CLINICAL SCIENCE

Serum testosterone, sex hormone-binding globulin and total calcium levels predict the calcaneal speed of sound in men

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OBJECTIVES: Variations in sex hormones and the calcium balance can influence bone health in men. The present study aimed to examine the relationship between the calcaneal speed of sound and biochemical determinants of bone mass, such as sex hormones, parathyroid hormones and serum calcium.

METHODS: Data from 549 subjects from the Malaysian Aging Male Study, which included Malay and Chinese men aged 20 years and older residing in the Klang Valley, were used for analysis. The subjects' calcaneal speed of sound was measured, and their blood was collected for biochemical analysis. Two sets of multiple regression models were generated for the total/bioavailable testosterone and estradiol to avoid multicollinearity.

RESULTS: The multiple regression results revealed that bioavailable testosterone and serum total calcium were significant predictors of the calcaneal speed of sound in the adjusted model. After adjustment for ethnicity and body mass index, only bioavailable testosterone remained significant; the total serum calcium was marginally insignificant. In a separate model, the total testosterone and sex hormone-binding globulin were significant predictors, whereas the total serum calcium was marginally insignificant. After adjustment for ethnicity and body mass index (BMI), the significance persisted for total testosterone and SHBG. After further adjustment for age, none of the serum biochemical determinants was a significant predictor of the calcaneal speed of sound.

CONCLUSION: There is a significant age-dependent relationship between the calcaneal speed of sound and total testosterone, bioavailable testosterone and sex hormone-binding globulin in Chinese and Malay men in Malaysia. The relationship between total serum calcium and calcaneal speed of sound is ethnicity-dependent.

KEYWORDS: Calcaneal Speed of Sound; Quantitative Ultrasound; Testosterone; Estradiol; Calcium; Parathyroid; Age; Men.

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INTRODUCTION

Osteoporosis is a systemic disease that is characterized by low bone density and deterioration of the bone microarchitecture, which lead to bone fragility and subsequent fractures (1). Men and women both suffer from osteoporosis, but the prevalence of fracture is lower in men than in women because men have a comparatively higher peak bone density and do not undergo a phase of accelerated bone loss (2). However, men suffer from greater morbidity and mortality after fracture than women (3). With the continual increase in lifespan, the burden of male osteoporosis on the healthcare system will continue to grow, especially in developing countries (4).

Bone mineral density (BMD) measurement using dual-energy X-ray absorptiometry (DXA) is the current gold standard for diagnosing osteoporosis (5). However, the cost and availability of DXA prevent its wide usage for osteoporosis screening in developing countries. Quantitative ultrasound (QUS) technology is an emerging technology that provides an alternative to DXA. It is relatively less costly, easier to handle, free of ionizing emission, portable and thus more accessible than DXA (6). Calcaneal speed of sound (SOS), which is a QUS index, has been shown to correlate strongly with bone density (7,8).

The role of testosterone in bone health has been confirmed by the observation that hypogonadal males have a lower BMD (9,10). The relationship between estradiol and bone health has also been shown in several ''natural experiments''. Males who express mutated estradiol receptors (11) or malfunctioning aromatase enzymes (12,13) have been

No potential conflict of interest was reported.

reported to experience abnormal bone growth and low bone density. This finding is further confirmed by experimental studies in which male subjects with suppressed expression of testosterone, estradiol, or both sex hormones exhibited higher bone turnover (14,15). Fewer studies have established a relationship between sex hormones and QUS indices.

Changes in calcium absorption can also contribute to osteoporosis in men. Studies have shown that there is a concurrent decline in BMD when calcium absorption is reduced in men (16). The decline in calcium absorption may trigger a feedback mechanism via the parathyroid (PTH) hormone, whereby bone resorption is increased to maintain the calcium balance in the blood (17). The relationship between PTH levels and BMD has been established (18). However, no study to date has attempted to correlate the variations in serum calcium and PTH levels with calcaneal QUS indices.

We previously indicated that the calcaneal SOS measured using a CM-200 sonometer (Furuno, Nishinomiya City, Japan) demonstrates an age-related decline in a healthy Malaysian male population (19). The present study aimed to explore whether the decline in calcaneal SOS is related to variations in biochemical determinants such as sex hormones, serum calcium and PTH levels in a population of healthy Malaysian men composed of men aged 20 years and older from two major ethnic groups, Chinese and Malays. The information from this study will provide a better understanding of the biochemical variables that influence QUS indices in men, thus enabling a wider application of the technology for osteoporosis screening. It will also help to identify potential areas of intervention that can prevent the progress of osteopenia and osteoporosis in aging men.

MATERIALS AND METHODS

Study design

The present study was conducted as part of the Malaysian Aging Male Study, which aimed to determine the nutritional, oxidative and bone health of healthy Malaysian men aged 20 years and older. It was a cross-sectional study, and subjects were recruited from September 2009 to September 2011. Purposive sampling was used, and subjects were recruited via advertisements in major newspapers, radio broadcasts, flyers and public announcements through community centers and religious facilities. The details of the study, including the specific inclusion and exclusion criteria, were clearly stated in the advertisement. The original sample size derived from the Malaysian Aging Male study was 840 subjects; 570 of these subjects consented to blood draws for biochemical testing, and their data were used for analysis. This study was approved by the Ethics Committee of Universiti Kebangsaan Malaysia Medical Center (UKMMC), Research Project Code: UKM-AP-TKP-09-2009 and FF-376-2010.

Subjects

The subjects who volunteered for this study were males aged 20 years and older of Malay and Chinese ethnicity who resided in the Klang Valley of Peninsular Malaysia (Kuala Lumpur, Shah Alam, Klang, Petaling Jaya, Gombak). All of the subjects were screened using a detailed demographic questionnaire and their previous medical records. Physical examinations and medical history interviews were conducted by qualified physicians. Subjects with the following conditions were excluded: 1) mobility impairment (requiring walking aids); 2) bone fracture six months prior to screening; 3) major systemic diseases affecting bone metabolism, such as osteoporosis, osteomalacia, osteogenesis imperfecta, rickets, Paget's disease, hyper/hypocalcemia and hyper/hypoparathyroidism; and 4) taking medications known to affect bone metabolism, such as testosterone, thyroid hormones, thiazide, diuretics, glucocorticoids, bisphosphonate, anticonvulsants and lithium. All of the subjects received detailed information regarded the study, and written consent was obtained.

Body anthropometric measurements

The subjects' weight in light clothing and without shoes was determined using a standardized balance beam scale and was recorded to the nearest 0.1 kg. The subjects' standing height without shoes was determined using a portable stadiometer and was recorded to the nearest 0.1 cm. The subjects' body mass index (BMI) was calculated using the formula BMI (kg/m^2) = body weight (kg)/height squared (m^2).

Calcaneal speed of sound measurement

The subjects' calcaneal SOS was determined using a CM-200 sonometer (Furuno, Noshinomiya City, Japan), which measured the speed of sound (SOS) passing through the subject's calcaneus as a determinant of bone health status. The CM-200 is a gel-coupled (dry) system that consists of two transducers. The subjects were required to place their right foot on the foot patch, which was adjusted to their foot size. The sound waves emitted from one transducer were transmitted through the calcaneus and received by another transducer. The signal was then analyzed and sent to the computer for storage and display. Three readings with repositioning were obtained for each subject. All measurements were performed by a trained technician. The instruments were calibrated prior to each screening session, and quality control was conducted using a phantom. The short-term in-vivo coefficient of variation for the device was approximately 0.1%.

Laboratory assays

All of the subjects were required to fast for at least eight hours before attending the screening sessions. During the fasting period, they were not allowed to consume any food or beverages except plain water. Venipuncture was performed between 08:30 and 10:30. The blood was collected in plain tubes, and the serum was extracted. Part of the serum was sent immediately for total testosterone, total estradiol, total calcium, inorganic phosphate and albumin assays. The remaining serum was stored at -70℃ for sex hormone-binding globulin (SHBG) and intact parathyroid (PTH) level measurement. The storage period for the serum was one to six months. Total testosterone and total estradiol levels were measured using an ADVIA Centaur immunoassay system (Siemens Healthcare Diagnostics, Illinois, USA) based on competitive immunoassay with direct chemiluminescent technology. The free and bioavailable fractions of testosterone and estradiol were calculated using methods previously described by Södergård et al. (1982) (20). Total calcium, inorganic phosphate and albumin were measured with the ADVIA 2400 (Siemens Healthcare Diagnostics, Illinois, USA) using colorimetric methods. SHBG and PTH were

measured using solid phase enzyme-linked immunosorbent assay (ELISA) kits based on the sandwich principle (IBL International, Hamburg, Germany). The manufacturers' test principles and procedures were followed.

Data analysis

The normality of the data was determined using the Shapiro-Wilk test. A $\log_{10}$ transformation was attempted for the SHBG and PTH levels, which reverted to normal; thus, the $\log_{10}$ values were used for analysis. However, the estradiol levels remained skewed after conventional transformation methods were attempted. Therefore, the estradiol levels were recoded into tertiles as 'low', 'moderate', and 'high' levels for analysis. Normally distributed data were presented as the mean (standard deviation [SD]) and skewed data were presented as the median (interquartile range [IQR]). Age, body anthropometry, calcaneal SOS and serum biochemical determinants were compared between the Chinese and Malay subjects using independent t-tests for normal data and Mann-Whitney U-tests for skewed data. A univariate analysis with adjustment for confounding variables, such as age and BMI, was performed when necessary. A multiple linear regression was performed to evaluate the relationship between the calcaneal SOS value and biochemical determinants. Two separate models were generated for the total/bioavailable testosterone and total/bioavailable estradiol to prevent multicollinearity. Estradiol levels were entered into the regression models as dummy variables, using the 'low' level as the reference group to which the 'moderate' and 'high' estradiol level groups were compared. For continuous data, the standardized coefficient beta (β) explained the extent of variation in calcaneal SOS when predictors of interest changed by 1 SD, whereas for dichotomous data (dummy variables), β explained the standardized difference of the group in comparison to the reference group. Significance was set at $p<0.05$. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) Version 16.0 (SPSS Inc., Chicago, USA).

RESULTS

A total of 570 subjects completed the required screening procedures, calcaneal SOS measurement, body anthropometric measurements and blood collection. After adjustment for missing values and outliers, data from 549 subjects (96.32%) were available for analysis. Two hundred forty subjects (43.7%) were Malay, and 309 subjects (56.3%) were Chinese. The subjects' ages ranged from 20 to 83 years, with a mean of 46.1 years (SD = 15.1 years).

The Malay subjects were significantly younger than the Chinese subjects were ($p<0.05$). They also had a significantly higher weight and shorter stature, thus presenting a higher BMI than the Chinese subjects ($p<0.05$). The difference in calcaneal SOS values between the two ethnic groups was not significant after adjustment for age and BMI ($p>0.05$). The Malay subjects also had significantly higher total serum calcium and inorganic phosphate levels than the Chinese subjects ($p<0.05$), and the significance persisted after adjustment for age. The differences in all sex hormones (total and bioavailable fractions), intact PTH and SHBG levels between the two ethnic groups were not significant ($p<0.05$; Table 1).

Multiple regression analyses revealed that bioavailable testosterone ($\beta = 0.132$, $p<0.05$) and serum total calcium ($\beta = 0.091$, $p<0.05$) were significant predictors of the SOS in men. However, after adjustment for BMI and ethnicity, only bioavailable testosterone ($\beta = 0.162$, $p<0.05$) was a significant predictor of the SOS in the study population, and the serum total calcium was marginally not significant ($\beta = 0.081$, $p = 0.07$). After further adjustment for age, the relationship between the calcaneal SOS value and all biochemical determinants was not significant (Table 2).

Table 1 - Characteristics of the study population, stratified by ethnicity.

Ethnicity	Malays	Chinese	Total
Variable	Mean (SD)	Mean (SD)	Mean (SD)
Age	45.29 (17.38)*	46.80 (13.08)	46.14 (15.11)
Height	165.98 (6.37)*	168.42 (6.31)	167.36 (6.45)
Weight	71.54 (14.36)*	69.06 (12.54)	70.14 (13.40)
BMI	25.94 (4.78)*	24.31 (3.89)	25.02 (4.37)
Calcaneal SOS	1521.30 (27.94)	1515.80 (26.82)	1518.24 (27.43)
Total T	19.18 (6.94)9	18.76 (6.39)	18.94 (6.63)
Bioavailable T	11.09 (3.66)	10.62 (3.46)	10.82 (3.56)
Total calcium	2.29 (0.12)*	2.23 (0.10)	2.26 (0.11)
Inorganic phosphate	1.12 (0.15)*	1.08 (0.15)	1.10 (0.15)
	Median (IQR)	Median (IQR)	Median (IQR)
SHBG	38.63 (28.04)	41.12 (28.31)	39.69 (28.00)
Total E_2	87.50 (86.00)	82.00 (112.50)	86.00 (101.00)
Bioavailable E_2	60.65 (57.15)	55.13 (77.64)	58.03 (68.91)
Intact PTH	44.58 (21.55)	43.43 (22.34)	43.96 (22.00)

Abbr: SD, standard deviation; IQR, interquartile range; BMI, body mass index; E_2, estradiol; PTH, parathyroid hormone; SOS, calcaneal SOS; T, testosterone.*Indicates significant differences between Malay and Chinese subjects ($p<0.05$). Normally distributed data are presented as the mean (standard deviation), whereas skewed variables are presented as the median (interquartile range).

Multiple regression was repeated using total testosterone, total estradiol and SHBG as predictors. Total testosterone ($\beta = 0.120$, $p<0.05$) and SHBG ($\beta = -0.149$, $p<0.05$) were significant predictors of SOS in the study population. The relationship between total serum calcium and calcaneal SOS was marginally not significant ($\beta = 0.081$, $p = 0.068$). The significance for total testosterone ($\beta = 0.156$, $p<0.05$) and SHBG ($\beta = 0.132$, $p<0.05$) persisted after adjustment for BMI and ethnicity. However, after further adjustment for age, none of the biochemical determinants was a significant predictor of calcaneal SOS ($p>0.05$).

DISCUSSION

Bone mineral density (BMD) has been associated with variations in sex hormones. A cross-sectional study conducted by Khosla et al. (1998) revealed that in males, the BMD at various sites correlated significantly with the bioavailable testosterone, total estradiol and bioavailable estradiol, but not with total testosterone. A multiple regression model in the same study also indicated that bioavailable estradiol and non-bioavailable estradiol were significant determinants of BMD (21). This finding was confirmed by Araujo et al. (2008), who showed that after various adjustments, total estradiol and free estradiol but not testosterone levels were significantly correlated with BMD in males. However, the bioavailable fraction of sex hormones was not considered in their study (22).

The calcaneal SOS has been shown to reflect bone mineral density, but its association with sex hormones remains

Table 2 - Results of a stepwise multiple regression between calcaneal speed of sound values and biochemical determinants.

Predictors	Standardized regression coefficient (β)		
	Unadjusted	Adjusted for ethnicity and BMI	Adjusted for ethnicity, BMI and age
1 Bioavailable T	0.132*	0.162*	0.044
Serum total calcium	0.091*	0.081	-0.006
Intact PTH	-0.056	-0.069	-0.038
Moderate *vs.* low bioavailable E_2	0.079	0.068	0.062
High *vs.* low bioavailable E_2	0.061	0.058	0.018
2 Total T	0.120*	0.156*	0.046
SHBG	-0.149*	0.132*	0.045
Serum total calcium	0.081	0.074	-0.011
Intact PTH	-0.066	-0.077	-0.042
Moderate *vs.* total E_2	0.082	0.078	0.067
High *vs.* low total E_2	0.063	0.066	0.031

Abbr: E_2, estradiol; T, testosterone; SHBG, sex hormone-binding globulin.
*Indicates $p>0.05$. For continuous data, the standardized coefficient beta (β) explained the extent of the variation in calcaneal SOS when the predictor of interest changed by 1 SD, whereas for dichotomous data (dummy variables), β explained the standardized difference of the group compared with the reference group.

uncertain. A study conducted by Gennari et al. (2003) revealed that after adjustment for BMI and age, the calcaneal SOS correlated significantly with estradiol levels (total, bioavailable and free fractions), but not with testosterone levels (total, bioavailable and free fractions) (23). In comparison, Kuchuk et al. (2007) found significant differences in the calcaneal SOS value for subjects in the highest quartile of bioavailable testosterone compared with lower quartiles; however, similar results were not found for the bioavailable estradiol level, even though the men in the lowest quartile for bioavailable estradiol had a lower BMD and higher bone turnover (24). Vanderschueren et al. (2010) found that the associations between calcaneal SOS and free and bioavailable testosterone, sex-hormone binding globulin levels and estradiol levels (total, bioavailable and free fractions) in 3,141 European males were significant, but no significant association was found between calcaneal SOS and total testosterone (25).

In this study, calcaneal SOS measured with the CM-200 was moderately correlated with BMD ($r = 0.68$) (26). The calcaneal SOS was significantly associated with bioavailable testosterone in the unadjusted model; however, it was not independent of age (when adjusted for age, the relationship became insignificant). This finding is different from that of Vanderschueren et al. (2010), who found that the relationship between calcaneal SOS and bioavailable testosterone was significant when the analysis was adjusted for age (25). Most studies on the association between calcaneal SOS and total testosterone indicate a nonsignificant relationship (23,25); however, in the present study, the relationship between total testosterone and calcaneal SOS was significant. The association of total and free estradiol levels with calcaneal SOS was generally positive but not significant. In general, the subjects in the lowest tertile for estradiol levels had insignificantly lower calcaneal SOS values than subjects in the higher tertiles. This finding is similar to that of Kuchuk et al., who reported an insignificant relationship between SOS and bioavailable estradiol. Kuchuk et al. also reported a significant relationship between the broadband attenuation of sound and bioavailable testosterone (24), which suggests that bioavailable estradiol may exert effects on bone components that are undetectable using SOS. The level of sex hormone-binding globulin was significantly and inversely related to the calcaneal SOS in this study. Sex hormone-binding globulin reduces the bioavailability of sex hormones; therefore, it has a negative impact on bone health (27) that has been shown in previous studies in which lower SHBG levels in men appeared to offer protection against osteopenia (28), and men with higher SHBG levels had greater fracture risk (29).

Variations in calcium absorption play an important role in bone loss in men. Previous studies indicated a concurrent decline of calcium absorption and BMD in men (16) that may result from a decrease in renal function, which in turn reduces the renal secretion of calcitriol and leads to the malabsorption of calcium. Consequently, PTH secretion is induced, and bone resorption occurs (30). Other causes of calcium imbalance include decreased absorption of vitamin D (31) and a decreased intestinal response to calcitriol (32).

Most studies have established a significant relationship between BMD and PTH levels (18,33). Studies examining the association between calcaneal SOS and PTH or serum calcium levels are scarce. In the present study, calcaneal SOS values correlated with total serum levels but not with PTH level. The transient increase in PTH levels in our subjects was mild and may not have caused bone mass variations that were detectable with the QUS technique. It should be noted that previous findings related to the association between BMD and PTH levels were established at sites other than the calcaneus (for example, the hip (34) and the femoral neck (33)). Hence, it is uncertain how much PTH variations affect bone mass at the calcaneus. The positive and significant association between serum total calcium level and calcaneal SOS suggests that other underlying causes mediate the relationship between calcium balance and calcaneal SOS value. A high-protein diet has been hypothesized to increase calcium excretion (35), but this possibility was not examined in the present study. Calcium excretion has also been shown to increase with age (36), and its effects may not be compensated for, thus creating a continuous negative calcium balance in the body that stimulates bone resorption.

Several limitations must be considered in the interpretation of this study's results. The sampling method used in the present study was a purposive, nonrandomized sampling method; consequently, substantial selec-

tion bias may have been introduced during recruitment, and the results should be generalized with caution. The QUS device used, the CM-200, generates only one QUS index: calcaneal SOS. Other indices, such as broadband attenuation of sound and stiffness index, were not evaluated. The true calcium absorption and excretion were not measured in the present study; consequently, the calcium balance observed was based on the serum total calcium level alone and may not be adequate. Calcitriol levels and dietary calcium consumption among the subjects were not investigated in the present study; consequently, the variations in calcium level could not be fully explained. We experienced substantial difficulty in comparing the regression model generated in the present study with the literature because previous studies did not report their linear regression procedures in detail. Multicollinearity issues created by entering the total and bioavailable or free sex hormones into the same regression model were also apparent in previous studies. In the present study, the total and bioavailable sex hormones were entered into two separate regression models so that the results presented are valid and unaffected by multicollinearity.

In conclusion, SHBG and total and bioavailable testosterone are significantly associated with calcaneal SOS values in men, and the relationships are age-dependent. The relationship between total serum calcium and calcaneal SOS is ethnicity-dependent. Pharmacologic interventions for the age-dependent deterioration of bone health in men should aim to maintain optimal bioavailable testosterone and calcium levels.

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AUTHOR CONTRIBUTIONS

Chin KY contributed to subject recruitment, calcaneal speed of sound measurement, research implementation and the writing of the manuscript. Soelaiman IN planned the research, provided critical review and gave final approval of the manuscript. Mohamed IN provided the critical statistical review of the manuscript. Ngah WZ contributed to the research construction and obtained ethics approval, financial support and supervised the project.

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