Contents available at **PubMed**
www.anmm.org.mx

PERMANYER

Gac Med Mex. 2015;151:678-86

GACETA MÉDICA DE MÉXICO

ORIGINAL ARTICLE

Bone mineral density and its association with body composition and metabolic biomarkers of insulin-glucose axis, bone and adipose tissue in women

Edna J. Nava-González[1], Ricardo M. Cerda-Flores[2], Pedro A. García-Hernández[3], Gloria A. Jasso-de la Peña[3], Raul A. Bastarrachea[4] and Esther C. Gallegos-Cabriales[2]*

[1]Center of Research in Nutrition and Public Health, Faculty of Public Health and Nutrition, Universidad Autónoma de Nuevo León, [2]Subdirection of Post-degree and Research, Faculty of Nursing, Universidad Autónoma de Nuevo León, [3]Department of Endocrinology, Hospital Universitario de Monterrey "Dr. José Eleuterio González", Monterrey, N.L., México; [4]Department of Genetics, Texas Biomedical Research Institute, San Antonio, Tx., USA

Abstract

Introduction: *There are few studies integrating the common causes of osteoporosis and obesity (disorders of body composition). A first step is to investigate correlations between their biological phenotypes to determine their common integrative physiology.* **Objective:** *To correlate the variation of bone mineral density with phenotypes of body composition and biomarkers of bone physiology, insulin-glucose axis, and adipose tissue.* **Methods***: Cross-sectional study of 75 women (aged 18-45 years). Measurements: Body mass index, waist, fat mass, lean mass (dual-energy X-ray absorptiometry), glucose, insulin, osteocalcin, leptin, tumor necrosis factor alpha. Statistical analysis: multivariate general linear model, SPSS v.22, $p < 0.05$.* **Results:** *Age: 32.08 ± 7.33. Bone mineral content multivariate general linear model 1 with two phenotypes excluded (glucose, insulin): osteocalcin ($\beta = -0.228$, $p = 0.011$), lean mass ($\beta = 0.606$, $p = 0.001$) and fat mass ($\beta = 1.237$, $p = 0.001$) in 62.0%. The bone mineral density multivariate general linear model 2 with three phenotypes excluded (body mass index, glucose, tumor necrosis factor alpha): insulin ($\beta = 0.250$, $p = 0.024$), osteocalcin ($\beta = -0.362$, $p = 0.001$), lean mass ($\beta = 0.512$, $p = 0.001$) and fat mass ($\beta = 0.701$, $p = 0.001$) in 46.3%.* **Conclusions:** *Results show that body composition with an increased lean mass is beneficial to bone. This study reaffirms the importance of performing regular exercise to prevent muscle loss.*
(Gac Med Mex. 2015;151:678-86)

Corresponding author: *Edna J. Nava-González, edna.navag@uanl.mx*

KEY WORDS: *Body composition. Bone density. Metabolic biomarker.*

Introduction

Demographic transition and body composition disorders

Due to the demographic transition experienced by the country with a trend towards population aging, with a resulting increase in specific, complex, common and highly prevalent chronic-degenerative diseases (osteoporosis, obesity and diabetes), future epidemiological impact of these conditions is expected to be considerably elevated, both in terms of costs and negative consequences on quality of life, disability and premature death[1]. Today, these 3 pathologies are referred to and grouped together as body composition disorders, since their pathophysiology entails alterations of the insulin-glucose axis, adipose tissue metabolism and

Correspondence:
*Edna J. Nava-González
Ave. Dr. Eduardo Aguirre Pequeño y Yuriria
Col. Mitras Centro, C.P. 64460, Monterrey, N.L., México
E-mail: edna.navag@uanl.mx

Date of reception: 19-06-2014
Date of acceptance: 22-01-2015

bone mineral turnover[2,3]. Therefore, it is both necessary and significant to develop research projects to further study their common links.

Body composition disorders were conceived in the past as mutually excluding and independent from each other. Currently, these conditions are known to share some distinctive common traits, since all of them have a genetic basis that interacts with environmental influences and, once occurred, they are associated with significant morbidity and mortality. These conditions are considered serious public health problems and, from the molecular point of view, can be identified as secondary to dysregulation of a common precursor cell[4]. These chronic conditions have many similarities in their pathogenesis and the most important include a genetic predisposition to develop them. The least integrated in the concept that comprises body composition disorders is osteoporosis, as the links associating diabetes and obesity have been extensively studied[5,6].

Osteoporosis, preceded by bone mineral density (BMD) decrease has become recently a focus of research in Mexico, since estimates are that 1 in every 12 women and 1 in every 4 men older than 50 years will suffer a hip fracture in the remaining years of her/his life[7]. The costs for the treatment of hip fractures in Mexico in 2006 were higher than USD 97 million, which represents an important burden for the health system[8]. BMD is a clinical predictor for osteoporosis and this condition constitutes a critical factor in the risk for fratures in adulthood[9].

Interactions between bone, adipose tissue and pancreas

Knowledge on the mechanisms that control body fat mass deposits and bone mineral turnover molecular pathways has substantially advanced. Shared regulation and common cell communication pathways between the hypothalamus, adipose tissue and bone marrow have been reported to exist, a situation that leads to speculate about a biological interaction between bone and adipose tissue[10]. These mechanisms appear to modulate osteocyte and adipose cell metabolism, through inter-regulated molecular pathways involving the sympathetic nervous system activity, the system that regulates hunger and satiety, the insulin-glucose axis, energy balance and skeletal system remodeling[11].

Glucose metabolism and insulin physiological effects are importantly affected when there is metabolic alteration in the communication between bone and adipose tissue[12,13]. From the clinical point of view, most patients with diabetes exhibit obesity and sedentarism. This type of patients usually has an enlarged body size and has been observed to experience fractures more frequently[14]. However, the most important factor on the influence of an altered insulin-glucose axis is the effect of vascular microcirculation, which is common in type 2 diabetes[15]. Women with type 2 diabetes have been found to experience a higher proportion of fractures, especially in the hip, than women without diabetes, as demonstrated in a prospective study named The Study of Osteoporotic Fracture[14]. In addition, bone loss has been documented to be much higher in patients with poor control of their glucose levels than in those with diabetes and good metabolic control[14,15].

Recent literature has reported that the effects of the gene-environment interaction on molecular biomarkers, as those involved in bone mineral turnover would be, interfere in the biology of bone tissue by modulating calcium replacement and bone integrity maintenance, ultimately regulating bone mass closely linked to the use of energy through the adipose tissue, food intake and insulin physiological actions[16,17]. Additionally, it is important to further know the biological response to environmental stimuli[18]. An example is deficient nutrient intake, which is helpful to understand how an observable behavior triggers a pathological process, such as bone demineralization.

The purpose of this study is to determine the normal quantitative variation with regard to BMD in healthy adult women, associating it with phenotypes determined by measurements of body composition and circulating biomarkers corresponding to bone physiology (bone mineral turnover determined by osteocalcin), and to insulin-glucose axis and adipose tissue activity, in order to establish deleterious health consequences, such as osteoporosis, diabetes and obesity, in the context of body composition disorders.

Material and methods

Design

This is a descriptive, cross-sectional, correlational study. The methodologic design comprised the selection of female voluntary participants, data collection procedures, inclusion and exclusion criteria, statistical analyses plan and ethical considerations, including the informed consent. Analyses of biochemical phenotypes were performed, including specific metabolic biomarkers that indicate bone mineral turnover (fasting

circulating osteocalcin measurements), actions by the adipose tissue (fasting leptin and TNF-α) and insulin-glucose axis metabolism (fasting glucose and insulin). Anthropometric phenotypes and body composition measurements (waist circumference, body mass index [BMI], % of fat, lean mass and total fat) were also carried out, as well as BMD measurements by dual-energy X-ray absorptiometry (DEXA).

Population, sampling and sample

The population of interest was comprised by women between 18 and 45 years of age. Sampling was considered non-probabilistic, since it was carefully selected to include healthy women by direct invitation and through social networks. The sample size (n = 75) has been calculated with the nQuery Advisor program, with a calculation power of 80% at a 0.05 level of significance[19,20]. The recruitment took place at the Universidad Autónoma de Nuevo León (UANL). Healthy women with regular menses participated in the study. The participants were referred to the Center of Research in Nutrition and Public Health, UANL, for measurements relevant to the investigation. Close before their appointment for measurements, it was made sure that the patients had no signs or symptoms of acute conditions and/or were not consuming any medication by questioning prior to any protocol procedure. All participants in this study previously signed an informed consent form. Participants with chronic conditions (polycystic ovary syndrome, arterial hypertension, thyroid disease), pregnant women, breastfeeding women, women who had given birth to a child in less than one year, women who had undergone hysterectomy, women on hormone replacement therapy, and especially women with an established diagnosis of osteopenia or bone disease were excluded. The procedure to determine whether any participant had any of the above-mentioned diagnoses was based on the history taken at the moment they were invited to participate, and also through the clinical skills of the healthcare professional in charge of this process.

Measurements

Measurements were made for height, weight, waist circumference, body composition and BMD by DEXA.

- Height. This measurement was performed following standardized procedures with the participant standing up straight, back against the wall; the head, scapulae and buttocks in touch with the stadiometer, without shoes and heels together. A stadiometer of the Brand SECA, model 274, with minimum measurement capacity of 60 to 220 cm and 0.1 cm accuracy was used.
- Weight. The measurement was carried out through standard procedure checking for the scale to be on zero. The equipment used was an electronic scale of the brand SECA, model 874. The employed cutoff points for weight-height ratio (BMI) were 18.50-24.99 kg/m^2, which is considered normal range; 25-29.9 kg/m^2, which corresponds to overweight; 30-34.9 kg/m^2, to grade 1 obesity; 35-39.9 kg/m^2, to grade 2 obesity, and for extreme or morbid obesity, a BMI higher than or equal to 40 kg/m^2 [21].
- Waist circumference. For perimeter measurements, standardized methods were used, taking the narrowest level between the lowest rib edge and the iliac crest, with a flexible steel tape with 1.5 m minimum length, calibrated in cm with millimetric gradation of the Lufkin brand. An excess of visceral fat (> 88 cm waist circumference in women) is known as central obesity and is associated with the development of type 2 diabetes, dyslipidemia and cardiovascular conditions[22].
- Body composition and BMD by DEXA. Full-body bone density was measured by DEXA. Body fat, lean mass and total and regional BMD were measured in the anteroposterior (AP) spine (L1-L4), right proximal femur (total hip, femoral neck, trochanter) and right forearm. The measurements were carried out once with the Lunar Prodigy Advance equipment (GE Lunar Radiation Corp, Madison WI). The duration of this measurement was approxomately 15 minutes, with the participant in the supine position (AP examination). The Encore 11.4 software reported the body composition estimates: lean mass (kg), fat mass (kg), fat percentage, BMD (g/cm^2) and BMI (kg/m^2). The bone density results were expressed as standardized T- and Z-scores, which are clinically used to predict fracture risk. The BMD report in premenopausal women uses preferably the Z-score. A Z-score of -2.0 or lower is defined as “below expectations for age range” and a Z-score higher than -2.0 is “within expectations for age range”[23].

Biochemical measurements

The analysis of metabolic biomarkers was carried out in the clinical analyses laboratory certified by the Health Council of the University Hospital.

- Glucose and insulin. Circulating glucose and insulin determinations are a direct reflection of liver and muscle carbohydrate metabolism molecular and metabolic control. Glucose was determined using the glucose oxidase method with spectrophotometry, and insulin was determined using the immunoassay technique in the Luminex-100 System. A level from 70 to under 100 mg is regarded as normal; from 100 to 125 mg/dl means there is a fasting glucose alteration, known as pre-diabetes. This increses the risk for type 2 diabetes. A level of 126 mg/dl or higher is diagnostic of diabetes[24]. The cutoff point used for insulin is 15 µU/ml[25].
- TNF-α and leptin. The immunoassay technique was used with the Luminex-100 system through a multiplexed XY platform (Luminex®) with calibration microspheres for the report of readings with the magnetic bands MilliPlex™ software to obtain the TNF-α and leptin plasma concentrations. Leptin represents the main biological and metabolic aspect of adipocyte dysfunction or adequate function. Elevated TNF-α is indicative of the presence of deletereius chronic subclinical inflammation[26].
- Osteocalcin. The ELISA technique was used with available specific commercial reagents. Osteocalcin is a biochemical marker representative of the osteoblast-osteoclast function and bone mineral turnover balance[27].

Data analysis

The associations of body composition phenotypes and metabolic parameters with BMD and bone mineral content (BMC) were analyzed with Pearson's correlation coefficient in order to identify bivariate relationships. Multivariate linear regression analysis was used to measure the strength of the relationship between body composition and metabolic parameters with BMD and BMC. For the analysis of results, descriptive statistics (means, standard deviations [SD], frequencies and percentages) were used through the SPSS V.22 software. This protocol was submitted fo review for approval by the Ethics and Biosafety Commission, as well as by the Research Commission of the Nursing Faculty of the UANL.

Results

The sample was structured with 75 female participants with an average of 32.08 years of age (SD = 7.93; 19-45 years), 69.3% were born in the State of Nuevo León, 84% had college education, 64% worked as employees, out of which 62.7% received a salary and, in 52%, marital status was married. Average age at menarch was 12.15 years (SD = 1.42; 9-15 years). Additionally, 12% were smokers, 64% referred drinking alcohol socially with an average consumption of 1.55 (SD = 1.58) alcoholic beverages per occasion. An average of 1.23 pregnancies (SD = 1.35) and 1.15 children born at term (SD = 1.21) were reported by 53%. 4% referred having suffered from gestational diabetes. Oral contraceptives were used by 10.7%, out of which 5.3% had used them for more than 1 year. Dietary supplements were consumed by 36% (9.3% multivitamins, 6% omega 3, 2.7% calcium, vitamin D and C, 2.6% folic acid). Previous fractures had been suffered by 12% at an average age of 21.44 years (SD = 12.85) (wrist, fingers, ankle, arm, skull and ribs).

General descriptive characteristics about genotypes that influence on BMD are reported in table 1, including body composition and circulating biomarkers associated with bone and metabolic physiology of the study population. Included phenotypes were divided to compare female participants in 2 groups according to being < 30 (n = 32) or ≥ 30 years of age (n = 43), since the literature has described this age to be when bone mass maximum peak is reached[28]. Weight, waist circumference, body fat percentage and quantity in kg and leptin levels were significantly higher in the > 30-year age group. The difference in osteocalcin levels was significant between both groups, with decreased measurements in the group of women older than 30 years. No significant differences were detected between BMC (g) and total and regional BMD (g/cm^2).

General descriptive characteristics of phenotypes that influence on BMD are reported on table 2, including body composition and circulating biomarkers associated with bone and metabolic physiology of the study population. These phenotypes were divided according to the percentage of body fat (BF%), with a cutoff point selected according to the BF% mean in our study population, to reports of investigations that classify its excess with the presence of deleterious effects for health[29,30], and to a good number of studies that suggest that excessive body fat produces unfavorable effects on BMD[31,32]. Insulin and leptin levels were significantly more elevated in the group with higher BF%, as was BMI, waist circumference, fat mass in kg and BMC (g). The difference between osteocalcin levels was significant between both groups, with decreased measurements in the group of women with larger amounts of body fat.

Table 1. Descriptive characteristics of the study population with regard to age

Phenotypes	< 30-year group (n = 43)	SD	> 30-year group (n = 43)	SD	t	p-value
Age (years)	23.81	2.51	38.23	3.94	–18.18	.001*
Glucose (mg/dl)	87.78	10.45	91.93	15.56	–1.30	.196
Insulin (μU/ml)	8.33	4.32	9.14	6.87	–0.58	.561
Osteoclacin (ng/ml)	14.01	4.31	10.90	4.06	3.18	.002*
Leptin (pg/ml)	15,589.31	11265.07	25,434.01	16326.80	–2.92	.005*
TNF-α (pg/ml)	7.22	4.17	12.89	23.16	–1.36	.177
Weight (kg)	62.54	12.15	69.74	13.08	–2.43	.017*
Height (cm)	160.62	6.52	160.00	5.97	0.44	.657
BMI (kg/m^2)	24.24	4.63	27.17	4.40	–2.79	.007*
Waist C. (cm)	73.56	9.21	82.28	10.66	–3.69	.001*
Lean Mass (kg)	36.19	4.70	37.18	5.61	–0.80	.424
Fat Mass (kg)	23.08	10.27	29.07	9.68	–2.57	.012*
Fat %	37.09	10.34	43.03	7.45	–2.83	.006*
BMC (g)	2.431	0.332	2.460	0.346	–0.35	.724
Total BMD (g/cm^2)	1.142	0.058	1.148	0.071	–0.44	.657
Spine BMD (g/cm^2)	1.193	0.107	1.205	0.135	–0.41	.680
Femur BMD (g/cm^2)	1.006	0.107	0.996	0.110	0.39	.695
Forearm BMD (g/cm^2)	0.843	0.107	0.848	0.055	–0.43	.667

t: test; BMC: bone mass content; BMD: bone mass density.
*p < .05.

For the analyses of relationships between the study phenotypes, their normality was determined with the Kolmogorov-Smirnov statistical test with the Lilliefors correction[33], and only 7 phenotypes were found to be normally distributed: BF%, lean mass, BMC, total BMD, spine BMD, femur BMD and osteocalcin.

Tables 3 and 4 list the continuous phenotypes in order to identify bivariate relationships with BMD. A significant direct correlation was observed with body composition phenotypes: BMI, waist, fat mass (kg) and lean mass, as well as a significant indirect correlation with osteocalcin.

An adjusted multivariate general linear model (MGLM) was used, where independent variables were: BMI, waist, lean mass, fat mass, glucose, insulin, osteocalcin, leptin and TNF-α, and dependent variables were BMC and BMD. Table 5 shows that the BMC-dependent variable model was significant and is explained with the osteocalcin, leptin, BMI, waist, lean mass and fat mass phenotypes in 60.7%. Based on the largest-sized p-value, the independent phenotypes glucose and insulin were successively removed using the backward technique and, this way, the multivariate model is explained with the osteocalcin, leptin, waist, lean mass and fat mass phenotypes in 62.0%. Table 6 shows that the total BMD dependent variable model was equally significant, and it is explained with the insulin, osteocalcin, leptin, waist, lean mass and fat mass phenotypes in 42.7%. Subsequently, the independent phenotypes BMI, glucose and TNF-α were successively removed, always using the backward technique, and this way this model is explained with the insulin, osteocalcin, leptin, waist, lean mass and fat mass phenotypes in 46.3%. The negative relationships that were expressed as standardized β-coefficients were osteocalcin, leptin, BMI and waist circumference, with BMC, as well as total BMD, as dependent phenotypes.

Table 2. Descriptive characteristics of the study population with regard to body fat percentage

Phenotypes	< 40% Fat Group (n = 35)	SD	> 40% Fat Group (n = 40)	SD	t	p
Age (years)	30.34	8.35	33.60	7.36	–1.80	.076
Glucose (mg/dl)	87.14	10.48	92.80	15.64	–1.81	.074
Insulin (μU/ml)	6.83	4.18	10.51	6.66	–2.81	.006*
Osteoclacin (ng/ml)	14.02	3.96	10.66	4.25	3.52	.001*
Leptin (pg/ml)	11,505.57	7739.95	29,745.53	14901.87	–6.51	.001*
TNF-α (pg/ml)	7.81	5.26	12.80	23.89	–1.20	.231
Weight (kg)	58.60	7.86	73.73	12.81	–6.10	.001*
Height (cm)	160.85	5.81	159.75	6.52	0.80	.426
BMI (kg/cm^2)	22.58	2.16	28.84	4.44	–7.70	.001*
Waist C. (cm)	71.86	7.09	84.43	10.32	–6.04	.001*
Lean Mass (kg)	37.33	5.40	36.26	5.10	0.87	.385
Fat Mass (kg)	18.29	4.49	33.71	8.38	–9.70	.001*
Fat %	32.29	5.80	47.67	4.77	–12.34	.001*
BMC (g)	2.361	0.321	2.523	0.339	–2.10	0.39
Total BMD (g/cm^2)	1.134	0.069	1.156	0.061	–1.41	.163
Spine BMD (g/cm^2)	1.183	0.142	1.215	0.104	–1.09	.276
Femur BMD (g/cm^2)	0.984	0.116	1.015	0.100	–1.25	.213
Forearm BMD (g/cm^2)	0.844	0.100	0.848	0.051	–0.34	.732

t: test; BMC: bone mass content; BMD: bone mass density.
*p < .05.

Table 3. Correlations of total BMD and body composition phenotypes

Phenotypes	Total BMD	BMI	Waist	Fat%	Fat kg	Lean mass
Total BMD	1					
BMI	.395†	1				
Waist	.322†	.888†	1			
Fat%	.147	.786†	.711†	1		
Fat kg	.367†	.944†	.873†	.888†	1	
Lean mass	.477†	.349†	.401†	-.172	.247*	1

*p < .05

Discussion

The purpose of this study is to explain the BMD quantitative normal variation, in order to determine the influence of body composition and metabolic biomarkers in healthy adult women. This research was focused on studying 3 metabolic axes (insulin-glucose axis, adipose tissue, bone mineral turnover), trying to identify patterns that allow for common pathways in the etiopathogenesis of obesity, diabetes and osteoporosis to be established. The importance of preserving bone integrity in order to regulate bone mass through

Table 4. Correlations of total BMD and metabolic biomarker phenotypes

Variable	Total BMD	Glucose	Insulin	Leptin	TNF-α	Osteocalcin
Glucose	.022	1				
Insulin	.175	.427†	1			
Leptin	.033	.287*	.569†	1		
TNF-α	.085	.011	-.026	.029	1	
Osteocalcin	–.389†	–.004	-.239*	–.286*	–.072	1

*p < .05.
†p < .01.

Table 5. Predictive model of BMC as dependent phenotype with body composition and metabolic biomarkers

	Model 1		Model 2		Model 3	
Phenotypes	β	**p**	β	**p**	β	**p**
Glucose	–.036	.666	X		X	
Insulin	.166	.111	.149	.108	X	
Osteocalcin	–.215	.021	–.221	.013	–.228	.011*
Leptin	–.308	.012	–.319	.006	–.238	.023*
TNF-α	.120	.124	.132	.077	.133	.078
BMI	–.552	.045	–.523	.044	–.450	.080
Waist	–.507	.012	–.519	.006	–.462	.013*
Lean mass	.609	.001	.615	.001	.606	.001*
Fat mass	1.395	.001	1.357	.001	1.237	.001*

*p < .05.
Note. Phenotypes removed: glucose, insulin.
GLM1: (F = 9.78; p = .001), adjusted r^2 = .607. GLM2: (F = 16.675; p = 001), adjusted r^2 = .629. GLM3: (F = 18.230; p = .001) adjusted r^2 = .620.

Table 6. Predictive model of total BMD as dependent phenotype with body composition and metabolic biomarkers

	Model 1		Model 2		Model 3		Model 4	
Phenotypes	β	**p**	β	**p**	β	**p**	β	**p**
Glucose	–.086	.396	–.085	.384	X		X	
Insulin	.288	.023	.289	.015	.251	.022	.250	.024*
Osteocalcin	–.342	.003	–.348	.001	–.356	.001	–.362	.001*
Leptin	–.299	.041	–.304	.029	–.313	.025	–.313	.025*
TNF-a	.085	.364	.090	.301	.087	.318	X	
BMI	–.059	.085	X		X		X	
Waist	–.593	.015	–.582	.009	–.572	.010	–.584	.008*
Lean mass	.553	.001	.524	.001	.517	.001	.512	.001*
Fat mass	.748	.023	.692	.001	.686	.001	.701	.001*

*p < .05.
Note. Phenotypes removed: BMI, glucose, TNF-a.
GLM1: (F = 5.247; p = .001), adjusted r^2 = .427. GLM2: (F = 8.029; p = .001), adjusted r^2 = .461. GLM3: (F = 8.968; p = .001) adjusted r^2 = .463. GLM4: (F = 10.102; p = .001) adjusted r^2 = .463.

bone mineral turnover was able to be documented. This class of bone biological regulations share common pathways with adipose tissue and insulin-glucose axis energy homeostasis (involving muscles and pancreas), and thus, the measurement of their biological effectors, known as metabolic biomarkers, is a reflection of their integrative biology[34]. These metabolic biomarkers represent the genetical secondary response to environmental influence, and their measurement appears to be an adequate strategy to determine the way risk-behaviors resulting from a given lifestyle influence on the susceptibility for the development of these metabolic disorders, which result in pathological consequences for health when they occur.

The descriptive results indicate that this women's age group is within normal variation of clinical risk parameters for the development of diabetes, determined by glucose, insulin, anthropometric measurements and body composition, as well as BMD and BMC. According to the methodological design, the objectives were met, since the intention was to have a group of healthy women available. This is the main premise of the research model: trying to find in an age group with an adequate health profile with predisposition, within that normal variation range, to present patterns of susceptibility for the development of the contemplated pathologies.

With regard to the body composition phenotypes, 46.6% of the women had a BMI consistent with overweight and obesity, and according to the WHO standard classification by age, 37.4% had moderate-high risk for developing diabetes owing to their waist circumference, and 81.3% had BF% higher than 32%, which is considered diagnostic of obesity. Metabolic parameters indicate that 18.7% had glucose levels higher than 100 mg/dl, which is currently considered as pre-diabetes, 10.7% had insulin levels higher than 15 μU/ml, with these percentages falling within the risk prevalences foreseen in our population. Osteocalcin levels were below the normal range in 5.3% of the women.

The bivariate correlations between total BMD and all 5 body composition phenotypes (BMI, waist, BF%, fat in kg and lean mass) reveal that, in these women, the highest correlation was found between BMD and lean mass ($r = 0.47$), followed by BMI ($r = 0.39$), with the lowest correlation being with BF% ($r = 0.14$), which was statistically non-significant. This is consistent with other reports in the literature[35]. Most part of weight gain towards adulthood is well known to be at fat expense. Ohumura et al.[36] demonstrated that fat mass increases gradually from 40 to 59 years of age, whereas lean mass remains relatively constant throughout life. Therefore, when people's weight increases, there is a strong possibility for fat mass to be the important factor in such weight increase. In the results of that study, as in ours, BF% was not significantly correlated with BMD. Takada et al.[37] reported that if there is weight gain, this is ineffective in influencing on BMD, unless this gain includes lean mass increase[38]. In this context, increasing or maintaining lean mass appers to be the most important factor to acquire and maintain BMD within normal limits. Our data revealed an important correlation between lean mass and BMD. The bivariate correlations between total BMD and all 5 phenotypes associated with metabolic biomarkers (glucose, insulin, leptin, TNF-α, osteocalcin) report correlations with no statistical significance, except for osteocalcin, which had a significant indirect correlation ($r = -0.38$), indicating that there is consistency with the findings on BMD and body composition and metabolic biomarker phenotypes, suggesting that the higher the weight and amount of fat, the higher the insulin and TNF-α, and lower osteocalcin.

The body of data obtained through multivariate analyses with methodology used for simultanious comparison of these variables enabled us to acquire further understanding on the studied phenomenon. Hence, the statistically significant results allow for normal variation of BMC and BMD in these women to be explained according to body mass (waist, fat mass and lean mass) and metabolic biomarker (insulin, leptin and osteocalcin) phenotypes that deeply influence on their biology. The relationships that determined the highest power with standardized multivariate regression coefficients between total BMD and body composition were represented by lean mass ($\beta = .512$, $p = .001$) and fat mass ($\beta = .701$), $p = .011$). Accordingly, between BMC and body composition, lean mass ($\beta = .606$, $p = .001$) and fat mass ($\beta = 1.23$, $p = .001$) relationships were significant. These finding appears to explain that women in our study population who had larger amounts of fat mass were exposed to its deleterious effects on bone mineral turnover. Moreover, it is to be assumed that lean mass defines the protecting effect towards BMD.

There are several reasons that make the study of the relationship between body composition, fat mass distribution, BMD and their metabolic biomarkers important. Perhaps the two most important aspects are focused on predicting the risk of fracture and undestanding its clinical-observational aspects, as in the case of this study, through multivariate regression coefficients

applied to statistical analyses. Body composition, fat mass amount and distribution, and lean mass measurements are shown to explain most part of both BMD and BMC variation. From the point of view of patient care and support with regard to prevention, knowing that fat excess is not beneficial to the bone is important, as suggested by this and other studies. Our results also demonstrate that body composition with larger amounts of lean mass is beneficial to the bone. Therefore, the translation of this investigation into clinical-practical aspects reaffirms the importance of recommending regular physical exercise, since this activity prevents muscle mass loss and increases mechanisms that strengthen the skeleton.

References

1. Rivera JA, Barquera S, Campirano F, et al. Epidemiological and nutritional transition in Mexico: rapid increase of non-communicable chronic diseases and obesity. Public Health Nutr. 2002;5:113-22.
2. Rosen CJ, Klibanski A. Bone, fat, and body composition: evolving concepts in the pathogenesis of osteoporosis. Am J Med. 2009;122(5):409-14.
3. Bastarrachea R, Fuenmayor R, Brajkovich I, et al. Entendiendo las causas de la obesidad a través de la biología celular del adipocito. Revista Venezolana de Endocrinología Metabólica. 2005;3(3):20-9.
4. Zipori D. The stem state: mesenchymal plasticity as a paradigm. Curr Stem Cell Res Ther. 2006;1:95-102.
5. DeFronzo RA. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. Diabetologia. 2010;53(7):1270-87.
6. Aguilar-Salinas CA, Rojas R, Gomez-Perez FJ, et al. The metabolic syndrome: a concept hard to define. Arch Med Res. 2005;36(3):223-31.
7. Clark P, Lavielle P, Franco-Marina F, et al. Incidence rates and life-time risk of hip fractures in Mexicans over 50 years of age: a population-based study. Osteoporos Int. 2005;16(12):2025-30.
8. Clark P, Carlos F, Barrera C, et al. Direct costs of osteoporosis and hip fracture: an analysis for the Mexican healthcare system. Osteoporos Int. 2008;19(3):269-76.
9. Lazcano-Ponce E, Tamayo J, Díaz R, et al. Tendencias de correlación para la densidad mineral ósea en mujeres mexicanas: pruebas de predisposición familiar. Salud Pública Mex. 2009;51 Supl 1:S93-9.
10. Rosen CJ. Bone remodeling, energy metabolism, and the molecular clock. Cell Metab. 2008;7:7-10.
11. Rosen CJ, Klibanski A. Bone, fat, and body composition: evolving concepts in the pathogenesis of osteoporosis. Am J Med. 2009;122(5): 409-14.
12. Zillikens MC, Uitterlinden AG, van Leeuwen JP, et al. The role of body mass index, insulin, and adiponectin in the relation between fat distribution and bone mineral density. Calcif Tissue Int. 2010;86(2):116-25.
13. Boyanov M, Bakalov D, Boneva Z. Bone mineral density in men with and without the metabolic syndrome. Aging Male. 2009;12(2-3):62-5.
14. Schwartz AV, Sellmeyer DE, Ensrud KE, et al. Older women with diabetes have an increased risk of fractures: a prospective study. J Clin Endocrinol Metab. 1997;86:32-8.
15. Ma L, Oei L, Jiang L, et al. Association between bone mineral density and type 2 diabetes mellitus: a meta-analysis of observational studies. Eur J Epidemiol. 2012;27(5):319-32.
16. Rosen CJ, Bouxsein ML. Mechanisms of disease: is osteoporosis the obesity of bone? Nat Clin Pract Rheumatol. 2006;2(1):35-43.
17. Nava-González EJ, de la Garza-Casas YE, Salazar-Montalvo RG, et al. Relationship among anthropometric and gluco-metabolic parameters, bone mineral density and endometriosis. Rev Med Inst Mex Seguro Soc. 2013;51(5):522-31.
18. Rebbeck TR. The contribution of inherited genotype to breast cancer. Breast Cancer Res. 2002;4(3):85-9.
19. Kolassa JE. A Comparison of Size and Power Calculations for the Wilcoxon Statistic for Ordered Categorical Data. Stat Med. 1995;14:1577-81.
20. Ralston SH. Genetic control of susceptibility to osteoporosis. J Clin Endocrinol Metab.
21. Gregory RS, Handelsman Y, Pezalla EJ, et al. Practical perspectives on the management of overweight and obesity. Am J Manag Care. 2014;20(3 Suppl):S64-75.
22. Janssen I, Katzmarzyk PT, Ross R. Body mass index, waist circumference, and health risk: evidence in support of current National Institutes of Health guidelines. Arch Intern Med. 2002;162(18):2074-9.
23. Kanis JA, Melton LJ III, Christiansen C, et al. The diagnosis of osteoporosis. J Bone Miner Res. 1994;9:1137-41.
24. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33:S62-9.
25. Lee JM, Okumura MJ, Davis MM, et al. Prevalence and determinants of insulin resistance among U.S. adolescents. Diabetes Care. 2006;29(11):2427-32.
26. Corica F, Allegra A, CorsonelloA, et al. Relationship between plasma leptin levels and the tumor necrosis factor-alpha system in obese subjects. Int J Obes. 1999;23:355-60.
27. Delmas PD, Eastell R, Garnero P, et al. The use of biochemical markers of bone turnover in osteoporosis. Committee of Scientific Advisors of the International Osteoporosis Foundation. Osteoporos Int. 2000;11 (Suppl 6):S2-17.
28. Warming L, Hassager C, Christiansen C. Changes in bone mineral density with age in men and women: a longitudinal study. Osteoporos Int. 2002;13(2):105-12.
29. Seidell JC, Flegal KM. Assessing obesity: classification and epidemiology. Br Med Bull. 1997;53(2):238-52.
30. Okorodudu DO, Jumean MF, Montori VM, et al. Diagnostic performance of body mass index to identify obesity as defined by body adiposity: a systematic review and meta- analysis. Int J Obes (Lond). 2010;34(5):791-9.
31. Hsu YH, Venners SA, Terwedow HA, et al. Relation of body composition, fat mass, and serum lipids to osteoporotic fractures and bone mineral density in Chinese men and women. Am J Clin Nutr. 2006;83:146-54.
32. Bhupathiraju SN, Dawson-Hughes B, Hannan MT, et al. Centrally located body fat is associated with lower bone mineral density in older Puerto Rican adults. Am J Clin Nutr. 2011;94:1063-70.
33. Salvador Figueras M, Gargallo P. Análisis Exploratorio de Datos. Disponible en: 5campus.com, Estadística <http://www.5campus.com/leccion/aed, 2003.
34. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;69:89-95.
35. Tunenari T, Tsutsumi M, Ohno K, et al. Age- and gender-related changes in body composition in Japanese subjects. J Bone Miner Res. 1993;8(4):397-402.
36. Ohmura A, Kushida K, Yamazaki K, et al. Bone density and body composition in Japanese women. Calcif Tissue Int. 1997;61:117-22.
37. Takada H, Washino K, Iwata H. Risk factor for low bone mineral density among females: the effect of lean body mass. Prev Med. 1997;26:633-8.
38. Kim H, Tanaka K, Amagai H, et al. Age-related changes of body composition by dual- energy X-ray absorptiometry in Japanese men and women (in Japanese). Environ Health Prev Med. 2009;14:46-51.