD from QUS measures was demonstrated in South East Asian women in the United States (Lauderdale, 2001). In contrast, among Asian premenopausal women (Vu *et al.*, 2005) increased height was associated with increased SOS, but not with change in BUA, while other studies have shown positive associations between height and both BUA and SOS (Gregg *et al.*, 1997).

Age at menarche

The association of increased QUS measures with earlier onset of menarche was consistently present in our black women however among our mixed race women, there was

no trend in association between age of menarche and QUS measures and further stratified analysis according to three BMI strata did not alter this.

A number of cross sectional DEXA studies of premenopausal women demonstrated a similar inverse relationship with age at menarche (Tudor-Locke and McColl 2000), although these authors did caution that these effects may not generalize to all populations. Increased bone mass associated with early onset of menarche may be attributed to the trophic effect on bone due to longer exposure to estrogens, however it can also happen that a later age at menarche is the result of underlying and unreported hormonal irregularities. In these cases bone mineral would be directly influenced to a greater extent than would otherwise occur in the normal hormonal environment.

In some of the European studies (Babaroutsi *et al.*, 2005) the inverse association with age at menarche was present for all 3 QUS measures, while other European and Saudi Arabian studies showed associations with BUA but not with SOS and QUI (Adami *et al.*, 2004, Saardi *et al.*, 2003). In Asian Americans (Lauderdale *et al.*, 2001) no relationship was present.

Parity

Our mixed-race parous women had lower BUA, SOS and QUI values than nulliparous women. For parous black participants SOS and QUI were lower but not BUA. There is currently no consistent reported evidence that parity is associated with reduced BMD as measured by DEXA (Tudor-Locke and McColl 2000).

During pregnancy estrogen levels rise, and then decrease postpartum and during prolonged lactation. Thus over an extended period in premenopausal women, estrogen levels vary

with numbers of pregnancies. These variations will affect BMD accordingly (Karlsson *et al.*, 2001).

In other QUS studies, some have demonstrated inverse associations of parity with QUI (Adami *et al.*, 2004), while others have shown similar inverse associations with SOS but not with BUA (Vu *et al.*, 2005, Saardi *et al.*, 2003).

Physical Activity

Our study demonstrated a positive association for primary school physical activity measured in total impact hours with QUS measures in mixed race but not in black women. The benefits of current high impact endurance activities on BMD determined by DEXA have been repeatedly demonstrated (Tudor-Locke and McColl 2000), but the evidence for associations between BMD and historical activity is inconclusive.

Historical physical activity is notoriously difficult to capture accurately due to difficulty with recall. Difficulties in cross sectional studies also stem from indistinct definitions of current versus historical activity as well as the use of a plethora of different methods for quantifying physical activity. The instrument used to quantify and evaluate historic physical activity in a study population needs to be appropriate to the environment of the participants. Our study reported on a specific range of physical activities during primary school years in an attempt to quantify historical activity in a defined and accurate way. In this, we were guided by a previous study of South African black and mixed race women, where the physical activities shown to be most strongly associated with BMD were walking, walking with loads and sport activities (Micklesfield *et al.*, 2003). Despite much higher levels of primary school physical activity in our black women than in our mixed race women, the latter group showed a stronger association with all QUS measures.

The positive effect of current physical activity on BUA and SOS has been demonstrated in South African white women (Micklesfield *et al.*, 2005) and premenopausal European, Asian and American women (Barabroutsi *et al.*, 2005, Vu *et al.*, 2005, Gregg *et al.*, 1999, 1997). However, for historic physical activity some researchers have shown a relationship with QUS measures (Vu *et al.*, 2005, Adami *et al.*, 2004 Micklesfield *et al.*, 2003), while others have not (Micklesfield *et al.*, 2005).

Education

In our study we found no association of QUS measures with education level in either study group. Similarly, most DEXA studies found associations with educational levels to be either weak or absent (Alver *et al.*, 2007).

Educational level is an imperfect measure for socio economic status, however it is often a good predictor for chronic diseases known to be associated with poverty. Unfortunately DEXA studies addressing the association between BMD and socioeconomic status tend to be limited to postmenopausal women or to have small and biased samples (Alver *et al.*, 2007). Despite these limitations there is some evidence for lower BMD in populations of low socioeconomic status (Gur *et al.*, 2004).

Most reports failed to show an association of QUS bone measures with educational level with the exception of a study on Vietnamese women in which a lower educational level was associated with reduced SOS (Vu *et al.*, 2005).

Calcium Intake

As with education, we found no association of QUS measures with calcium intake in either study group. Some cross sectional DEXA studies have suggested a small consistent association between calcium intake and bone mineral status which was most common in

younger women, particularly among those whose calcium intake is low (Tudor-Locke & McColl 2000). In QUS studies, the associations have been inconsistent (Stewart A, 2006, Barbaroutsi *et al.*, 2005, Adami *et al.*, 2004).

Alcohol Intake

As with education and calcium intake, we found no association of QUS measures with alcohol intake in either study group.

DEXA studies have shown no association or negative associations of moderate alcohol consumption patterns with BMD (Tudor-Locke & McColl 2000). In the case of alcoholism, the adverse impact on bone is well documented and reported to be severe, however at the opposite extreme, a small alcohol intake has been shown in some studies to be protective for BMD as compared with nondrinkers (Bainbridge *et al.*, 2004). This effect is illustrated in the well-documented, although controversial, “j-shaped” curve for association between alcohol intake and chronic disease (Connor J, 2006).

The majority of QUS studies in other populations have been inconclusive in demonstrating any consistent effect of alcohol drinking intake on bone health (Gregg *et al.*, 1999, 1997).

Smoking

Our results suggest that mixed race women smokers have a lower SOS than non-smokers but there was no association evident with any of the QUS measures for non-smokers, ex-smokers and current smokers of cigarettes in black women. The prevalence of ever-smokers in the mixed race women was high (68%), however the numbers of cigarettes smoked and numbers of smoking years was relatively low thus generating a limited range of response for evaluation of a possible association. Prevalence of ever-smokers among our black participants was low (10%). Smoking is hypothesized to affect BMD by altering

the steroid hormonal environment, but again reviews in literature are inconsistent as to how reliable the evidence is for this effect. Tudor Locke (2000) argues that there is sufficient evidence to support an association with BMD measured by DEXA.

The ESOPQ QUS study (Adami *et al.*, 2004) found an adverse effect of smoking on Ultrasound QUI, however a recent meta-analysis (In Babaroutsi *et al.*, 2005) showed no consistent effect of smoking on QUS measures. This is generally supported in other reports (Micklesfield *et al.*, 2005, Adami *et al.*, 2004, Gregg *et al.*, 1999). Specifically, no association was demonstrated in American premenopausal women (Gregg *et al.*, 1999), nor in Europeans (Kroke *et al.*, 2000).

6.2 Strength of association with risk factors.

Our analysis is restricted to premenopausal women as a result of the aim of the original study which was to evaluate the effects of injectable contraceptive use on bone mass. Studies comparing pre- and post menopausal women have repeatedly demonstrated the less obvious relationship between risk factors and QUS measurements in younger women in whom only a small number of measured risk factors showed association (Stewart A, 2006 Saadi *et al.*, 2003, Kroke *et al.*, 2000). In comparison, postmenopausal bone loss was accelerated and early or cumulative life-style influences tended to make their appearance more obvious during this process.

6.3 Normative data for black and mixed race women

There are no previous studies describing QUS measures for black and mixed race premenopausal women in South Africa. Comparative data for factors related to QUS measures in South African women are limited to a study of 187 white women (Micklesfield *et al.*, 2005) which showed BMD estimated by QUS to be associated with

age and current physical activity, but not with parity, calcium intake, smoking or alcohol use.

Our age-adjusted means for black and mixed race women were derived from a sufficiently large sample to be considered as reliable normative data for premenopausal women from these populations (Tables 6 and 7). We considered the two study groups separately as both the risk factor distributions and the adjusted QUS outcomes were significantly different. In addition, our study was not designed specifically to assess ethnic differences as different Sahara machines were used at the two sites. Nonetheless population comparisons are made in the public health literature and the plea for population specific databases for QUS measures is often heard (Krieg M-A *et al.*, 2008, Gluer, 2006, Hough, 2003).

In order to compare our results for black and mixed race women, it is necessary to bear in mind that different QUS machines were used for each group. We measured differences between the two machines on three occasions in the course of our study, and analysis of this combined data showed no significant differences between the two machines for BUA, but significantly higher SOS readings for the device used for mixed race women. We therefore conclude that the lower adjusted means in every age category for BUA in our mixed race women suggested that these women do indeed have a marginally lower bone mass than their black counterparts. The similar lower results in the majority of categories for SOS supported this.

Differences for ethnic groups in BMD and in the strength of association between QUS and various risk factors have been reported elsewhere. Thus South African black and mixed-race children under the age of 12 had higher QUS measures than white children.

(Micklesfield *et al.*, 2004), and African–American women aged 45 -53 had a substantially higher SOS, but no difference in BUA, than white women (Gregg *et al.*, 1999).

Whether or not ethnic differences are based on genetic or lifestyle differences or on investigator bias, additional normative data on population- specific QUS values and comparative QUS associations are needed. These will assist in evaluating QUS as a clinical tool and as a useful instrument in epidemiological studies, particularly in resource- poor settings where DEXA is not accessible.

6.4 QUS and DEXA measures: The question of validity

In our small study on 14 individuals in which we measured BMD using DEXA, the correlation coefficient for femoral neck and lumbar spine was 0.78 and for total hip and lumbar spine it was 0.88. In other DEXA studies, the between site correlation for spine and hip BMD is reported to be in the region of 0.77. (Gluer and Hans, 1999) This correlation increased in studies on excised bone specimens ($r=0.9$, Gluer 2006).

We also compared DEXA to QUS measures and showed a lower correlation between lumbar spine BMD and QUS measures than between hip BMD and QUS. Similar lower correlations between lumbar spine DEXA and QUS as compared to femoral neck measurements is reported frequently (Krieg *et al.*, 2008).

In other studies the magnitude of the correlation coefficient for ultrasonically derived measurement of bone mineral density (BMD) at the calcaneum, and spinal or hip BMD as measured by DEXA has ranged from 0.29 to 0.88 (Prins *et al.*, 1998). This variation stemmed in part from intra- individual variation due to varying BMD at different anatomical sites, as well as from differences between the two methods of measurement. Given that precision errors occurred in both DEXA (Error ~ 0.5 to 1 T-score, Gluer 2006)

and QUS (Reproducibility error = 2.5 – 3.5 % (WHO, 1994) and therefore were compounded in comparative studies; these could explain the modest correlation between the two measurement techniques (Gluer, 2006). Further differences could be explained by the QUS measurement itself as QUS measures other elements of bone strength related to trabecular architecture and structural anisotropy, that are not dependent on BMD (Gluer 2006, Bauer *et al.*,1997).

Notwithstanding all the reports of varying strengths of correlation, it is the measure of agreement rather that dictates whether or not two measurement methods can be used interchangeably. As noted earlier the ISCD has an official position on this matter that is revised annually according to the most recent evidence (International Society for Clinical densitometry, 2008). From our study results, the Bland Altman analyses confirmed that QUS and DEXA methodologies are not sufficiently in agreement to be substituted for each other.

6.5 Short and Long Term precision

Short term precision.

Our tests of repeated measurements on a single individual by 1 operator on the same machine reflected operator technical expertise and machine stability, given that the ambient temperature and soft tissue properties over the course of the trial (approximately two hours) were stable. These trails replicate the method used by Hologic to generate their product specifications (Hologic Users guide 1998) and narrow the source of variability down to operator technique and machine function. The mean and range for coefficients of variation from 6 other studies reporting precision for the Sahara machine are 4.1% (2.7-5.0) for BUA, 0.3% (0.2 – 0.4) for SOS and 2.6 % (1.6-3.5) for QUI (Krieg M-A *et al.*, 2008).

Following training, our precision error was within these ranges. These results ensured that we could assume operator proficiency of an international standard and that the Ultrasound devices were in full working order at the start of the study.

Long term precision

For the two Sahara machines used in the study, QC was performed on a daily basis and monthly means and standard deviations were monitored for long term trends. Transducer pads were changed when necessary and recalibration was performed when readings were consistently on the low or high side. We felt assured that the built in QC safety limits combined with our monitoring processes ensured a sufficient level of precision to be confident of the quality of our study results.

6.6 Strengths and Limitations of the study

The major strength of this study was the large sample size. This allowed for efficient statistical power in the multivariable analyses. In addition, little interviewer and machine variability and careful QC monitoring contributed to high levels of precision in the study. Limitations were that we were not able to carry out DEXA measurements on our study subjects to confirm our results due to distance from study centers with a DEXA machine and the expense that this would have incurred. Another limitation is the lack of age data for the various school epochs that we used to classify periods of physical activity. Since bone responses to physical activity are greatest during the growth phase, and as the age during which our participants attended primary school was very variable, information on age on finishing primary school might have been useful by allowing us to link historical physical activity to biological age.

7. CONCLUSION

This study examined QUS measures in a large population of South African black and mixed race premenopausal women in relation to known risk factors for BMD as measured by DEXA. For the most part, associations with risk factors were similar for both study groups. However black women had higher QUS values after adjusting for confounders than mixed race women, and there were differences between the study groups for the associations of QUS measures with age at menarche and with primary school physical activity. In general, however, we demonstrated that measures in this study had similar associations to those shown in DEXA studies on premenopausal women, and to those reported in QUS studies in other populations.

As all women in our study were premenopausal and less than 44 years old the rapid age related bone loss associated with menopause had not commenced, the strength of association with the risk factors was moderate. QUS measures slightly different qualities of bone than does DEXA and further studies comparing its associations with risk factors to those associated with DEXA measures will increase the usefulness of this highly portable and easily accessible device. DEXA is the more precise instrument, yet in resource-poor settings where cost and logistical difficulties preclude any such studies from being carried out, QUS may be considered a suitable tool to assess bone quality in large epidemiological studies. However it is well noted that QUS may not be used for monitoring bone status in individuals taking medications that could influence their bone health.

8. REFERENCES

1. Adami S, Giannini S, Giorgino R, Isaia GC, Maggi S, Sinigaglia L, Filipponi P, Crepaldi G. 2004 Effect of age, weight and lifestyle factors on calcaneal quantitative ultrasound in women: the ESOPO study. *Calcif Tissue Int* 74:317-21.
2. Alexandersen P, de Terlizzi F, Tankó LB, Bagger YZ, Christiansen C. 2005 Comparison of quantitative ultrasound of the phalanges with conventional bone densitometry in healthy postmenopausal women. *Osteoporos Int*. 16:1071-1078.
3. Alver K, Sjøgaard AJ, Falch JA and Meyer HE. 2007 The Oslo Health Study: Is bone mineral density higher in affluent areas? *BMC International Journal for Equity in Health* 6:19
4. Babaroutsi E, Magkos F, Manios Y, Sidossis LS. 2005 Lifestyle factors affecting heel ultrasound in Greek females across different life stages. *Osteoporos Int* 16:552–561.
5. Bainbridge KE, Sowers M, Lin X, Harlow SD. 2004 Risk factors for low bone mineral density and the 6-year rate of bone loss among and perimenopausal women. *Osteoporos Int*. 15: 439-446.
6. Bauer DC, Gluer CC, Cauley JA, Vogt TM, Ensrud KE, Genant HK, Black DM 1997 Broadband ultrasound attenuation predicts fractures strongly and independently of densitometry in older women. A prospective study. Study of Osteoporotic Fractures Research Group. *Arch Intern Med* 157:629–634.
7. Bland JM & Altman DG. 1986 Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*: 1 (8476):307-310.
8. Camozzi V, De Terlizzi F, Zangari M, Luisetto G. 2007 Quantitative bone ultrasound at phalanges and calcaneus in osteoporotic postmenopausal women: influence of age and measurement site. *Ultrasound Med Biol* 33(7):1039-1045.
9. Cetin A, Erturk H, Celiker R, Sivri A, Hascelik Z. 2001 The role of quantitative ultrasound in predicting osteoporosis defined by dual X-ray absorptiometry. *Rheumatol Int* 20:55–59.
10. Chappard C, Camus E, Lefebvre F, Guillot G, Bittoun J, Berger G & Laugier P. 2000 Evaluation of error bounds on calcaneal speed of sound caused by surrounding soft tissue. *J Clin Densitom* 3: 121-31.
11. Connor J. 2006 The life and times of the J-shaped curve. *Alcohol* 41(6):583-584.
12. Conradie M, du Plessis M, Hough FS. 2005 A Comparative Study of the Determinants of Bone Strength and the Propensity to falls in Black and White

South African women – A Preliminary report on Bone Mineral density (BMD) and Fracture Prevalence. JEMDSA 10(1): 40.

13. Cummings SR, Black DM, Nevitt MC, Browner W, Cauley J, Ensrud K, Genant HK, Palermo L, Scott J, Vogt TM. 1993 Calcaneum vs hip Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group. *Lancet* 341(8837):72-75.
14. Daniels ED, Pettifor JM, Schnitzler CM, Russell SW Patel DN. 1995 Ethnic differences in bone density in female South African nurses. *J Bone Miner Res* 10: 359 - 367.
15. El-Desouki MI, Sherafzal MS, Othman SA 2005 Comparison of bone mineral density with dual energy x-ray absorptiometry, quantitative ultrasound and single energy Xray absorptiometry. *Saudi Med J*. 26(9):1346-1350.
16. Frost ML, Blake GM, Fogelman I. 1999 Contact quantitative ultrasound: an evaluation of precision, fracture discrimination, age-related bone loss and applicability of the WHO criteria. *Osteoporos Int* 10: 441–449.
17. Frost ML, Blake GM, Fogelman I. 2000 Does quantitative ultrasound imaging enhance precision and discrimination? *Osteoporos Int* 11: 425–433.
18. Frost ML, Blake GM, Fogelman I. 2001 Quantitative ultrasound and bone mineral density are equally strongly associated with risk factors for osteoporosis. *J Bone Miner Res* 16: 406–416.
19. Gemalmaz A, Discigil G, Sensoy N, Basak O. 2007 Identifying osteoporosis in a primary care setting with quantitative ultrasound: relationship to anthropometric and lifestyle factors. *J Bone Miner Metab* 25:184–192.
20. Glüer CC. 2006 Quantitative Ultrasound - it is time to focus research efforts. *Bone*. 40(1): 9-13
21. Gluer CC. 2008 A New Quality of Bone Ultrasound Research. *IEEE transactions on ultrasonics, ferroelectrics, and frequency control*. 55: 1524 – 1528.
22. Glüer CC, Hans D. 1999 How to use ultrasound for risk assessment: a need for defining strategies. *Osteoporos Int*. 9:193-5.
23. Grampp S, Genant HK, Mathur A, Lang P, Jergas M, Takada M, Glüer CC, Lu Y, Chavez M. 1997 Comparisons of noninvasive bone mineral measurements in assessing age-related loss, fracture discrimination, and diagnostic classification. *J Bone Miner Res* 12(5): 697-711.

24. Groothausen J, Siemer H, Kemper HCG, Twisk J, Welten DC. Influence of peak strain on lumbar bone mineral density: an analysis of 15-year activity in young males and females. *Pediatric Exerc Sci.* 9:159-173.
25. Gregg EW, Kriska AM, Salamone LM, Roberts MM, Anderson SJ, Ferrell RE, Kuller LH, Cauley JA 1997 The epidemiology of quantitative ultrasound: a review of the relationships with bone mass, osteoporosis and fracture risk. *Osteoporos Int.* 7: 89-99.
26. Gregg EW, Kriska AM, Salamone LM, Wolf RL, Roberts MM, Ferrell RE, Anderson SJ, Kuller LH, Cauley JA. 1999 Correlates of quantitative ultrasound in the Women's Healthy Lifestyle Project. *Osteoporos Int* 10:416–424.
27. Gur A, Sarac AJ, Nas K & Cevik R. 2004 The relationship between educational level and bone mineral density in postmenopausal women . *BMC Fam Pract.* 5: 18.
28. Hans D, Dargent-Molina P, Schott AM, Sebert JL, Cormier C, Kotzki PO, Delmas PD, Pouilles JM, Breart G, Meinuer PJ. 1996 Ultrasonographic heel measurements to predict hip fracture in elderly women: the EPIDOS prospective study. *Lancet* 348:511–514.
29. Hans D, Wacker W, Genton L, Paris E, Le-Floch C, Slosman D. 2002 Longitudinal quality control methodology for the quantitative ultrasound Achilles+ in clinical trial settings. *Osteoporos Int.* 13(10):788-795.
30. Hans D & Krieg M-A. 2008 The Clinical Use of Quantitative Ultrasound (QUS) in the Detection and Management of Osteoporosis. *IEEE transactions on ultrasonics, ferroelectrics, and frequency control.* 55: 1529 – 1538.
31. Hough S. 2003 Population differences in parameters of bone and mineral metabolism – the African paradox. *SAJCN* 16(3): 77-78.
32. Huopio J, Kroger H, Honkanen R, Jurvelin J, Saarikoski S, Alhava E. 2004 Calcaneal ultrasound predicts early postmenopausal fractures as well as axial BMD. A prospective study of 422 women. *Osteoporos Int* 15:190–195.
33. Ikeda Y & Iki M. 2004 Precision control and seasonal variations in quantitative ultrasound measurement of the calcaneus. *J Bone Miner Metab* 22: 588-93.
34. International Society for Clinical Densitometry. 2008 Official positions. Available at <http://www.iscd.org/Visitors/positions/OP-Index.cfm>
35. Karlsson C, Obrant KJ and Karlsson M. 2001 Pregnancy and Lactation Confer Reversible Bone Loss in Humans. *Osteoporos Int* 12:828–834.

36. Khan, AA and Syed ZS. 2004 Bone Densitometry in Premenopausal Women: synthesis and Review. *Journal of Clinical Densitometry* 7: 85-92.
37. Krieg M-A, Barkmann R, Gonelli S, Stewart A, Bauer, DC, barquero L, Kaufman J, Lorenc R, Miller PD, OLSzynski W, Poiana C, Schott A-M, Lewiecki EM & Hans D. 2008 Position Statement: Quantitative Ultrasound in the Management of Osteoporosis: the 2007 ISCD Official Positions. *Journal of Clinical Densitometry: Assessment of skeletal Health*, 11: 163-187.
38. Kriska AM, Sandler RB, Cauley JA, Laporte RE, Ham DL, Pambianco G. 1988 The assessment of historical physical activity and its relation to adult bone parameters. *Am J Epidemiol* 127: 1053-1063.
39. Kroke A, Klipstein-Grobusch K, Bergmann MM, Weber K, Boeing H. 2000 Influence of body composition on quantitative ultrasound parameters of the os calcis in a population-based sample of pre- and postmenopausal women. *Calcif Tissue Int* 66: 5-10.
40. Langton CM, Langton DK. 1997 Male and female normative data for ultrasound measurement of the calcaneus within the UK adult population. *Br J Radiol* 70: 580–585.
41. Langton CM, Langton DK. 2000 Comparison of bone mineral density and quantitative ultrasound of the calcaneus: site-matched correlation and discrimination of axial BMD status. *Br J Radiol* 73:31–35.
42. Langton CM and Njeh CF. 2008 The Measurement of Broadband Ultrasonic Attenuation in Cancellous Bone—A Review of the Science and Technology. *IEEE transactions on ultrasonics, ferroelectrics, and frequency control*. 55:1546 – 1554.
43. Lappa V, Dontas IA, Trovas G, Constantelou E, Galanos A, Lyritis GP. 2007 Quantitative ultrasound is better correlated with bone mineral density and biochemical bone markers in elderly women. *Clin Rheumatol* 26(7): 1067-73.
44. Lauderdale DS, Salant T, Han KL, Tran PL. 2001 Life-course predictors of ultrasonic heel measurement in a cross-sectional study of immigrant women from Southeast Asia. *Am J Epidemiol* 153(6):581-6.
45. Lopez-Rodriguez F, Mezquita-Raya P, de Dios LJ, Escobar-Jimenez F, Munoz-Torres M. 2003 Performance of quantitative ultrasound in the discrimination of prevalent osteoporotic fractures in a bone metabolic unit. *Bone (NY)* 32:571–578.
46. Mazess RB and Barden HS. 1991 Bone density in premenopausal women: effects of age, dietary intake, physical activity, smoking, and birth-control pills. *Am J Clin Nutr*. 1991 53(1):132-142.

47. McLean RR, Hannan MT, Epstein BE, Bouxsein ML, Cupples LA, Murabito J, Kiel DP. 2000 Elderly cohort study subjects unable to return for follow-up have lower bone mass than those who can return. *Am J Epidemiol* 151(7):689-692.
48. Micklesfield L, Rosenberg L, Cooper D, Hoffman M, Kalla A, Stander I, Lambert E. 2003 Bone mineral density and lifetime physical activity in South African women. *Calcif Tissue Int.* 73(5):463-469.
49. Micklesfield LK, Zielonka EA, Charlton KE, Katzenellenbogen L, Harkins J, Lambert EV. 2004 Ultrasound bone measurements in pre-adolescent girls: interaction between ethnicity and lifestyle factors. *Acta Paediatr* 93(6):752-758.
50. Micklesfield LK, van der Merwe L, Lambert EV. 2005 Lifestyle questionnaire to evaluate risk for reduced bone mineral density in women. *Clin J Sport Med.* 15(5):340-348.
51. Micklesfield LK, Norris SA, Nelson DA, Lambert EV, Van der Merwe L & Pettifor JM. 2007 Comparisons of body Size, Composition, and Whole Body Mass between North American and South African Children. *J Bone Miner Res* 22: 1-9.
52. Moyad MA. 2003 Osteoporosis: a rapid review of risk factors and screening methods. *Urol Oncol.* 21(5):375-9.
53. Nayak S, Olkin I, Hau Liu H, Grabe M, Gould MK, Allen IE, Owens DK and Bravata DM. 2006 Meta-Analysis: Accuracy of Quantitative Ultrasound for Identifying Patients with Osteoporosis *Annals of Internal Medicine* 144(11): 832-841
54. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis and Therapy. 2001 *JAMA* 285(6): 785-795.
55. Njeh CF, Fuerst T, Diessel E & Genant HK. 2001 Is quantitative ultrasound dependent on bone structure? A reflection. *Osteoporos Int* 12: 1-15.
56. Pocock NA, Babichev A, Culton N, Graney K, Rooney J, Bell D & Chu J. 2000 Temperature dependency of quantitative ultrasound. *Osteoporos Int* 11: 316-20.
57. Prins SH, Jorgensen HL, Jorgensen LV, Hassager C. 1998 The role of quantitative ultrasound in the assessment of bone: a review. *Clin Physiol* 18:3-17.
58. Rosenberg L, Zhang Y, Constant D, Cooper D, Kalla AA, Micklesfield L, Hoffman M. 2007 Bone status after cessation of use of injectable progestin contraceptives. *Contraception* 76(6):425-31.

59. Saadi HF, Reed RL, Carter AO, Dunn EV, Qazaq HS, Al-Suhaili AR. 2003 Quantitative ultrasound of the calcaneus in Arabian women: relation to anthropometric and lifestyle factors: *Maturitas* 44(3):215-23.
60. Sahara User's Guide. Document No 080-0631: Revision D. 1998 Hologic Inc, Waltham MA, USA: Section 1.14.
61. Stewart A, Felsenberg D, Eastell R, Roux C, Glüer CC, Reid DM. 2006 Relationship between risk factors and QUS in a European Population: The OPUS study. *Bone* 39(3):609-15.
62. Solomon L. 1979 Bone Density in aging Casucasian and African populations. *Lancet* 2: 1326-1330.
63. Töyräs J, Kröger H, Jurvelin JS. 1999 Bone properties as estimated by mineral density, ultrasound attenuation, and velocity. *Bone*. 25(6):725-31.
64. Tudor-Locke C, McColl RS. 2000 Factors related to variation in bone mineral status: a health promotion approach. *Osteoporos Int*. 11(1):1-24.
65. Vu TT, Nguyen CK, Nguyen TL, Le BM, NguyenTrung Le D, Bui TN, Nakamori M, Kunii D, Sakai T, Yamamoto S. 2005 Determining the prevalence of osteoporosis and related factors using quantitative ultrasound in Vietnamese adult women. *Am J Epidemiol* 161(9):824-830.
66. WHO Study Group. 2003 Prevention and management of osteoporosis. Technical report series 921, WHO, Geneva. Available at http://whqlibdoc.who.int/trs/who_trs_921.pdf
67. WHO Summary Meeting Report. 2004. Scientific group on the assessment of osteoporosis at primary health care level. 2004 available at http://www.nof.org/professionals/WHO_Osteoporosis_Summary.pdf
68. Yang, N-P, Jen I, Chuang S-Y, Chen S-H & Chou P. 2006 Screening for low bone mass with quantitative ultrasonography in a community without dual-energy X-ray absorptiometry: population-based survey. *BMC Musculoskeletal Disorders* 7:24.

APPENDIX 1

Table 13 : Adjusted Mean BUA, SOS and QUI in height categories, according to BMI strata

| BMI | Height | Coloured Participants Multivariable mean | | | | Black Participants Multivariable mean | | | |
|-------|-------------|---|-------|-------|-------|--|-------|-------|-------|
| | | N | BUA | SOS | QUI | N | BUA | SOS | QUI |
| < 25 | < 153 | 317 | 76.5 | 1567 | 102. | 52 | 75.5 | 1570 | 102.9 |
| | 153 – 157 | 235 | 74.8 | 1561 | 99.8 | 85 | 75.8 | 1561 | 100.2 |
| | 158 – 161 | 208 | 74.9 | 1557 | 98.2 | 136 | 78.0 | 1561 | 101.0 |
| | 162+ | 141 | 75.2 | 1557 | 98.2 | 183 | 77.0 | 1559 | 99.9 |
| | P for trend | | 0.237 | 0.000 | 0.001 | | 0.404 | 0.055 | 0.278 |
| 25-29 | < 153 | 204 | 77.6 | 1561 | 101.0 | 73 | 78.0 | 1566 | 102.9 |
| | 153 – 157 | 135 | 79.0 | 1559 | 100.8 | 138 | 81.9 | 1567 | 104.9 |
| | 158 – 161 | 108 | 78.4 | 1558 | 99.6 | 151 | 80.0 | 1563 | 102.5 |
| | 162+ | 60 | 79.6 | 1556 | 99.6 | 171 | 81.5 | 1559 | 101.8 |
| | P for trend | | 0.342 | 0.122 | 0.471 | | 0.224 | 0.020 | 0.225 |
| >30 | < 153 | 241 | 81.6 | 1566 | 104.5 | 86 | 83.8 | 1566 | 105.3 |
| | 153 – 157 | 121 | 80.0 | 1557 | 100.0 | 163 | 86.3 | 1570 | 108.3 |
| | 158 – 161 | 92 | 78.9 | 1552 | 97.7 | 179 | 84.4 | 1563 | 104.5 |
| | 162+ | 33 | 77.9 | 1560 | 100.4 | 181 | 84.9 | 1563 | 104.5 |
| | P for trend | | 0.047 | 0.000 | 0.001 | | 0.963 | 0.056 | 0.178 |

Control for age, education, age at menarche, number of live births, cigarettes smoking, alcohol use, calcium intake, physical activity..

Table 14: Adjusted Mean BUA, SOS and QUI in Physical activity categories, according to BMI strata

| BMI | Quartile of Primary school PA | Coloured Participants Multivariable mean | | | | Black Participants Multivariable mean | | | |
|-------|-------------------------------|---|-------|-------|-------|--|-------|-------|-------|
| | | N | BUA | SOS | QUI | N | BUA | SOS | QUI |
| < 25 | 1 (0 - 310) | 298 | 75.2 | 1561 | 99.7 | 61 | 74.5 | 1556 | 97.6 |
| | 2 (311 – 1118) | 288 | 75.3 | 1560 | 99.3 | 82 | 76.4 | 1562 | 101.1 |
| | 3 (1119 – 2582) | 244 | 75.1 | 1563 | 100.5 | 116 | 77.8 | 1560 | 100.7 |
| | 4 (2583 – 31579) | 71 | 78.4 | 1569 | 104.3 | 197 | 77.4 | 1562 | 101.3 |
| | P for trend | | 0.130 | 0.022 | 0.031 | | 0.286 | 0.329 | 0.282 |
| 25-29 | 1 (0 - 310) | 179 | 77.3 | 1556 | 98.7 | 82 | 81.1 | 1565 | 104.1 |
| | 2 (311 – 1118) | 149 | 78.7 | 1561 | 101.8 | 93 | 81.9 | 1568 | 105.5 |
| | 3 (1119 – 2582) | 128 | 80.5 | 1562 | 102.7 | 134 | 79.0 | 1560 | 101.2 |
| | 4 (2583 – 31579) | 51 | 76.2 | 1559 | 99.4 | 225 | 81.0 | 1562 | 102.5 |
| | P for trend | | 0.940 | 0.378 | 0.556 | | 0.892 | 0.156 | 0.236 |
| ≥30 | 1 (0 - 310) | 161 | 80.0 | 1560 | 101.2 | 93 | 82.2 | 1562 | 103.0 |
| | 2 (311 – 1118) | 165 | 79.4 | 1559 | 100.8 | 99 | 85.5 | 1565 | 105.6 |
| | 3 (1119 – 2582) | 111 | 81.7 | 1561 | 102.7 | 138 | 85.3 | 1566 | 106.2 |
| | 4 (2583 – 31579) | 50 | 82.7 | 1566 | 105.1 | 279 | 85.6 | 1566 | 106.2 |
| | P for trend | | 0.133 | 0.000 | 0.090 | | 0.212 | 0.315 | 0.246 |

Control for age, BMI, height, education, age at menarche, number of live births, cigarettes smoking, alcohol use, calcium intake.

Table 15: Adjusted Mean BUA, SOS and QUI in Age at menarche categories, according to BMI strata

| BMI | Age at Menarche | Coloured Participants | | | | Black Participants | | | |
|-------|-----------------|-----------------------|-------|-------|-------|--------------------|-------|-------|-------|
| | | N | BUA | SOS | QUI | N | BUA | SOS | QUI |
| < 25 | < 13 | 265 | 76.8 | 1564 | 101.8 | 37 | 78.7 | 1558 | 100.2 |
| | 13 – 14 | 375 | 75.1 | 1560 | 99.6 | 162 | 77.4 | 1562 | 101.1 |
| | 15 | 111 | 74.8 | 1561 | 99.8 | 96 | 76.8 | 1564 | 100.9 |
| | 16+ | 150 | 74.5 | 1560 | 99.1 | 117 | 75.2 | 1557 | 98.1 |
| | P for trend | | 0.113 | 0.134 | 0.108 | | 0.107 | 0.573 | 0.354 |
| 25-29 | < 13 | 149 | 78.4 | 1558 | 100.0 | 36 | 80.5 | 1561 | 102.1 |
| | 13 – 14 | 199 | 78.8 | 1560 | 100.8 | 218 | 80.5 | 1564 | 103.3 |
| | 15 | 68 | 76.9 | 1558 | 99.2 | 110 | 81.1 | 1563 | 103.0 |
| | 16+ | 90 | 78.4 | 1561 | 101.3 | 139 | 81.2 | 1562 | 102.5 |
| | P for trend | | 0.985 | 0.393 | 0.562 | | 0.665 | 0.689 | 0.895 |
| >30 | < 13 | 177 | 81.1 | 1561 | 102.2 | 47 | 88.8 | 1576 | 111.6 |
| | 13 – 14 | 197 | 79.6 | 1559 | 100.0 | 196 | 84.9 | 1566 | 105.8 |
| | 15 | 52 | 81.8 | 1566 | 104.4 | 134 | 83.9 | 1565 | 105.0 |
| | 16+ | 61 | 80.4 | 1559 | 101.1 | 173 | 84.0 | 1561 | 103.6 |
| | P for trend | | 0.728 | 0.617 | 0.633 | | 0.05 | 0.003 | 0.003 |

Control for age, BMI, height, education, number of live births, cigarettes smoking, alcohol use, calcium intake and physical activity.