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Feasibility of impact microindentation testing in population-based research

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ABSTRACT

Objectives The OsteoProbe® measures bone material strength index (BMSi) of cortical bone in living humans using impact microindentation (IMI). Research using this minimally-invasive technique is expanding yet, to-date, there have been no reports about its acceptability in the research setting. In this study we assessed the acceptability and feasibility of using the OsteoProbe® to assess men enrolled in the Geelong Osteoporosis Study.

Design Cross-sectional analysis of data collected in a population-based study.

Setting Barwon Statistical Division, south-eastern Australia, 2016-2018

Methods For 252 of 345 consecutive participants (ages 33-96 years), BMSi was measured using the OsteoProbe® at the mid-tibia. Immediately following measurement, each participant used a visual analogue scale (0-10) to rate the level of discomfort that was anticipated and experienced, their initial reluctance towards the measurement and their willingness to repeat measurement.

Results Reasons for non-measurement in 92 men were needle phobia (n=8), discomfort after first indentation (n=5), skin infections (n=21), excessive soft tissues around the mid-tibia region (n=56), inability to provide informed consent (n=2). Among 252 men who had IMI measures, the expectation for pain during measurement was low (1.54±1.56), as was actual pain experienced (0.38±0.71). Reluctance to undergo measurement was low (0.34±0.93). All participants indicated a willingness to have the measurement performed again. Mean (±SD) BMSi was 83.0±6.4 (range 62.3-93.0).

Conclusion In this study, the procedure was well accepted by participants suggesting that IMI testing with the OsteoProbe® is feasible in a research setting.

Keywords: Microidentation, bone material strength index, fractures, OsteoProbe.

Strengths and limitations of this study

- This is the first study to examine the acceptability and feasibility of the OsteoProbe® in a population-based study setting.
- We evaluated the associations between BMSi and age in the largest population-based sample of men so far.
- We cannot exclude the possibility of some bias in the BMSi outcome because of non-participation.

Introduction

The most widely used clinical measurement for ascertainment of fracture risk is bone mineral density (BMD) measured by dual- energy X-ray absorptiometry (DXA) (1). However, BMD does not fully explain fracture risk, as the largest absolute number of fragility fractures occur in people without severe deficits in BMD (2,3). Other determinants of bone strength such as bone geometry, microarchitecture and material properties are likely to contribute to fracture risk. Also, clinical factors such as a history of prior fracture, age, exposure to glucocorticoid and other medications, smoking and falls, can contribute independently to fracture risk, and these risk factors can be incorporated into fracture risk algorithms such as FRAX (4), the GARVAN algorithm (5) and the FRISK score (6). Therefore, the goal of much research is to develop techniques to better identify patients at risk of fracture. Using a novel device, the OsteoProbe®, to assess cortical bone material strength index (BMSi) in vivo, impact microindentation (IMI) is one such technique (7). The technical aspects of this device have been discussed in detail elsewhere. (7,8) Its output; the BMSi, is a unitless parameter derived as the ratio of the average indentation distance of repeated measurements made into a reference material, polymethylmetacrylate (PMMA), and into bone (8). The use of this device

in research is growing; and BMSi has reportedly distinguished between patients with different levels of fracture risk in some (9–12) but not all (13) studies, often independently of BMD. These studies have been limited to relatively small sample sizes and have often involved patients selected on the basis of disease.

Although these data suggest that BMSi might have clinical utility, future research is warranted, particularly using unselected, population-based samples, to assess the performance of IMI for identifying individuals at risk of fracture. As a new, minimally-invasive technology, it is important to gauge the acceptability of the IMI to better understand whether participant groups and volunteers are likely to undergo the procedure in a research setting. The aim of this study was to assess the acceptability and feasibility of the OsteoProbe® among men enrolled in the Geelong Osteoporosis Study (GOS).

METHODS

Patient and Public involvement

Study participants were selected at random from the general population and invited to participate; they did not represent a specific patient group. This observational study was designed as part of an ongoing program of research aimed at improving fracture risk assessment. While participants were not involved in developing the research question, we documented their participant experience by questionnaire. Results will be disseminated to participants via a research update which is distributed annually.

Source Population

The GOS is a population-based study situated in a defined region in south-eastern Australia, known as the Barwon Statistical Division (14). An age-stratified, randomly-selected cohort of 1540 men aged 20-97 years was recruited 2001-2006 using the electoral roll as the sampling frame. The cohort is being followed prospectively, with assessments every few years. (14) This analysis focuses on the first 345 men assessed as part of the 15-year follow-up. In conjunction with other clinical measures including bone densitometry, participants were approached to undergo IMI testing and complete a short questionnaire about their experience relating to the technique. The study was approved by the Human Research Ethics Committee at Barwon Health. All participants provided informed consent.

Bone material strength testing

IMI was performed by a trained operator using the OsteoProbe® (Active Life Scientific, Inc., CA, USA). The IMI procedure was explained to participants before measurements were made. Participants were positioned in decubitus supine position, with the leg to be measured rotated to orient the flat surface of the medial tibia diaphysis. The mid distance between the medial border of the tibia plateau and the medial malleolus was marked using a measuring tape. Following a disinfection of the area using a 70% isopropyl alcohol swab, 2% Lidocaine was administered by inserting a syringe both subcutaneously and in the periosteal surface. A sterile probe was then inserted at the marked mid diaphysis, piercing the skin and periosteum until reaching the bone cortex. While maintaining probe contact with the bone surface, as well as orienting the probe perpendicularly to the tibia surface, the outer housing of the device was slid towards the subject's leg to initiate a measurement. After the first measurement, the probe was moved to a new location, at least 2mm away from the prior measurement, to obtain another measurement. In this study, at least 11 indentations were

performed on each subject, of which the first measurement was systematically disregarded followed by 10 valid test indentations. A trained observer assisted the operator by ensuring that the probe was held perpendicular to the tibial surface. The procedure was conducted according to internationally-recognised recommendations for using the Osteoprobe RUO (15). Immediately following measurement, each participant completed a questionnaire that asked them to rate on a visual analogue scale (0-10) the level of pain that was anticipated, the level of pain that was experienced, their initial reluctance towards the measurement and their willingness to undergo the measurement again.

Statistical Analyses

Comparisons between participants included and excluded in analyses were identified using t-tests, and one-way ANOVA for differences in visual analogue scale responses across age groups. Pearson product moment correlation was used to test for a linear correlation between BMSi and age, weight, height and BMI. Statistical analyses were performed using Minitab (version 17; Minitab, State College, PA).

RESULTS

Of 345 potential participants, exclusions were: needle phobia (n=8), skin infections (n=21), excessive soft tissues around mid-tibia region (n=56), due to discomfort (pressure, no pain) after the first indentation (n=5), unable to provide informed consent (n=2). One participant had IMI after skin infection had been treated. Therefore, 252 participants were included in the subsequent analyses.

Table 1 shows characteristics of study participants included and excluded in the analyses respectively. Participants ranged in age from 33 to 96 years.

BMSi ranged from 62.3 to 93.0. Mean BMSi±SD values for age groups 30-49, 50-69 and 70+ years were 81.9±5.4, 83.7±6.2 and 81.6±6.7, respectively. Acceptability scores were similar across all ages (Table 2, Figure 1). The expectation for pain during OsteoProbe® measurement was low, as was actual pain experienced and initial reluctance to undergo measurement. Acceptability of the OsteoProbe® measurement was high; all 252 participants who had a successful measurement indicated a willingness to undergo the measurement again. No adverse events were reported.

There were no correlations detected between BMSi and age (r= 0.070, p=0.270), height (r=0.068, p= 281) or weight (r= -0.078, p= 0.215); however, there was a negative correlation between BMSi and BMI (r=-0.135, p=0.032).

Table 1 Characteristics of the study participants included and excluded in analyses

Characteristics	Included (n=252)	Excluded (n=92)	p Value
Age (yr)	63.2 ± 12.6	65.5 ± 15.6	0.2
Weight (kg)	81.2 ± 10.9	92.9 ± 19.1	0.6
Height (cm)	174.3 ± 6.9	173.7± 10.3	0.0
Body Mass Index (kg/m^2)	26.7 ± 3.1	30.8 ± 7.8	0.0

Data shown as mean (±SD)

Table 2: Feasibility scores for the whole group and according to age

	ALL ages	30-49 yr	50-69 yr	70+ yr	p Value
***Expectation for Pain**	1.5 ± 1.6	2.0 ± 1.7	1.6 ± 1.6	1.2 ± 1.5	0.1
***Actual pain experienced**	0.4 ± 0.7	0.4 ± 0.8	0.4 ± 0.8	0.3 ± 0.47	0.4
***Level of reluctance**	0.3± 0.9	0.6 ± 1.0	0.4 ± 1.0	0.2 ± 0.6	0.1

*Questionnaire results for 0-10 visual analogue scale

DISCUSSION

In this study, we observed a high acceptability of IMI; their reluctance to undergo the measurement was low and a majority indicated a willingness to undergo the measurement again.

Although IMI is a minimally invasive procedure, with indentations on the scale of micrometres, a valid concern is the acceptability of the procedure in a research setting, particularly in studies that rely on participants/volunteers from the general population. We report that testing with the device is feasible among participants in our population-based study. The most common reason for exclusion was excessive soft tissues around the mid-tibia. Other reasons for exclusions were skin infections, needle phobia, inability to provide informed consent and discomfort after the first indentation, but these affected only one-tenth of the study participants. Other potential contraindications include prior clinical or stress fracture in the tibia diaphysis, focal tibial lesions and Paget's disease (15); however, to the best of our knowledge, no studies have reported the actual number of exclusions based on these criteria.

In studies using IMI, only two instances of adverse effects have been reported in the literature, one associated with reaction to local anaesthetic and one mild skin infection, in more than 1300 measured individuals (15). To our knowledge, we are the first to document the acceptability of the procedure. Given that the anterior surface of the tibial is pre-treated with a local anaesthetic delivered by subcutaneous injection through the skin and around the periosteum and that the probe of the device is inserted through the skin, subcutaneous soft tissue and periosteum into the cortical bone, it would seem likely that the procedure might seem unpleasant for some study participants. High rates of refusal would introduce bias. However, our results indicate that IMI is generally acceptable, at least for the participants of our study implying that the technique shows promise as a measure of bone material properties in a practical, safe and convenient manner.

Furthermore, we investigated participants from a wide age range and observed similar feasibility scores across age groups ranging from 30-49, 50-69 to 70 years and older. The men in the oldest category tended to report a lower expectation for pain, experienced less pain and were less reluctant to undergo the measurement, but these differences were not statistically significant.

In this study, we did not observe a correlation between BMSi and age, corresponding to two studies by Duarte Sosa et al. in which no association was detected between age and BMSi in 42 Norwegian and 46 Spanish women (16) and among 30 women with previous stress fractures and 30 normal controls (17). By contrast, an inverse association between age and BMSi has been reported for 90 patients (male and female) with low bone mass, ($r=-0.539$; $p<0.001$) and in a case-control study of 48 acromegaly patients and 44 controls (male and female), there was a positive association for patients with acromegaly ($r=0.291$, $p = 0.045$) and an inverse relationship for the controls ($r=-0.457$, $p=0.002$) (18). The reason for lack of

consistency in results is not clear but likely reflects differences in study design and study populations.

Moreover, no correlations of BMSi with height, weight were found but we observed a negative correlation between BMSi and BMI. Similarly, Sundh et al. (19) reported a negative correlation (r=-0.17, p=0.01) between BMSi and BMI in a population-based study of 202 women between 75 and 80 years of age, and Rudang et al. (13) reported a weak inverse correlation (r=-0.14, p=0.04) between BMSi and weight in a population-based cohort of 211 women between 75 and 80 years of age, and, in accordance with our findings, there was no association with height.

To our knowledge, this is the first study to examine the acceptability and feasibility of the OsteoProbe® in a population-based study. Unlike most of the previous studies, this study is population-based and not selected on the basis of disease status. The outcome will thus be relevant for the general population. In this study, one operator conducted the IMI measurements and an observer was present to ensure the procedure was performed according to the standardised procedure.

However, we acknowledge the following limitations. IMI could not be performed in individuals with substantial amounts of soft tissue around the mid-tibia region, nor in individuals with skin disorders or infections in at the site of measurement and this may have biased our results. It should be noted that there are also drawbacks with other technologies. For example, a weight limitation (typically 120kg or 300lb) and narrow bed width (~60 cm) necessitates exclusion of large individuals from assessment with DXA (20). Furthermore, individuals with spinal abnormalities and those affected by devices such as plates, screws, silicone implants and prostheses can compromise the interpretation of DXA scans (21–24). In

our study, we investigated men only, and recognise that the observations may not be generalisable to women or other populations.

In conclusion, IMI was well accepted by participants suggesting that testing with the OsteoProbe® is feasible in a research setting. Further assessment of the clinical utility of this technology for assessing fracture risk is warranted and currently in progress.

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Competing Interests

PGR is supported by Deakin University Postgraduate Industry Research Scholarship. KLH-K is supported by an Alfred Deakin Postdoctoral Research Fellowship. AD-P owns shares of Active Life Scientific, Inc., the manufacturer of the RPI device. MAK and JAP are recipients of grants from the NHMRC and Amgen-GSK OA-ANZBMS.

Authors' contributions

PGR performed the indentation measurements in the presence of another trained observer (KLH-K) and drafted the manuscript. KLH-K assisted with taking measurements and administered questionnaires. AD-P assisted with training to use the OsteoProbe device and provided advice on measurement technique. MAK and JAP conceived and designed the study. JAP secured ethics approval. All authors interpreted the data, guided and reviewed the manuscript. All authors read and approved the final manuscript.

Data sharing statement

The datasets used and analysed during the current study are available from the corresponding author on reasonable request. Requests can be sent to gos@barwonhealth.org.au.

Ethics approval and consent to participate

This project has been approved by Human Research Ethics Committee at Barwon Health. All participants gave written informed consent to participate in the study.

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Figure 1: An interval plot of 0-10 Visual Analogue scale results, according to age

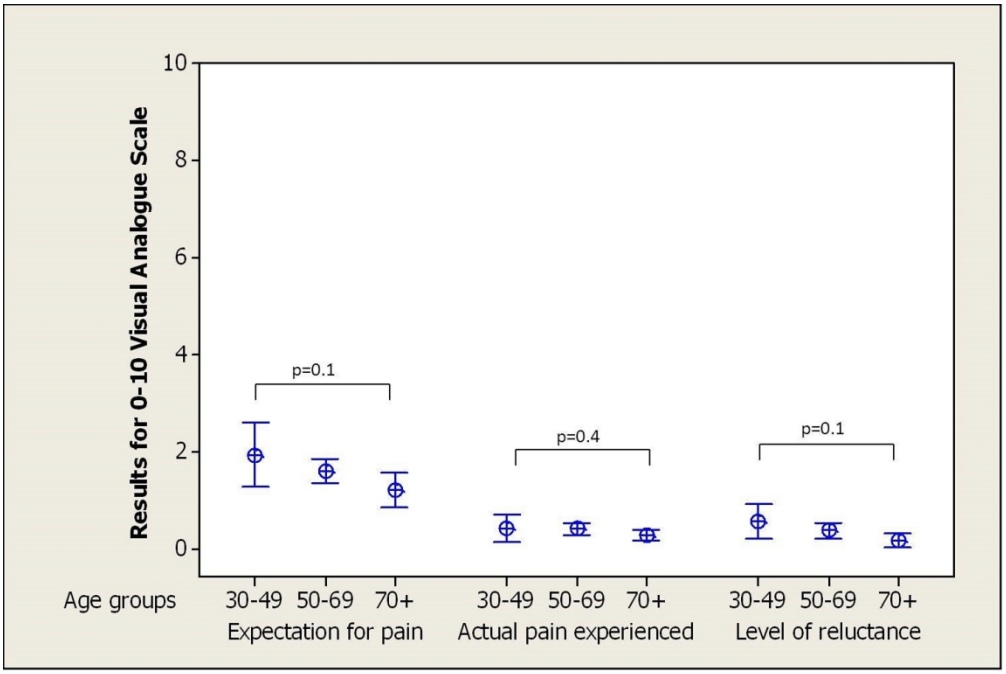

Figure 1: An interval plot of 0-10 Visual Analogue scale results, according to age

127x84mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	3-4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-6
Bias	9	Describe any efforts to address potential sources of bias	4-6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	N/A
		(*e*) Describe any sensitivity analyses	N/A

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6-7
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	7
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6-7
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Setting Barwon Statistical Division, south-eastern Australia, 2016-2018

Methods For 252 of 345 consecutive participants (ages 33-96 years), BMSi was measured using the OsteoProbe® at the mid-tibia. Immediately following measurement, each participant used a visual analogue scale (0-10) to rate the level of discomfort that was anticipated and experienced, their initial reluctance towards the measurement and their willingness to repeat measurement.

Results Reasons for non-measurement in 92 men were needle phobia (n=8), discomfort after first indentation (n=5), skin infections (n=21), excessive soft tissues around the mid-tibia region (n=56), inability to provide informed consent (n=2). Among 252 men who had IMI measures, the expectation for pain during measurement was low (1.54±1.56), as was actual pain experienced (0.38±0.71). Reluctance to undergo measurement was low (0.34±0.93). All participants indicated a willingness to have the measurement performed again. Mean (±SD) BMSi was 83.0±6.4 (range 62.3-93.0).

Conclusion In this study, the procedure was well accepted by participants suggesting that IMI testing with the OsteoProbe® is feasible in a research setting.

Keywords: Microidentation, bone material strength index, fractures, OsteoProbe.

Strengths and limitations of this study

- This is the first study to examine the feasibility and tolerability of the OsteoProbe® in a population-based study setting.
- We evaluated the associations between BMSi and age in the largest population-based sample of men so far.
- We cannot exclude the possibility of some bias in the BMSi outcome because of non-participation.

Introduction

The most widely used clinical measurement for ascertainment of fracture risk is bone mineral density (BMD) measured by dual- energy X-ray absorptiometry (DXA) (1). However, BMD does not fully explain fracture risk, as the largest absolute number of fragility fractures occur in people without severe deficits in BMD (2,3). Other determinants of bone strength such as bone geometry, microarchitecture and material properties are likely to contribute to fracture risk. Also, clinical factors such as a history of prior fracture, age, exposure to glucocorticoid and other medications, smoking and falls, can contribute independently to fracture risk, and these risk factors can be incorporated into fracture risk algorithms such as FRAX (4), the GARVAN algorithm (5) and the FRISK score (6). Therefore, the goal of much research is to develop techniques to better identify patients at risk of fracture. Using a novel device, the OsteoProbe®, to assess cortical bone material properties in vivo, impact microindentation (IMI) is one such technique (7). The OsteoProbe® measures Bone Material Strength Index (BMSi). This parameter quantifies how well a bone resists microindentation. BMSi is defined as 100 times the ratio of the indentation distance from the impact to a calibration material, PMMA (poly methyl methacrylate), divided by the indentation distance from the impact into

the bone. As the probe indents the bone, it induces microfractures. The more easily the bone is fractured, the deeper the probe indents and the lower the BMSi. The technical aspects of this device have been described in detail in the initial scientific instrumentation papers (7, 8). The use of this device in research is growing; and BMSi has reportedly distinguished between patients with different levels of fracture risk in some (9–12) but not all (13) studies, often independently of BMD. These studies have been limited to relatively small sample sizes and have often involved patients selected on the basis of disease.

Although these data suggest that BMSi might have clinical utility, future research is warranted, particularly using unselected, population-based samples, to assess the performance of IMI for identifying individuals at risk of fracture. As a new, minimally invasive technology, it is important to gauge the tolerability of the IMI to better understand whether participant groups and volunteers are likely to undergo the procedure in a research setting. The aim of this study was to assess the feasibility and tolerability of the OsteoProbe® among men enrolled in the Geelong Osteoporosis Study (GOS).

METHODS

Patient and Public involvement

Study participants were selected at random from the general population and invited to participate; they did not represent a specific patient group. This observational study was designed as part of an ongoing program of research aimed at improving fracture risk assessment. While participants were not involved in developing the research question, we documented their participant experience by questionnaire. Results will be disseminated to participants via a research update, which is distributed annually. **Source Population**

The GOS is a population-based study situated in a defined region in south-eastern Australia, known as the Barwon Statistical Division (14). An age-stratified, randomly-selected cohort of 1540 men aged 20-97 years was recruited 2001-2006 using the electoral roll as the sampling frame. The cohort is being followed prospectively, with assessments every few years (14). This analysis focuses on the first 345 men assessed as part of the 15-year follow-up. In conjunction with other clinical measures including bone densitometry, participants were approached to undergo IMI testing and complete a short questionnaire about their experience relating to the technique. The study was approved by the Human Research Ethics Committee at Barwon Health (00/56-E7). All participants provided informed consent.

Bone material strength testing

IMI was performed by a trained operator using the OsteoProbe® (Active Life Scientific, Inc., CA, USA). The IMI procedure was explained to participants before measurements were made. They were informed that the procedure is a new technique that might assess the resistance of bones to fractures by inducing micro fractures on a small area of the tibia. Furthermore, participants were told the procedure is minimally invasive and does not affect the ability of the individual to walk immediately after. They were then given the option to participate or not participate in the study.

Participants who chose to participate in the study were positioned in decubitus supine position, with the leg to be measured rotated to orient the flat surface of the medial tibia diaphysis. The mid distance between the medial border of the tibia plateau and the medial malleolus were marked using a measuring tape. Following a disinfection of the area using a 70% isopropyl alcohol swab, 2% Lidocaine was administered by inserting a syringe both subcutaneously and in the periosteal surface. A sterile probe was then inserted at the marked

mid diaphysis, piercing the skin and periosteum until reaching the bone cortex. While maintaining probe contact with the bone surface, as well as orienting the probe perpendicularly to the tibia surface, the outer housing of the device was slid towards the subject's leg to initiate a measurement. After the first measurement, the probe was moved to a new location, at least 2mm away from the prior measurement, to obtain another measurement. In this study, at least 11 indentations were performed on each subject, of which the first measurement was systematically disregarded followed by 10 valid test indentations. A trained observer assisted the operator by ensuring that the probe was held perpendicular to the tibial surface. The procedure was conducted according to internationally-recognised recommendations for using the Osteoprobe RUO (15). Immediately following measurement, each participant completed a questionnaire that asked them to rate on a visual analogue scale (0-10) the level of pain that was anticipated, the level of pain that was experienced, their initial reluctance towards the measurement and their willingness to undergo the measurement again. The visual analogue scale is a valid, reliable and simple tool used to assess variations in pain intensity (16,17)

Statistical Analyses

Comparisons between participants included and excluded in analyses were identified using t-tests, and one-way ANOVA for differences in visual analogue scale responses across age groups. Pearson product moment correlation was used to test for a linear correlation between BMSi and age, weight, height and BMI. Statistical analyses were performed using Minitab (version 17; Minitab, State College, PA).

RESULTS

Of 345 potential participants, exclusions were: needle phobia (n=8), existing skin infections (n=21), excessive soft tissues around mid-tibia region (n=56), due to discomfort (pressure, no pain) after the first indentation (n=5), unable to provide informed consent (n=2). One participant had IMI after skin infection had been treated. Therefore, 252 participants were included in the subsequent analyses.

Table 1 shows characteristics of study participants included and excluded in the analyses respectively. Participants ranged in age from 33 to 96 years.

BMSi ranged from 62.3 to 93.0. Mean BMSi±SD values for age groups 30-49, 50-69 and 70+ years were 81.9±5.4, 83.7±6.2 and 81.6±6.7, respectively. The average BMI of participants excluded due to soft tissues was 33.4 ± 5.6.

Tolerability scores were normally distributed and similar across all ages (Table 2, Figure 1). The expectation for pain during OsteoProbe® measurement was low, as was actual pain experienced and initial reluctance to undergo measurement. Tolerability of the OsteoProbe® measurement was high; all 252 participants who had a successful measurement indicated a willingness to undergo the measurement again. No adverse events were reported.

There were no correlations detected between BMSi and age (r= 0.070, p=0.270), height (r=0.068, p= 0.281) or weight (r= -0.078, p= 0.215); however, there was a negative correlation between BMSi and BMI (r=-0.135, p=0.032).

Table 1 Characteristics of the study participants included and excluded in analyses

Characteristics	Included (n=252)	Excluded (n=92)	*p* Value
Age (yr)	63.2 ± 12.6	65.5 ± 15.6	0.163
Weight (kg)	81.2 ± 10.9	92.9 ± 19.1	<0.001
Height (cm)	174.3 ± 6.9	173.7± 10.3	0.983
Body Mass Index (kg/m^2)	26.7 ± 3.1	30.8 ± 7.8	<0.001

Data shown as mean (±SD)

Table 2: Tolerability scores for the whole group and according to age

	ALL ages	30-49 yr	50-69 yr	70+ yr	*p* Value
***Expectation for Pain**	1.5 ± 1.6	2.0 ± 1.7	1.6 ± 1.6	1.2 ± 1.5	0.070
***Actual pain experienced**	0.4 ± 0.7	0.4 ± 0.8	0.4 ± 0.8	0.3 ± 0.47	0.462
***Level of reluctance**	0.3± 0.9	0.6 ± 1.0	0.4 ± 1.0	0.2 ± 0.6	0.136

*Questionnaire results for 0-10 visual analogue scale

DISCUSSION

In this study, we observed a high tolerability of IMI; reluctance to undergo the measurement was low and while there may be a potential concern for possible harm to the bone, all participants who had a successful measurement indicated a willingness to undergo the measurement again.

Although IMI is a minimally invasive procedure, with indentations on the scale of micrometres, a valid concern is the tolerability of the procedure in a research setting,

particularly in studies that rely on participants/volunteers from the general population. We report that testing with the device is feasible among participants in our population-based study. The most common reason for exclusion was excessive soft tissues around the mid-tibia. Other reasons for exclusions were skin infections, needle phobia, inability to provide informed consent and discomfort after the first indentation, but these affected only one-tenth of the study participants. Other potential contraindications include prior clinical or stress fracture in the tibia diaphysis, focal tibial lesions and Paget's disease (15); however, to the best of our knowledge, no studies have reported the actual number of exclusions based on these criteria.

In studies using IMI, only two instances of adverse effects have been reported in the literature, one associated with reaction to local anaesthetic and one mild skin infection, in more than 1300 measured individuals (15). To our knowledge, we are the first to document the tolerability of the procedure. Given that the anterior surface of the tibial is pre-treated with a local anaesthetic delivered by subcutaneous injection through the skin and around the periosteum and that the probe of the device is inserted through the skin, subcutaneous soft tissue and periosteum into the cortical bone, it would seem likely that the procedure might seem unpleasant for some study participants. High rates of refusal would introduce bias. However, our results indicate that IMI is generally acceptable, at least for the participants of our study implying that the technique shows promise as a measure of bone material properties in a practical, safe and convenient manner**.**

Furthermore, we investigated participants from a wide age range and observed similar tolerability scores across age groups ranging from 30-49, 50-69 to 70 years and older. The men in the oldest category tended to report a lower expectation for pain, experienced less pain and were less reluctant to undergo the measurement, but these differences were not statistically significant.

In this study, we did not observe a correlation between BMSi and age, corresponding to two studies by Duarte Sosa et al. in which no association was detected between age and BMSi in 42 Norwegian and 46 Spanish women (18) and among 30 women with previous stress fractures and 30 normal controls (19). By contrast, an inverse association between age and BMSi has been reported for 90 patients (male and female) with low bone mass, (r=-0.539; p<0.001) and in a case-control study of 48 acromegaly patients and 44 controls (male and female), there was a positive association for patients with acromegaly (r=0.291, p = 0.045) and an inverse relationship for the controls (r=-0.457, p=0.002) (20). The reason for lack of consistency in results is not clear but likely reflects differences in study design and study populations.

Moreover, no correlations of BMSi with height, weight were found but we observed a negative correlation between BMSi and BMI. Similarly, Sundh et al. (21) reported a negative correlation *(r*=-0.17, *p*=0.01) between BMSi and BMI in a population-based study of 202 women between 75 and 80 years of age, and Rudang et al. (13) reported a weak inverse correlation *(r*=-0.14, *p*=0.04) between BMSi and weight in a population-based cohort of 211 women between 75 and 80 years of age, and, in accordance with our findings, there was no association with height.

To our knowledge, this is the first study to examine the tolerability and feasibility of the OsteoProbe® in a population-based study. Unlike most of the previous studies, this study is population-based and not selected on the basis of disease status. The outcome will thus be relevant for the general population. In this study, one operator conducted the IMI measurements and an observer was present to ensure the procedure was performed according to the standardised procedure.

However, we acknowledge the following limitations. IMI could not be performed in individuals with substantial amounts of soft tissue around the mid-tibia region, nor in individuals with skin disorders or infections in at the site of measurement and this may have biased our results. It should be noted that there are also drawbacks with other technologies. For example, a weight limitation (typically 120kg or 300lb) and narrow bed width (~60 cm) necessitates exclusion of large individuals from assessment with DXA (22). Furthermore, individuals with spinal abnormalities and those affected by devices such as plates, screws, silicone implants and prostheses can compromise the interpretation of DXA scans (23–26). In our study, we investigated men only, and recognise that the observations may not be generalisable to women or other populations.

In conclusion, IMI was well accepted by participants suggesting that testing with the OsteoProbe® is feasible in a research setting. Further assessment of the clinical utility of this technology for assessing fracture risk is warranted and currently in progress.

Funding Statement

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Competing Interests

PGR is supported by Deakin University Postgraduate Industry Research Scholarship. KLH-K is supported by an Alfred Deakin Postdoctoral Research Fellowship. AD-P owns shares of Active Life Scientific, Inc., the manufacturer of the RPI device. MAK and JAP are recipients of grants from the NHMRC and Amgen-GSK OA-ANZBMS.

Authors' contributions

PGR performed the indentation measurements in the presence of another trained observer (KLH-K) and drafted the manuscript. KLH-K assisted with taking measurements and administered questionnaires. AD-P assisted with training to use the OsteoProbe device and provided advice on measurement technique. MAK and JAP conceived and designed the study. JAP secured ethics approval. All authors interpreted the data, guided and reviewed the manuscript. All authors read and approved the final manuscript.

Data sharing statement

The datasets used and analysed during the current study are available from the corresponding author on reasonable request. Requests can be sent to gos@barwonhealth.org.au.

Ethics approval and consent to participate

This project has been approved by Human Research Ethics Committee at Barwon Health. All participants gave written informed consent to participate in the study.

Acknowledgements

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Figure 1: An interval plot of 0-10 Visual Analogue scale results, according to age

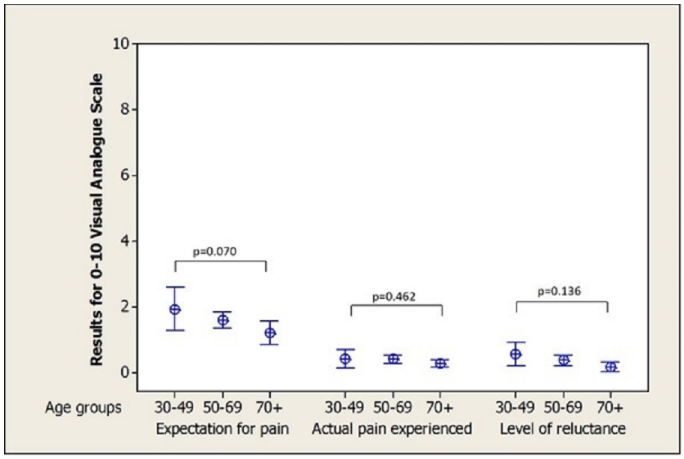

Figure 1: An interval plot of 0-10 Visual Analogue scale results, according to age

101x135mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	3-4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-6
Bias	9	Describe any efforts to address potential sources of bias	4-6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	N/A
		(*e*) Describe any sensitivity analyses	N/A

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6-7
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	7
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6-7
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Feasibility and Tolerability of Bone Impact Microindentation Testing: a cross-sectional, population-based study in Australia

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Feasibility and Tolerability of Bone Impact Microindentation Testing: a cross-sectional, population-based study in Australia

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ABSTRACT

Objectives The OsteoProbe® measures bone material strength index (BMSi) of cortical bone in living humans using impact microindentation (IMI). Research using this minimally-invasive technique is expanding yet, to-date, there have been no reports about its feasibility in the research setting. In this study, we assessed the feasibility and tolerability of using the OsteoProbe® in men enrolled in the Geelong Osteoporosis Study.

Design Cross-sectional analysis of data collected in a population-based study.

Setting Barwon Statistical Division, south-eastern Australia, 2016-2018

Methods For 252 of 345 consecutive participants (ages 33-96 years), BMSi was measured using the OsteoProbe® at the mid-tibia. Immediately following measurement, each participant used a visual analogue scale (0-10) to rate the level of discomfort that was anticipated and experienced, their initial reluctance towards the measurement and their willingness to repeat measurement.

Results Reasons for non-measurement in 92 men were needle phobia (n=8), discomfort after first indentation (n=5), skin infections (n=21), excessive soft tissues around the mid-tibia region (n=56), inability to provide informed consent (n=2). Among 252 men who had IMI measures, the expectation for pain during measurement was low (1.54±1.56), as was actual pain experienced (0.38±0.71). Reluctance to undergo measurement was low (0.34±0.93). All participants indicated a willingness to have the measurement performed again. Mean (±SD) BMSi was 83.0±6.4 (range 62.3-93.0).

Conclusion In this study, the procedure was well accepted by participants suggesting that IMI testing with the OsteoProbe® is feasible in a research setting.

Keywords: Microidentation, bone material strength index, fractures, OsteoProbe.

Strengths and limitations of this study

- This is the first study to examine the feasibility and tolerability of the OsteoProbe® in a population-based study setting.
- We evaluated the associations between BMSi and age in the largest population-based sample of men so far.
- We cannot exclude the possibility of some bias in the BMSi outcome because of non-participation.

Introduction

The most widely used clinical measurement for ascertainment of fracture risk is bone mineral density (BMD) measured by dual- energy X-ray absorptiometry (DXA) (1). However, BMD does not fully explain fracture risk, as the largest absolute number of fragility fractures occur in people without severe deficits in BMD (2,3). Other determinants of bone strength such as bone geometry, microarchitecture and material properties are likely to contribute to fracture risk. Also, clinical factors such as a history of prior fracture, age, exposure to glucocorticoid and other medications, smoking and falls, can contribute independently to fracture risk, and these risk factors can be incorporated into fracture risk algorithms such as FRAX (4), the GARVAN algorithm (5) and the FRISK score (6). Therefore, the goal of much research is to develop techniques to better identify patients at risk of fracture. Using a novel device, the OsteoProbe®, to assess cortical bone material properties in vivo, impact microindentation (IMI) is one such technique (7). The OsteoProbe® measures Bone Material Strength Index (BMSi). This parameter quantifies how well a bone resists microindentation. BMSi is defined as 100 times the ratio of the indentation distance from the impact to a calibration material, PMMA (poly methyl methacrylate), divided by the indentation distance from the impact into

the bone. As the probe indents the bone, it induces microfractures. The more easily the bone is fractured, the deeper the probe indents and the lower the BMSi. The technical aspects of this device have been described in detail in the initial scientific instrumentation papers (7, 8). The use of this device in research is growing; and BMSi has reportedly distinguished between patients with different levels of fracture risk in some (9–12) but not all (13) studies, often independently of BMD. These studies have been limited to relatively small sample sizes and have often involved patients selected on the basis of disease.

Although these data suggest that BMSi might have clinical utility, future research is warranted, particularly using unselected, population-based samples, to assess the performance of IMI for identifying individuals at risk of fracture. As a new, minimally invasive technology, it is important to gauge the tolerability of the IMI to better understand whether participant groups and volunteers are likely to undergo the procedure in a research setting. The aim of this study was to assess the feasibility and tolerability of the OsteoProbe® among men enrolled in the Geelong Osteoporosis Study (GOS).

METHODS

Patient and Public involvement

Participants received an information and consent form describing the research, but they were not involved in the design or conduct of the study. All participants provided written consent. Study findings are disseminated to participants via a research update, which is distributed annually.

Source Population

The GOS is a population-based study situated in a defined region in south-eastern Australia, known as the Barwon Statistical Division (14). An age-stratified, randomly-selected cohort of 1540 men aged 20-97 years was recruited 2001-2006 using the electoral roll as the sampling frame. The cohort is being followed prospectively, with assessments every few years (14). This analysis focuses on the first 345 men assessed as part of the 15-year follow-up. In conjunction with other clinical measures including bone densitometry, participants were approached to undergo IMI testing and complete a short questionnaire about their experience relating to the technique. The study was approved by the Human Research Ethics Committee at Barwon Health (00/56-E7).

Bone material strength testing

IMI was performed by a trained operator using the OsteoProbe® (Active Life Scientific, Inc., CA, USA). The IMI procedure was explained to participants before measurements were made. They were informed that the procedure is a new technique that might assess the resistance of bones to fractures by inducing micro fractures on a small area of the tibia. Furthermore, participants were told the procedure is minimally invasive and does not affect the ability of the individual to walk immediately after. They were then given the option to participate or not participate in the study.

Participants who chose to participate in the study were positioned in decubitus supine position, with the leg to be measured rotated to orient the flat surface of the medial tibia diaphysis. The mid distance between the medial border of the tibia plateau and the medial malleolus were marked using a measuring tape. Following a disinfection of the area using a

70% isopropyl alcohol swab, 2% Lidocaine was administered by inserting a syringe both subcutaneously and in the periosteal surface. A sterile probe was then inserted at the marked mid diaphysis, piercing the skin and periosteum until reaching the bone cortex. While maintaining probe contact with the bone surface, as well as orienting the probe perpendicularly to the tibia surface, the outer housing of the device was slid towards the subject's leg to initiate a measurement. After the first measurement, the probe was moved to a new location, at least 2mm away from the prior measurement, to obtain another measurement. In this study, at least 11 indentations were performed on each subject, of which the first measurement was systematically disregarded followed by 10 valid test indentations. A trained observer assisted the operator by ensuring that the probe was held perpendicular to the tibial surface. The procedure was conducted according to internationally-recognised recommendations for using the Osteoprobe RUO (15). Immediately following measurement, each participant completed a questionnaire that asked them to rate on a visual analogue scale (0-10) the level of pain that was anticipated, the level of pain that was experienced, their initial reluctance towards the measurement and their willingness to undergo the measurement again. The visual analogue scale is a valid, reliable and simple tool used to assess variations in pain intensity (16,17)

Statistical Analyses

Comparisons between participants included and excluded in analyses were identified using t-tests, and one-way ANOVA for differences in visual analogue scale responses across age groups. Pearson product moment correlation was used to test for a linear correlation between BMSi and age, weight, height and BMI. Statistical analyses were performed using Minitab (version 17; Minitab, State College, PA).

RESULTS

Of 345 potential participants, exclusions were: needle phobia (n=8), existing skin infections (n=21), excessive soft tissues around mid-tibia region (n=56), due to discomfort (pressure, no pain) after the first indentation (n=5), unable to provide informed consent (n=2). One participant had IMI after skin infection had been treated. Therefore, 252 participants were included in the subsequent analyses.

Table 1 shows characteristics of study participants included and excluded in the analyses respectively. Participants ranged in age from 33 to 96 years.

BMSi ranged from 62.3 to 93.0. Mean BMSi±SD values for age groups 30-49, 50-69 and 70+ years were 81.9±5.4, 83.7±6.2 and 81.6±6.7, respectively. The average BMI of participants excluded due to soft tissues was 33.4 ± 5.6.

Tolerability scores were normally distributed and similar across all ages (Table 2, Figure 1). The expectation for pain during OsteoProbe® measurement was low, as was actual pain experienced and initial reluctance to undergo measurement. Tolerability of the OsteoProbe® measurement was high; all 252 participants who had a successful measurement indicated a willingness to undergo the measurement again. No adverse events were reported.

There were no correlations detected between BMSi and age ($r = 0.070$, $p = 0.270$), height ($r = 0.068$, $p = 0.281$) or weight ($r = -0.078$, $p = 0.215$); however, there was a negative correlation between BMSi and BMI ($r = -0.135$, $p = 0.032$).

Table 1 Characteristics of the study participants included and excluded in analyses

Characteristics	Included (n=252)	Excluded (n=92)	*p* Value
Age (yr)	63.2 ± 12.6	65.5 ± 15.6	0.163
Weight (kg)	81.2 ± 10.9	92.9 ± 19.1	<0.001
Height (cm)	174.3 ± 6.9	173.7± 10.3	0.983
Body Mass Index (kg/m^2)	26.7 ± 3.1	30.8 ± 7.8	<0.001

Data shown as mean (±SD)

Table 2: Tolerability scores for the whole group and according to age

	ALL ages	30-49 yr	50-69 yr	70+ yr	*p* Value
***Expectation for Pain**	1.5 ± 1.6	2.0 ± 1.7	1.6 ± 1.6	1.2 ± 1.5	0.070
***Actual pain experienced**	0.4 ± 0.7	0.4 ± 0.8	0.4 ± 0.8	0.3 ± 0.47	0.462
***Level of reluctance**	0.3± 0.9	0.6 ± 1.0	0.4 ± 1.0	0.2 ± 0.6	0.136

*Questionnaire results for 0-10 visual analogue scale

DISCUSSION

In this study, we observed a high tolerability of IMI; reluctance to undergo the measurement was low and while there may be a potential concern for possible harm to the bone, all

participants who had a successful measurement indicated a willingness to undergo the measurement again.

Although IMI is a minimally invasive procedure, with indentations on the scale of micrometres, a valid concern is the tolerability of the procedure in a research setting, particularly in studies that rely on participants/volunteers from the general population. We report that testing with the device is feasible among participants in our population-based study. The most common reason for exclusion was excessive soft tissues around the mid-tibia. Other reasons for exclusions were skin infections, needle phobia, inability to provide informed consent and discomfort after the first indentation, but these affected only one-tenth of the study participants. Other potential contraindications include prior clinical or stress fracture in the tibia diaphysis, focal tibial lesions and Paget's disease (15); however, to the best of our knowledge, no studies have reported the actual number of exclusions based on these criteria.

In studies using IMI, only two instances of adverse effects have been reported in the literature, one associated with reaction to local anaesthetic and one mild skin infection, in more than 1300 measured individuals (15). To our knowledge, we are the first to document the tolerability of the procedure. Given that the anterior surface of the tibial is pre-treated with a local anaesthetic delivered by subcutaneous injection through the skin and around the periosteum and that the probe of the device is inserted through the skin, subcutaneous soft tissue and periosteum into the cortical bone, it would seem likely that the procedure might seem unpleasant for some study participants. High rates of refusal would introduce bias. However, our results indicate that IMI is generally acceptable, at least for the participants of our study implying that the technique shows promise as a measure of bone material properties in a practical, safe and convenient manner.

Furthermore, we investigated participants from a wide age range and observed similar tolerability scores across age groups ranging from 30-49, 50-69 to 70 years and older. The men in the oldest category tended to report a lower expectation for pain, experienced less pain and were less reluctant to undergo the measurement, but these differences were not statistically significant.

In this study, we did not observe a correlation between BMSi and age, corresponding to two studies by Duarte Sosa et al. in which no association was detected between age and BMSi in 42 Norwegian and 46 Spanish women (18) and among 30 women with previous stress fractures and 30 normal controls (19). By contrast, an inverse association between age and BMSi has been reported for 90 patients (male and female) with low bone mass, (r = -0.539; p<0.001) and in a case-control study of 48 acromegaly patients and 44 controls (male and female), there was a positive association for patients with acromegaly (r = 0.291, p = 0.045) and an inverse relationship for the controls (r = -0.457, p = 0.002) (20). The reason for lack of consistency in results is not clear but likely reflects differences in study design and study populations.

Moreover, no correlations of BMSi with height, weight were found but we observed a negative correlation between BMSi and BMI. Similarly, Sundh et al. (21) reported a negative correlation (r = -0.17, p = 0.01) between BMSi and BMI in a population-based study of 202 women between 75 and 80 years of age, and Rudang et al. (13) reported a weak inverse correlation (r = -0.14, p = 0.04) between BMSi and weight in a population-based cohort of 211 women between 75 and 80 years of age, and, in accordance with our findings, there was no association with height.

To our knowledge, this is the first study to examine the tolerability and feasibility of the OsteoProbe® in a population-based study. Unlike most of the previous studies, this study is

population-based and not selected on the basis of disease status. The outcome will thus be relevant for the general population. In this study, one operator conducted the IMI measurements and an observer was present to ensure the procedure was performed according to the standardised procedure.

However, we acknowledge the following limitations. IMI could not be performed in individuals with substantial amounts of soft tissue around the mid-tibia region, nor in individuals with skin disorders or infections in at the site of measurement and this may have biased our results. It should be noted that there are also drawbacks with other technologies. For example, a weight limitation (typically 120kg or 300lb) and narrow bed width (~60 cm) necessitates exclusion of large individuals from assessment with DXA (22). Furthermore, individuals with spinal abnormalities and those affected by devices such as plates, screws, silicone implants and prostheses can compromise the interpretation of DXA scans (23–26). In our study, we investigated men only, and recognise that the observations may not be generalisable to women or other populations.

In conclusion, IMI was well accepted by participants suggesting that testing with the OsteoProbe® is feasible in a research setting. Further assessment of the clinical utility of this technology for assessing fracture risk is warranted and currently in progress.

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Competing Interests

PGR is supported by Deakin University Postgraduate Industry Research Scholarship. KLH-K is supported by an Alfred Deakin Postdoctoral Research Fellowship. AD-P owns shares of Active Life Scientific, Inc., the manufacturer of the RPI device. MAK and JAP are recipients of grants from the NHMRC and Amgen-GSK OA-ANZBMS.

Authors' contributions

PGR performed the indentation measurements in the presence of another trained observer (KLH-K) and drafted the manuscript. KLH-K assisted with taking measurements and administered questionnaires. AD-P assisted with training to use the OsteoProbe device and provided advice on measurement technique. MAK and JAP conceived and designed the study. JAP secured ethics approval. All authors interpreted the data, guided and reviewed the manuscript. All authors read and approved the final manuscript.

Data sharing statement

The datasets used and analysed during the current study are available from the corresponding author on reasonable request. Requests can be sent to gos@barwonhealth.org.au.

Ethics approval and consent to participate

This project has been approved by Human Research Ethics Committee at Barwon Health. All participants gave written informed consent to participate in the study.

Acknowledgements

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Figure 1: An interval plot of 0-10 Visual Analogue scale results, according to age

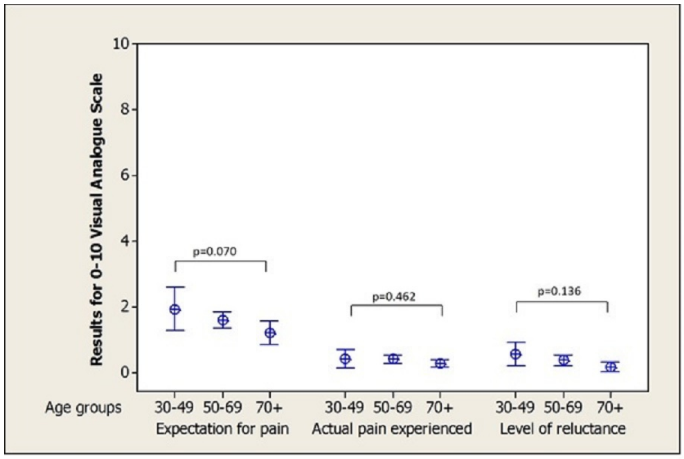

Figure 1: An interval plot of 0-10 Visual Analogue scale results, according to age

101x135mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	3-4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-6
Bias	9	Describe any efforts to address potential sources of bias	4-6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	N/A
		(*e*) Describe any sensitivity analyses	N/A

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6-7
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	7
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6-7
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Feasibility and Tolerability of Bone Impact Microindentation Testing: a cross-sectional, population-based study in Australia

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Feasibility and Tolerability of Bone Impact Microindentation Testing: a cross-sectional, population-based study in Australia

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ABSTRACT

Objectives The OsteoProbe® measures bone material strength index (BMSi) of cortical bone in living humans using impact microindentation (IMI). Research using this minimally-invasive technique is expanding yet, to-date, there have been no reports about its feasibility in the research setting. In this study we assessed the feasibility and tolerability of using the OsteoProbe® in men enrolled in the Geelong Osteoporosis Study.

Design Cross-sectional analysis of data collected in a population-based study.

Setting Barwon Statistical Division, south-eastern Australia, 2016-2018

Methods For 252 of 345 consecutive participants (ages 33-96 years), BMSi was measured using the OsteoProbe® at the mid-tibia. Immediately following measurement, each participant used a visual analogue scale (0-10) to rate the level of discomfort that was anticipated and experienced, their initial reluctance towards the measurement and their willingness to repeat measurement.

Results Reasons for non-measurement in 92 men were needle phobia (n=8), discomfort after first indentation (n=5), skin infections (n=21), excessive soft tissues around the mid-tibia region (n=56), inability to provide informed consent (n=2). Among 252 men who had IMI measures, the expectation for pain during measurement was low (1.54±1.56), as was actual pain experienced (0.38±0.71). Reluctance to undergo measurement was low (0.34±0.93). All participants indicated a willingness to have the measurement performed again. Mean (±SD) BMSi was 83.0±6.4 (range 62.3-93.0).

Conclusion In this study, the procedure was well accepted by participants suggesting that IMI testing with the OsteoProbe® is feasible in a research setting.

Keywords: Microidentation, bone material strength index, fractures, OsteoProbe.

Strengths and limitations of this study

- Feasibility and tolerability were assessed at the time of impact microindentation (IMI) testing.
- The sample was selected at random from the general population and not on the basis of disease status.
- One operator conducted the IMI measurements.
- This is the first study to report the actual number of IMI exclusions due to contraindications.
- The findings might not be generalizable to women or other populations.

Introduction

The most widely used clinical measurement for ascertainment of fracture risk is bone mineral density (BMD) measured by dual- energy X-ray absorptiometry (DXA) (1). However, BMD does not fully explain fracture risk, as the largest absolute number of fragility fractures occur in people without severe deficits in BMD (2,3). Other determinants of bone strength such as bone geometry, microarchitecture and material properties are likely to contribute to fracture risk. Also, clinical factors such as a history of prior fracture, age, exposure to glucocorticoid and other medications, smoking and falls, can contribute independently to fracture risk, and these risk factors can be incorporated into fracture risk algorithms such as FRAX (4), the GARVAN

algorithm (5) and the FRISK score (6). Therefore, the goal of much research is to develop techniques to better identify patients at risk of fracture. Using a novel device, the OsteoProbe®, to assess cortical bone material properties in vivo, impact microindentation (IMI) is one such technique (7). The OsteoProbe® measures Bone Material Strength Index (BMSi). This parameter quantifies how well a bone resists microindentation. BMSi is defined as 100 times the ratio of the indentation distance from the impact to a calibration material, PMMA (poly methyl methacrylate), divided by the indentation distance from the impact into the bone. As the probe indents the bone, it induces microfractures. The more easily the bone is fractured, the deeper the probe indents and the lower the BMSi. The technical aspects of this device have been described in detail in the initial scientific instrumentation papers (7, 8). The use of this device in research is growing; and BMSi has reportedly distinguished between patients with different levels of fracture risk in some (9–12) but not all (13) studies, often independently of BMD. These studies have been limited to relatively small sample sizes and have often involved patients selected on the basis of disease.

Although these data suggest that BMSi might have clinical utility, future research is warranted, particularly using unselected, population-based samples, to assess the performance of IMI for identifying individuals at risk of fracture. As a new, minimally invasive technology, it is important to gauge the tolerability of the IMI to better understand whether participant groups and volunteers are likely to undergo the procedure in a research setting. The aim of this study was to assess the feasibility and tolerability of the OsteoProbe® among men enrolled in the Geelong Osteoporosis Study (GOS).

METHODS

Patient and Public involvement

Patients were not involved in the planning and design of this study.

Source Population

The GOS is a population-based study situated in a defined region in south-eastern Australia, known as the Barwon Statistical Division (14). An age-stratified, randomly-selected cohort of 1540 men aged 20-97 years was recruited 2001-2006 using the electoral roll as the sampling frame. The cohort is being followed prospectively, with assessments every few years (14). This analysis focuses on the first 345 men assessed as part of the 15-year follow-up. In conjunction with other clinical measures including bone densitometry, participants were approached to undergo IMI testing and complete a short questionnaire about their experience relating to the technique. The study was approved by the Human Research Ethics Committee at Barwon Health (00/56-E7). All participants provided informed consent.

Bone material strength testing

IMI was performed by a trained operator using the OsteoProbe® (Active Life Scientific, Inc., CA, USA). The IMI procedure was explained to participants before measurements were made. They were informed that the procedure is a new technique that might assess the resistance of bones to fractures by inducing micro fractures on a small area of the tibia. Furthermore, participants were told the procedure is minimally invasive and does not affect the ability of the

individual to walk immediately after. They were then given the option to participate or not participate in the study.

Participants who chose to participate in the study were positioned in decubitus supine position, with the leg to be measured rotated to orient the flat surface of the medial tibia diaphysis. The mid distance between the medial border of the tibia plateau and the medial malleolus were marked using a measuring tape. Following a disinfection of the area using a 70% isopropyl alcohol swab, 2% Lidocaine was administered by inserting a syringe both subcutaneously and in the periosteal surface. A sterile probe was then inserted at the marked mid diaphysis, piercing the skin and periosteum until reaching the bone cortex. While maintaining probe contact with the bone surface, as well as orienting the probe perpendicularly to the tibia surface, the outer housing of the device was slid towards the subject's leg to initiate a measurement. After the first measurement, the probe was moved to a new location, at least 2mm away from the prior measurement, to obtain another measurement. In this study, at least 11 indentations were performed on each subject, of which the first measurement was systematically disregarded followed by 10 valid test indentations. A trained observer assisted the operator by ensuring that the probe was held perpendicular to the tibial surface. The procedure was conducted according to internationally-recognised recommendations for using the Osteoprobe RUO (15). Immediately following measurement, each participant completed a questionnaire that asked them to rate on a visual analogue scale (0-10) the level of pain that was anticipated, the level of pain that was experienced, their initial reluctance towards the measurement and their willingness to undergo the measurement again. The visual analogue scale is a valid, reliable and simple tool used to assess variations in pain intensity (16,17)

Statistical Analyses

Comparisons between participants included and excluded in analyses were identified using t-tests, and one-way ANOVA for differences in visual analogue scale responses across age groups. Pearson product moment correlation was used to test for a linear correlation between BMSi and age, weight, height and BMI. Statistical analyses were performed using Minitab (version 17; Minitab, State College, PA).

RESULTS

Of 345 potential participants, exclusions were: needle phobia (n=8), existing skin infections (n=21), excessive soft tissues around mid-tibia region (n=56), due to discomfort (pressure, no pain) after the first indentation (n=5), unable to provide informed consent (n=2). One participant had IMI after skin infection had been treated. Therefore, 252 participants were included in the subsequent analyses.

Table 1 shows characteristics of study participants included and excluded in the analyses respectively. Participants ranged in age from 33 to 96 years.

BMSi ranged from 62.3 to 93.0. Mean BMSi±SD values for age groups 30-49, 50-69 and 70+ years were 81.9±5.4, 83.7±6.2 and 81.6±6.7, respectively. The average BMI of participants excluded due to soft tissues was 33.4 ± 5.6.

Tolerability scores were normally distributed and similar across all ages (Table 2, Figure 1). The expectation for pain during OsteoProbe® measurement was low, as was actual pain experienced and initial reluctance to undergo measurement. Tolerability of the OsteoProbe® measurement was high; all 252 participants who had a successful measurement indicated a willingness to undergo the measurement again. No adverse events were reported.

There were no correlations detected between BMSi and age (r = 0.070, p = 0.270), height (r = 0.068, p = 0.281) or weight (r = -0.078, p = 0.215); however, there was a negative correlation between BMSi and BMI (r = -0.135, p = 0.032).

Table 1 Characteristics of the study participants included and excluded in analyses

Characteristics	**Included (n=252)**	**Excluded (n=92)**	***p* Value**
Age (yr)	63.2 ± 12.6	65.5 ± 15.6	0.163
Weight (kg)	81.2 ± 10.9	92.9 ± 19.1	<0.001
Height (cm)	174.3 ± 6.9	173.7± 10.3	0.983
Body Mass Index (kg/m^2)	26.7 ± 3.1	30.8 ± 7.8	<0.001

Data shown as mean (±SD)

Table 2: Tolerability scores for the whole group and according to age

	ALL ages	30-49 yr	50-69 yr	70+ yr	*p* Value
***Expectation for Pain**	1.5 ± 1.6	2.0 ± 1.7	1.6 ± 1.6	1.2 ± 1.5	0.070
***Actual pain experienced**	0.4 ± 0.7	0.4 ± 0.8	0.4 ± 0.8	0.3 ± 0.5	0.462
***Level of reluctance**	0.3± 0.9	0.6 ± 1.0	0.4 ± 1.0	0.2 ± 0.6	0.136

*Questionnaire results for 0-10 visual analogue scale

DISCUSSION

In this study, we observed a high tolerability of IMI; reluctance to undergo the measurement was low and while there may be a potential concern for possible harm to the bone, all participants who had a successful measurement indicated a willingness to undergo the measurement again.

Although IMI is a minimally invasive procedure, with indentations on the scale of micrometres, a valid concern is the tolerability of the procedure in a research setting, particularly in studies that rely on participants/volunteers from the general population. We report that testing with the device is feasible among participants in our population-based study. The most common reason for exclusion was excessive soft tissues around the mid-tibia. Other reasons for exclusions were skin infections, needle phobia, inability to provide informed consent and discomfort after the first indentation, but these affected only one-tenth of the study participants. Other potential contraindications include prior clinical or stress fracture in the tibia diaphysis, focal tibial lesions and Paget's disease (15); however, to the best of our knowledge, no studies have reported the actual number of exclusions based on these criteria.

In studies using IMI, only two instances of adverse effects have been reported in the literature, one associated with reaction to local anaesthetic and one mild skin infection, in more than 1300 measured individuals (15). To our knowledge, we are the first to document the tolerability of the procedure. Given that the anterior surface of the tibial is pre-treated with a local anaesthetic delivered by subcutaneous injection through the skin and around the periosteum and that the probe of the device is inserted through the skin, subcutaneous soft tissue and periosteum into the cortical bone, it would seem likely that the procedure might seem unpleasant for some study participants. High rates of refusal would introduce bias. However, our results indicate that IMI is generally acceptable, at least for the participants of our study implying that the technique shows promise as a measure of bone material properties in a practical, safe and convenient manner**.**

Furthermore, we investigated participants from a wide age range and observed similar tolerability scores across age groups ranging from 30-49, 50-69 to 70 years and older. The men in the oldest category tended to report a lower expectation for pain, experienced less pain and were less reluctant to undergo the measurement, but these differences were not statistically significant.

In this study, we did not observe a correlation between BMSi and age, corresponding to two studies by Duarte Sosa et al. in which no association was detected between age and BMSi in 42 Norwegian and 46 Spanish women (18) and among 30 women with previous stress fractures and 30 normal controls (19). By contrast, an inverse association between age and BMSi has been reported for 90 patients (male and female) with low bone mass, (r = -0.539; p<0.001) and in a case-control study of 48 acromegaly patients and 44 controls (male and female), there was a positive association for patients with acromegaly (r = 0.291, p = 0.045) and an inverse

relationship for the controls *(r* = -0.457, *p* = 0.002) (20). The reason for lack of consistency in results is not clear but likely reflects differences in study design and study populations.

Moreover, no correlations of BMSi with height, weight were found but we observed a negative correlation between BMSi and BMI. Similarly, Sundh et al. (21) reported a negative correlation *(r* = -0.17, *p* = 0.01) between BMSi and BMI in a population-based study of 202 women between 75 and 80 years of age, and Rudang et al. (13) reported a weak inverse correlation *(r* = -0.14, *p* = 0.04) between BMSi and weight in a population-based cohort of 211 women between 75 and 80 years of age, and, in accordance with our findings, there was no association with height.

To our knowledge, this is the first study to examine the tolerability and feasibility of the OsteoProbe® in a population-based study. Unlike most of the previous studies, this study is population-based and not selected on the basis of disease status. The outcome will thus be relevant for the general population. In this study, one operator conducted the IMI measurements and an observer was present to ensure the procedure was performed according to the standardised procedure.

However, we acknowledge the following limitations. IMI could not be performed in individuals with substantial amounts of soft tissue around the mid-tibia region, nor in individuals with skin disorders or infections in at the site of measurement and this may have biased our results. It should be noted that there are also drawbacks with other technologies. For example, a weight limitation (typically 120kg or 300lb) and narrow bed width (~60 cm) necessitates exclusion of large individuals from assessment with DXA (22). Furthermore, individuals with spinal abnormalities and those affected by devices such as plates, screws, silicone implants and prostheses can compromise the interpretation of DXA scans (23–26). In our study, we

investigated men only, and recognise that the observations may not be generalisable to women or other populations.

In conclusion, IMI was well accepted by participants suggesting that testing with the OsteoProbe® is feasible in a research setting. Further assessment of the clinical utility of this technology for assessing fracture risk is warranted and currently in progress.

Funding Statement

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Competing Interests

PGR is supported by Deakin University Postgraduate Industry Research Scholarship. KLH-K is supported by an Alfred Deakin Postdoctoral Research Fellowship. AD-P owns shares of Active Life Scientific, Inc., the manufacturer of the RPI device. MAK and JAP are recipients of grants from the NHMRC and Amgen-GSK OA-ANZBMS.

Authors' contributions

PGR performed the indentation measurements in the presence of another trained observer (KLH-K) and drafted the manuscript. KLH-K assisted with taking measurements and administered

questionnaires. AD-P assisted with training to use the OsteoProbe device and provided advice on measurement technique. MAK and JAP conceived and designed the study. JAP secured ethics approval. All authors interpreted the data, guided and reviewed the manuscript. All authors read and approved the final manuscript.

Data sharing statement

The datasets used and analysed during the current study are available from the corresponding author on reasonable request. Requests can be sent to gos@barwonhealth.org.au.

Ethics approval and consent to participate

This project has been approved by Human Research Ethics Committee at Barwon Health. All participants gave written informed consent to participate in the study.

Acknowledgements

We thank Dr Peter Burks from Active Life Scientific, Inc. for his technical guidance

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Figure 1: An interval plot of 0-10 Visual Analogue scale results, according to age

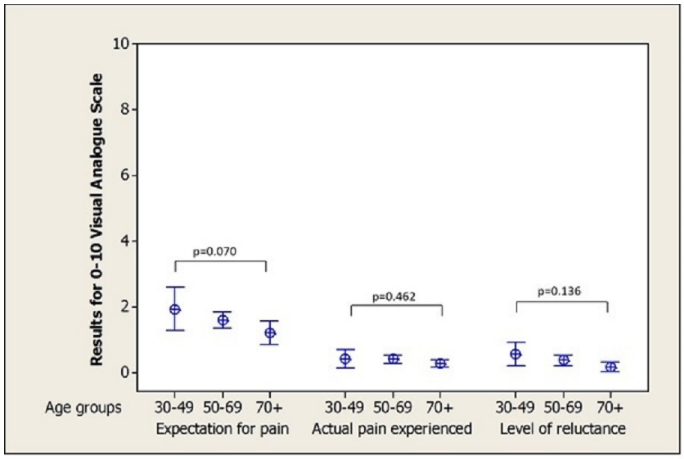

Figure 1: An interval plot of 0-10 Visual Analogue scale results, according to age

101x135mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	3-4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-6
Bias	9	Describe any efforts to address potential sources of bias	4-6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	N/A
		(*e*) Describe any sensitivity analyses	N/A

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6-7
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	7
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6-7
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.