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ORIGINAL ARTICLES

Understanding the physiological and functional consequences of menopause: The PROSALMEN study

Stefania Bandinelli¹, Fulvio Lauretani², Enrico Benvenuti¹, Annamaria Corsi¹, Maria Francesca De Marco¹, Benedetta Bartali¹, Giacomo Ruotolo³, Benedetta Miniati⁴, Claudio Macchi⁴, Cosimo R. Russo¹, Jack M. Guralnik⁵, and Luigi Ferrucci¹

¹Laboratory of Clinical Epidemiology, INRCA Geriatric Department, Firenze, ²Unit of Gerontology and Geriatric Medicine, University of Firenze, ³Laboratory of Lipid Metabolism and Prevention of Cardiovascular Diseases, S. Raffaele Hospital, Milano, ⁴Don Gnocchi Foundation, Pozzolatico, Firenze, Italy, ⁵Epidemiology, Demography and Biometry Laboratory, National Institute on Aging (NIH), Bethesda, MD, USA

ABSTRACT. Background and aims: Women live longer and are more often affected by disability and poor health than men. The mechanism underlying this sex-related "mortality-morbidity" paradox is still unclear but it has been suggested that the physiological and functional changes occurring during the menopausal transition play an important role. The aim of PROSALMEN (PROgetto SALute MENopausa: Health in Menopause Project) is to study in great detail how these changes affect the integrity and function of the physiologic subsystems that are relevant to the maintenance of an active and healthy life-style during the aging process. **Methods:** PROSALMEN is a cross-sectional comparison of age-matched pre- and post-menopausal women. Thirty post-menopausal women, aged 48-58 years, were enrolled in the study together with 30 age-matched pre-menopausal controls. A number of clinical, biological and functional parameters were collected assessing the integrity and level of function of the physiological subsystems that are important for mobility. Furthermore, we collected information on risk factors, medical conditions and symptoms that frequently develop or become clinically evident after menopause, including the most important elements of the classical post-menopausal syndrome. **Conclusions:** This rich dataset will be used to start dissecting the causal pathway leading from menopause to damages in the musculoskeletal

system and, in turn, to reduced physical function. The final goal is to understand how and to what extent changes in health behavior and pharmacological treatments in addition to hormone replacement therapy (HRT) may counteract these processes. (Aging Clin Exp Res 14: 170-177, 2002)

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INTRODUCTION

Demographic data of the last century have systematically shown higher mortality rates in men than in women in most countries (1, 2). Likewise, life expectancy at any age is greater in women, although the disparity between men and women tends to become smaller after the age of 90 years (3). The fact that women live longer than men is one of the most interesting paradoxes for those who study aging (4). The excess survival in women violates one of the major evolutionary principles since only men can still procreate in late life. Furthermore, most of the epidemiological studies demonstrating that women live longer than men also point out that independently of age, women are more often affected by disability and poor health status (5). According to some authors, this phenomenon is accounted for by the high prevalence in older women of chronic medical conditions that are highly symptomatic and disabling, but do not substantially affect

Key words: Aging, menopause, older women, physical performance, prevention, PROSALMEN Study.

Correspondence: L. Ferrucci, M.D., Ph.D., Laboratory of Clinical Epidemiology, INRCA Geriatric Department, Viale Michelangiolo 41, 50127 Firenze, Italy.

E-mail: ferrucci@dada.it

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the risk of death. Probably the best example of these conditions is osteoarthritis (6). On the contrary, older men are often affected by diseases that are less symptomatic and less likely to cause disability, but are frequently primary or precipitating causes of death, such as stroke or myocardial infarction (7). Recent data also suggest that in regard to disability, men are more susceptible to the detrimental effect of chronic conditions while women are somewhat more resistant (8).

Understanding the true underlying mechanism that explains the sex-related "mortality-morbidity" paradox may elucidate how genetic, environmental and behavioral factors influence trajectories of health and functional status over the aging process. Studies of the modifications in physiological parameters and other factors influencing the well-being of women going through menopause are part of this research agenda.

The study of menopause can be pursued with different research approaches. However, given the current lack of solid data, there is a great need for epidemiological studies conducted in samples representative of the general population. Traditionally, epidemiology deals with the relationship between risk factors and health-related outcomes such as disease, disability or death. While this certainly would be the ideal design to study the effect of menopause on active life expectancy and late life disability, implementing such a design is very difficult and expensive and would require a very long follow-up time. In fact, a large cohort of premenopausal women would have to be followed for at least 20 years before any meaningful result could be obtained. Epidemiological studies that have taken this approach to study the effect of menopause on bone mineral density are currently ongoing (9, 10).

AIM OF THE STUDY

An alternative approach which is taken by PROSALMEN (PROgetto SALute MENopausa: Health in Menopause Project) is an in-depth study of how the characteristic physiological changes occurring during the menopausal period affect the integrity and function of the physiological subsystems that are important for the maintenance of good health and a high level of physical performance. In fact, a modification of these physiological subsystems during the peri-menopausal period can be interpreted as the initial segment of a trajectory leading to a more or less accelerated loss of the physiological and functional reserve with aging. When this reserve drops below a certain threshold, physiological homeostasis becomes unstable, and frailty and disability are likely to occur.

PLAN OF THE STUDY

The hypothesis of the PROSALMEN study is that most of the pathophysiologic changes that lead to the excess of disability in older women compared to men, originate with, or at least are affected by, menopause. This hypothesis is strongly supported by what is known regarding the effect of sex hormones on aging bone and osteoporosis (11, 12). The typical disturbing symptoms that often occur over the perimenopausal period, such as hot flashes, are also related to the rapid decline in estrogen levels, and can be controlled with hormone replacement therapy (HRT). However, the effect of menopause on other important tissues, and the mechanism by which women experience other disturbing symptoms after menopause, such as weakness, joint pain in the upper limbs or restless legs at night, are still matter of discussion.

The main hypothesis of PROSALMEN is that the causal pathway for the excess of morbidity and disability in older women, compared to men, is a synergistic decline of muscle and bone mass due to an acceleration of the catabolic processes that is not counterbalanced by a comparable increment of the anabolic stimulus (13). The imbalance between catabolic and anabolic rates takes place during the menopausal period, but becomes clinically evident and functionally relevant only years later. A number of medical and non-medical factors modulate this process and are potential targets for intervention. Indeed, we focused PROSALMEN on muscle because there is evidence that muscle strength and mass can be improved by appropriate interventions even in very old persons (14).

In its current stage, PROSALMEN should be considered a pilot study whose main purpose is to collect the preliminary information needed to conduct a larger prospective survey. In practice, this pilot study is a cross-sectional comparison between age matched pre- and post-menopausal women. Depending on the results of this initial comparison, we plan to enlarge the pre-menopausal cohort and follow all the participants with serial assessments until they all become post-menopausal. The purpose of this second part of the study will be to validate the results obtained in the cross-sectional comparison in a longitudinal perspective and with an adequate sample size.

METHODS

Study population

For the first stage of PROSALMEN, we recruited 60 women aged 48-58 years; 30 had regular menstrual cycles, and 30 had had their last menstrual cycle at least 12 months prior to enrolment. The

age-range of 48-58 years was selected considering that the median age of menopause in Italy is 51 years (15). Exclusion criteria were: surgical menopause; severe diseases associated with high mortality in the following 2 years; pharmacological treatments that affect the activity of sex hormones (including hormone replacement therapy); disability in activities of daily living due to physical or cognitive problems; treatments or conditions that interact with bone and muscle metabolism.

Women living in the city of Firenze were invited to participate in the project during an educational wom-

en's health program organized in our department, and through public advertisements.

To improve the comparability between the two groups, each woman in post-menopause was paired with an age-matched woman (within a range of ± 3 years) in pre-menopause. Of the 30 pairs of women enrolled in the study, in 15 the age difference between the two members was within ± 1 year. The main characteristics of the study population are shown in Table 1. As expected by the design, the age of the two groups of women was substantially similar. More in-

Table 1 - Baseline characteristics of the women enrolled in the PROSALMEN study.

	Pre-menopause (N=30)	Post-menopause (N=30)	p*
GENERAL CHARACTERISTICS			
Age (years)			
Mean \pm SE	51.6 \pm 0.4	52.1 \pm 0.4	0.36
Range	48-55	50-58	
Smoking			
Smoker	10	11	0.42
Ex-smoker	7	5	
Never smoked	13	14	
BMI (Kg/m ²)			
Mean \pm SE	24.8 \pm 0.9	26.4 \pm 0.9	0.17
Range	17.0-33.9	19.4-36.4	
Age at menarche (years)			
Mean \pm SE	12.6 \pm 0.5	12 \pm 0.7	0.45
Range	10-17	9-14	
Age at menopause (years)			
Mean \pm SE		50	
Range		45-55	
School education (years)			
Mean \pm SE	11.2 \pm 3.1	11 \pm 3.2	0.81
Range	5-22	5-18	
Working	23 (76.6%)	17 (56.6%)	0.10
Exercising regularly	20 (66.7%)	21 (70.0%)	0.70
Bone densitometry in the last year	7 (23.3%)	6 (20.0%)	0.68
Mammography in the last year	14 (46.7%)	20 (66.7%)	0.19
Pap smear in the last year	15 (59.0%)	16 (53.3%)	0.72
HORMONES			
LH (mUI/mL)			
Mean \pm SE	12.1 \pm 1.7	30.5 \pm 2.1	<0.001
Range	1.0-32.3	14.5-52.0	
FSH (mUI/mL)			
Mean \pm SE	22.0 \pm 3.0	80.3 \pm 4.8	<0.001
Range	1.0-49.0	28.0-119.0	
SYMPTOMS			
Hot Flashes (at least 1/week)	3 (10.0%)	16 (53.3%)	<0.001
Palpitations (at least 1/week)	2 (6.7%)	11 (26.6%)	<0.01
Vaginal symptoms (dyspareunia dryness, discharge and itching)	2 (6.7%)	16 (53.3%)	<0.001
Hands accessional paresthesia	9 (30.0%)	13 (43.3%)	0.41

* χ^2 or ANOVA when appropriate.

terestingly, we found no statistical difference between the two groups concerning body mass index, smoking habit, years of formal education, age at menarche, occupation, and physical activity. The percentage of women who underwent bone densitometry, mammography and pap smear in the last year was also similar, suggesting that the women in the two groups were not substantially different regarding health behaviors. As expected, serum levels of LH and FSH, and the prevalence of symptoms characteristic of menopause were substantially and significantly different. These findings strengthen and validate our choice of allocating a woman to the pre- or post-menopausal group based on the menstrual cycle information she provided at the time of enrolment.

The INRCA Ethics Committee approved the study protocol, and all women agreed to participate after receiving an extensive description of the objectives of the project and the various stages of assessment. All women also gave the investigators permission to collect data from health-related administrative databases (hospital discharge records, use of drugs, etc.) and to store the biological specimens collected during the study for future use.

Measures

Recruitment of the study population started in January and was completed at the end of April 2001. All assessments were conducted in the outpatient clinic of our department. The initial evaluation included the collection of fasting blood and urine specimens from the first two morning hours, an electrocardiogram, a structured interview, a medical examination, and a comprehensive evaluation of eating habits performed by an expert dietician. The interview and the medical examination focused on problems typical of the peri-menopausal period, using a modified version of the questionnaire on menopausal symptoms that was originally developed in the context of the Women's Health Initiative (<http://www.nhlbi.nih.gov/whi/>). In particular, we collected information about the characteristics of current and past menstrual cycles, depressive symptoms, personal mastery, cognitive function, sleep problems, musculoskeletal pain, balance, parameters affecting lower limbs performance and urinary incontinence.

Furthermore, the following tests were performed:

- 1) A peripheral quantitative computerized tomography (PQCT) of the lower leg aimed at assessing muscle mass and parameters of bone structure and geometry (see Appendix);
- 2) An ultrasound color-coded-doppler examination of the carotid and vertebral arteries, and of the arterial and venous system of the lower limbs, including the measure of the ankle-brachial index.

- 3) An electromyography of the upper limbs with measures of motor and sensory nerve conduction velocity of the median and ulnar nerves.

Furthermore, in post-menopausal women we collected information on the presence and severity of symptoms which are characteristic of the peri-menopausal syndrome, such as hot flashes, pain, sleep problems, perception of weakness and fa-

Table 2 - Laboratory tests performed on the biological samples obtained in the PROSALMEN study.

1. Standard urinalysis
2. Complete blood cell count
3. Clinical chemistry
Glucose
BUN, Creatinine
AST, ALT, Gamma-GT
Uric acid
Iron
Ferritin, Transferrin
Sodium, Potassium
Chloride, Calcium
Magnesium
Total proteins
Protein electrophoresis
ESR, C-reactive protein
Fibrinogen and Prothrombin activity
Lpa
4. Endocrine-metabolic evaluation
Estrogens
FSH, LH
TSH, FT ₃ , FT ₄
Insulin, Glycated Hemoglobin
5. Bone and muscle metabolism*
PTH
25-OH Vitamin D
Bone Alkaline Phosphatase
Urinary NTx (27)**
Urinary Calcium (mg/dL), Phosphate (mg/dL), Creatinine (mg/dL), Hydroxyproline (mg/dL)
6. Lipid profile
Basal:
Total Cholesterol
LDL Cholesterol
HDL Cholesterol
Triglycerides
Lipid tolerance test* (see Methods):
Retinol Esters in "large" and "small" VLDL fractions
Triglycerides in "large" and "small" VLDL fractions

* On the day preceding the urine collection, the participant followed a diet without meat, fish and dairy foods; then, at 5:00 a.m. of the collection day, she voided and drank 3 glasses of water. Two hours later she emptied her bladder again and collected all the urine.

** The amino-terminal cross-linked telopeptide of type I collagen (NTx) was assayed using the immunoenzymatic test (EIA) OSTEOMARK. The bone-specific NTx molecule, provides a reliable measure of the rate of bone reabsorption.

tigue, restless legs especially at night and abnormal sensation.

The specimens of blood and urine were used to perform a series of laboratory examinations (Table 2) and in part to create a biological bank, including aliquots of plasma, serum, urine and purified DNA.

The structured interview included questions on health, socioeconomic status, and health behaviors. The evaluation of dietary intake included both an unstructured interview and the administration of the food frequency questionnaire that has been developed in the context of the EPIC project (16). History of weight change, specific dietary regimens and frequency of food intake outside the main meals were also investigated.

On the second and third visits, the participants underwent a comprehensive functional evaluation including performance-based measures of lower and upper limbs function, muscle strength (isometric and isokinetic) and power (17-19), and passive range of joint motion (20); a dynamic spirometric test was performed with portable equipment both at rest and while the participant was walking at maximum speed over a 400 m course (Table 3) (21).

Finally, the participants were administered a lipid tol-

erance test ("fat meal", Table 4). The purpose of this test was to evaluate to what extent the decline in the estrogenic level in menopause influences the activity of peripheral lipoprotein-lipase, thereby affecting the clearance of triglycerides after a meal containing mostly lipids. Recent studies have shown that the lipid tolerance test is a marker of risk for cardiovascular events that is more precocious and sensitive than a static evaluation of lipids at one point in time (22, 23).

All pre-menopausal women and the 15 post-menopausal women belonging to pairs closely matched for age (± 1 year) were given the lipid tolerance test. Following an overnight fast of at least 12 hours, an early morning blood sample was drawn and used for the baseline measurement of triglycerides, total-, HDL-, and LDL-cholesterol. The participants then received a lipid-rich standard meal (Table 4) that had to be consumed in 15 minutes; the meal was supplemented with 120,000 I.U. of Vitamin A (Arovit) to mark chylomicron-bound triglycerides, taking advantage of the bond existing between retinol-ester and triglycerides in the chylomicron core. Blood samples were subsequently collected after 2, 4, and 6 hours in sterile vacuum

Table 3 - Performance tests performed in the PROSALMEN study.

-
- A. Balance Test
 - B. "Five chair-stands"
 - C. Performance-based tests of walking:
 - Walking 4 m and 7 m at usual pace
 - Walking 4 m and 7 m as fast as possible
 - Walking 4 m within the limits of a 25 and 15 cm wide path, as fast as possible
 - Walking 7 m with steps as long as possible
 - Walking 7 m stepping over 2 obstacles without and with wearing sunglasses
 - Walking 7 m carrying a large package that does not allow view of the feet
 - "Talking while Walking" (7 m)
 - Collecting a specific object from the ground during a 7 m walk
 - Walking 400 m as fast as possible
 - Walking 60 m as fast as possible while wearing a heavy jacket that increases the weight of the participant woman by 15%.
 - D. Spirometry at rest and while the participant walked 400 m as fast as possible*
 - E. Shoulder mobility (Appley Test)
 - F. Measurement of passive range of motion of the hips, ankles, and shoulders
 - G. Measurement of leg muscle power by the "Power Rig" equipment (18)
 - H. Measurement of the isometric muscle strength by a manual dynamometer of four muscle groups of the lower limb (17)
 - I. Measurement of isokinetic muscle strength of the right leg**
-

* In PROSALMEN, O₂ consumption during exercise (VO₂ peak) was considered as a proxy measure of fitness. Oxygen consumption was assessed by a portable spirometer kit (K4b₂, COSMED, Italy), both at rest (3 minutes) and while the participant was walking as fast as possible for 400 m. The measure of VO₂ peak obtained with this method may be expressed as Metabolic Equivalents (METs, mL/min/kg). Moreover, the energy expenditure per minute (in Kcal/min), which provides an estimate of the energy requirement, was calculated (28).

** The isokinetic flexo-extension strength of the knee was performed by means of REV 9000, Tecnogym. Muscle strength was assessed during concentric isokinetic movements at a speed of 60°/s and 180°/s during 5 maximal repetitions. Furthermore, the total work of the flexor and extensor muscles was calculated during 30 repetitions of isometric maximal concentric contractions at a speed of 180°/s.

Table 4 - Food composition of the standard meal used for the lipid tolerance test.

FOODS AND BEVERAGES	AMOUNT
Focaccia ("White Pizza")	100 g
Stracchino (cream cheese)	100 g
Butter (spread on the focaccia)	20 g
Whole milk, fruit flavored yoghurt	125 g (1 can)
Toasted ground coffee, (one espresso cup, corresponding to about 6 g)	6 g (1 cup)
Whipped cream (to mix with the coffee)	50 g
MACRO-MICRONUTRIENTS and KCAL	
Total proteins	32.58 g / 13.4%*
Available glucids	60.33 g / 23.2%*
Total lipids	63.4 g / 64.4%*
Calories	973.8 Kcal
Cholesterol	181.8 mg
Total saturated fatty acids	35.7 g
Total monounsaturated fatty acids	24.5 g
Oleic acid	22.3 g
Total polyunsaturated fatty acids	4 g
Linoleic acid	3 g
Linolenic acid	0.9 g

* Percent by calories.

tubes containing 1% EDTA, and centrifuged at low speed (2000 rpm) for 10 minutes at room temperature. Plasma was separated, collected in aluminum wrapped sterile tubes, and stored at +4°C. The following lipoprotein fractions were separated within 24 hours by analytical ultracentrifugation: 1) plasma triglycerides; 2) retinol-ester contained in the

"large" VLDL fractions (60-400 Svedberg units) and in the "small" VLDL fractions (20-60 Svedberg units) of intestinal origin; 3) triglycerides in the "large" VLDL fractions (60-400 Svedberg units) and in the "small" VLDL fractions (20-60 Svedberg units) released from the liver in the early postprandial period. All measures were performed in the laboratory of lipid metabolism of the San Raffaele Institute (Milano, Italy).

THE ANALYTICAL PLAN OF PROSALMEN AND FUTURE DEVELOPMENTS

An overview of the measures performed in the PROSALMEN participants is shown in Table 5.

The collection of the parameters reported in the Table is complete, and an analytical database has been created and validated through a number of quality control checks.

In accordance with the design of the study, in the initial stage we intend to perform an age-matched analysis to test whether, independently of age, the parameters shown in Table 5, which are considered the main outcomes of the study, have a different distribution in pre- and post-menopausal women. In the second stage, we will introduce meaningful covariates in the analysis that may explain such a difference. Serum estrogen levels and their biological activity are certainly the most important explanatory variables, however, we expect that other behavioral factors, such as differences in dietary intake and current level of physical activity, may ex-

Table 5 - Summary of the measures obtained in the PROSALMEN study relevant for the main outcomes.

	Test	Description
Muscle system	1) Performance tests of the lower limbs	See Table 3
	2) Isometric strength of lower and upper limbs	See Table 3
	3) Leg muscle power	See Table 3
	4) Isokinetic strength of the lower limbs	See Table 3
	5) Muscle cross-sectional area/mass at the lower leg	See Appendix
	6) Nerve motor and sensitive conduction velocity*	See Table 2
Bone and joints	1) Trabecular and cortical bone density, mass and geometrical distribution	See Appendix
	2) Passive range of motion of joints of the lower limbs	See Table 3
	3) Bone catabolic rate (NTx and Nordin test)	
Cardiovascular system	1) EKG	
	2) Color-ultrasound examination of carotid and vertebral arteries and of the arterial and venous system of the lower limbs [#]	
	3) Lipid tolerance test	See Methods
	4) Static and dynamic spirometry	See Table 3

* Nerve conduction velocity of the median nerve was assessed through surface electromyography. The following parameters of nerve conduction were considered: sensory and motor nerve conduction velocity (m/s), amplitude (mV/micron); distal latency (msec).

[#] The equipment used to perform these assessments was an ultrasound color-coded-doppler equipped with a linear-array probe 7.5 MHz. OTEBiomedica.

plain some of the changes that are traditionally attributed to menopause. Were this hypothesis confirmed, it would provide scientific strength to the idea that changes in behavior may counteract, at least in part, the negative effect of menopause on the quality of life.

One of the objectives of PROSALMEN is to obtain a quantitative estimate of the "pure" effect of menopause on trabecular and cortical bone mass, muscle mass, physical performance, and other related biological parameters, such as sensitivity to insulin, and mechanisms affecting the clearance of serum lipids. The potential to obtain this information is intrinsic to the study design and is also one of the major strengths of PROSALMEN, which will be reinforced as longitudinal data become available. A number of studies have pointed out that while the level of sex hormones declines rapidly during the peri-menopausal period, changes in these parameters are somewhat delayed and, therefore, their strict dependence on the level of sex hormones is questionable (24). For example, the decline in cortical bone mass and density (the two most important parameters affecting bone mechanical strength) becomes evident mostly after 60 years of age (25). On the contrary, recent data suggest that the acceleration of sarcopenia starts long before menopause, and its rate of decline does not substantially change with menopause (26).

PROSALMEN is a small but very focused study that may help understand how the physiological changes occurring during menopause affect the quality of aging in older women. We plan to use this information to design a larger multicenter survey looking at potential targets of intervention, based on the hypothesis that the current approach to menopause, mostly limited to hormone replacement therapy, should be complemented with other pharmacological and non-pharmacological strategies.

As mentioned above, PROSALMEN is a preliminary study mostly aimed at confirming our "a priori" hypothesis and at providing estimates that can be used to design a definitive study on a much larger number of participants. The limitations of this preliminary study are intrinsic to both the design and the small sample size. The core question that we want to address is "What parameters differentiate two women of the same age but, respectively, in pre- and post-menopausal state?". To respond to this question, we used a pair-matching design, therefore providing an adequate control for each post-menopausal woman enrolled in the study population. We assume that the difference between the members in each pair is independent of the age that charac-

terizes that specific pair. As a consequence, we cannot test whether the effect of menopause is different across the spectrum of early to late menopause. In future, much larger studies, this hypothesis could be addressed by a stratified sample including the same number of pairs in each age strata.

APPENDIX

Lower leg Peripheral Quantitative Computerized Tomography (pQCT)

Lower leg pQCT was performed in all the study participants by means of a recent generation device (XCT 2000, Stratec Medizintechnik, Pforzheim, Germany). The subjects were seated in front of the apparatus with the right leg extended, positioned inside the gantry of the device. The distal end of the tibia (the tibiotalar joint cleft), identified using a pQCT longitudinal scout view, was used as an anatomical marker for the identification of the measurement sites. The length of the tibia had been previously assessed as the distance between the medial knee joint cleft and the medial malleolus (both identified by manual palpation) while the participant was lying supine. Standard 2.5 mm thick transverse scans were obtained at 4% of the tibial length (measured from the distal end of the tibia) where trabecular bone is most abundant, and at 38% of the tibial length, where the cortical shell is usually thicker than 2.5 mm, thus allowing an accurate detection of the bone boundaries. The cross-sectional images obtained from the pQCT were analyzed using the BonAlyse software (BonAlyse Oy, Jyväskylä, Finland, <http://www.bonalyse.com>) a software for processing pQCT scans that automatically identifies bone tissue (cortical and trabecular), and assesses its density and geometry. Different tissues in the analysis were separated according to different density thresholds. In particular, areas with density values above 710 mg/cm³ were considered as "cortical bone" (29), while areas with density values between 180 and 710 mg/cm³ were considered as "trabecular bone". The following bone parameters were derived from the pQCT images: *total bone density* (mg/cm³); *trabecular bone density* (mg/cm³); *cortical bone density* (mg/cm³); *total bone area* (mm²); and *cortical bone area* (mm²).

The parameters described so far can be considered as the density and geometry determinants of bone compressive and tensile strength. In order to extend our assessment to the mechanical competence against bending and torsional loads, composite parameters, which take into account the characteristics and the spatial distribution of bone material, were calculated:

Minimum moment of inertia (mg/cm): was calculated at the 38% site as a density weighted moment of inertia, according to the following formula:

$$I = \int a^2 r^2 \rho$$

where "I" is the minimum moment of inertia; "a" is the voxel area; "r" is the distance between each voxel and the neutral axis of the section; "ρ" is the density of the voxel that is considered (30). The minimum moment of inertia provides an estimate of the minimal resistance to bending of the examined section (31).

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