Giner M[1,2], Montoya MJ[3], Miranda C[1], Vázquez MA[3], Miranda MJ[1], Pérez-Cano R[1,3]
1 Unidad de Osteoporosis - Servicio Medicina Interna - Hospital Universitario Virgen Macarena - Sevilla (España)
2 Departamento de Citología Normal y Patológica - Facultad de Medicina - Universidad de Sevilla - Sevilla (España)
3 Departamento de Medicina - Facultad de Medicina - Universidad de Sevilla - Sevilla (España)

Influence of obesity on microarchitecture and biomechanical properties in patients with hip fracture

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Correspondence: Mercedes Giner - Avda. Dr. Fedriani, s/n - 41009 Sevilla (Spain)
e-mail: merce_giner@yahoo.es

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Summary

Introduction: Obesity and osteoporosis (OP) are two very prevalent diseases in our society today. The effect of obesity on bone quality is currently a subject under discussion.
Objective: To assess the effect of body weight on the microstructure and biomechanical properties of trabecular bone biopsies from the proximal end of the femur in patients with hip fracture fragility.
Material and Methods: Cross-sectional study of 16 patients with hip fracture. The 2 groups are divided according to their BMI: (A) normal weight individuals and (B) those with obesity. We collected biopsies of cancellous bone from the femoral head and assessed biochemical determinations (PTH, 25 (OH) vitamin D and IGF-1), bone remodeling markers (PINP, CTX), bone mass (BMD neck and total hip), bone microstructure and biomechanical study (μCt). Statistical analysis: Student's t test (SPSS 22.0) significance $p<0.05$.
Results: All patients had hip BMD in osteoporotic range. The obese group had higher levels of PTH and lower IGF-1, vitamin D and PINP. We found no differences in the parameters related to bone metabolism. The obese group showed better indices reaching microstructural significance: increased bone volume (BV/TV: 36.6±12.7 vs 19.4±11.4%, BS/TV: 5.5±1.1 vs 3.9±1.3%), higher trabecular number (Tb.N: 1.6±0.4 vs 1,01±0,4), greater trabecular width (Tb.Th: 0.22±0.003 vs 0.17±0.05) and lower trabecular separation (Tb.Sp: 0.51±0.12 vs 0.66±0.16). Biomechanical parameters confirm greater strength of trabecular bone in obese patients.
Conclusion: Obesity may be a protective factor of bone quality in the femoral region and has less effect on bone mineral density.

Key words: *osteoporosis, obesity, microstructure trabecular bone, biomechanics, bone mineral density, bone turnover markers.*

Introduction

Obesity and osteoporosis (OP) are diseases that have presented in epidemic form in recent decades. Both are of multifactorial etiology and chronic. They have a significant rate of morbimortality in developed countries[1,2]. The relationship between them has been studied extensively from different points of view, including epidemiological, clinical and basic research, and different links have been proposed, such as: 1) both are influenced by genetic and environmental factors, or the interaction between both; 2) aging is associated, with a high incidence of bone loss and adiposity in the bone marrow; 3) both bone remodeling and obesity are regulated by a complex of adipocytokines and hormones; 4) physical activity improves these two diseases; and 5) adipocytes and osteoblasts are derived from common progenitors[3].

Obesity is determined by an imbalance in which caloric intake exceeds consumption over a prolonged period[3,4] and constitutes a risk factor for hypertension, dyslipidemia, diabetes mellitus, cardiovascular disease and some forms of tumors[5].

Osteoporosis is a metabolic bone disease characterized by decreased bone strength due to a reduction in bone quantity and/or quality, which leads to an increased risk of spontaneous and traumatic fractures[6].

Traditionally, obesity has been considered a protective factor of bone loss and osteoporosis, because of the positive relationship between body weight, or body mass index (BMI), with bone mineral density (BMD), which is one of the main determinants of osteoporotic fracture risk[7]. This beneficial effect of body weight on BMD has been mainly related to increased bone formation due to mechanical loading, as well as the contribution of hormones derived from fatty tissue and its action on bone metabolism[6,8]. It has been shown that the incidence of hip fractures is decreased in subjects with a high BMI[9]. Likewise, low BMI (below 18 kg/m^2) reportedly presents a risk factor for osteoporotic fracture[10]. More recent evidence, however, indicates that while overweight (BMI between 25-29 kg/m^2) protects against OP, obesity (BMI >30) interferes with bone health[11]. Thus, fractures in children have been associated with alterations in body composition, such as an increase in adiposity and bone structure[12] and a risk of osteoporosis and non-vertebral fractures has been reported in subjects with the highest proportion of body fat, regardless of weight[11]. This has led researchers to suggest that the relationship between body mass index and obesity and the risk of frailty fracture and BMD is complex. A meta-analysis published in 2014 showed that more than 80% of osteoporosis fractures (including the hip) were found in women with a BMI <30 kg/m^2 [13] and in obese women, a higher prevalence of fractures of the proximal humerus and ankle[14,15].

Changes in parameters related to bone metabolism, such as insufficient levels of vitamin D and elevated PTH, have also been reported in obesity, along with markers of bone reshaping of formation and resorption[16], which points once again to a negative effect of fatty tissue on the bone. In addition, energy metabolism is closely linked to the osteoblastic response to insulin regulating homeostasis and bone remodeling. In stages of normaglycemia, insulin stimulates osteoblastogenesis and the production of RANKL inducing an increase in bone turnover. On the other hand, the release to the environment of decarboxylated osteocalcin regulates insulin production in an endocrine manner[17].

The effect of obesity on bone quality is currently under debate and very few studies have evaluated the microstructure and properties of bone in this condition. Thus, an inverse relationship between fat mass and the Trabecular Bone Score (TBS), assessed in the lumbar spine, was observed in premenopausal women with obesity, whereas there was no relationship with the structural analysis of the hip (HSA)[18]. A reduction of cortical bone has also been reported, due to a greater porosity, and yet an increase in volumetric trabecular BMD analyzed by peripheral computed tomography[19] and a negative correlation of cortical bone properties, resistance indexes (by microindentation), with BMI and total fat mass[20].

Our aim was to assess the effect of body weight on the microstructure and biomechanical properties of trabecular bone from femoral proximal extremity biopsies of obese patients versus subjects with normal weight who present fragility hip fracture.

Material and methods

1. Study design and subjects included

The present research is an experimental, analytical and transversal study of cases and controls. It was approved by the Virgen Macarena University Hospital Ethics Committee and informed, written consent was obtained from all participants. All included patients agreed to donate their bone samples for the study.

Patients have been included randomly. They entered our hospital's Unit of Clinical Management of Traumatology to be operated on for hip arthroplasty due to fracture or osteoporosis due to fragility or fall in height below that of the individual without acceleration mechanism, all were over 65 years of age. We included 16 patients divided into two groups according to BMI: 7 subjects formed group A with BMI <25, and 9 had group B with BMI >30.

We excluded patients taking medication with influence on bone metabolism (antiresorptive, osteoformers, corticoids and anticonvulsants) and/or vitamin D or calcium supplementation. The clinical history, densitometric, istomorphometric and biomechanical determinations were carried out.

2. Clinical data

A medical history was carried out that included data related to parentage; birthdate; anthropometric measures: weight (kg); height (cm) and body mass index (BMI) (weight in kg/height in m^2). History of osteoporosis and previous fractures were also included.

3. Biochemical determinations

Biochemical determinations, based on the serum samples, performed at the Biochemistry Service of the Virgen Macarena University Hospital (Seville).

Parameters included were: glucose, glycosylated hemoglobin (HbA1c), calcium, phosphorus, insulin-like growth factor 1 (IGF-I), total alkaline phosphatase (FAT), carboxyl terminal telopeptide of type I collagen (β-CrossLaps) and amino-terminal propeptide of collagen type I (P1NP) in autoantibody ADVIA 2400 (Siemens). Vitamin D [25 (OH) D_3] and PTH were determined by chemiluminescent immunoassay (CLIA) on the CP ADVIA Centaur Immunoassay (Siemens).

4. Assessment of bone mass

We measured bone mineral density (BMD) of the lumbar spine (L2-L4) and hip (total hip and femoral neck) by Dual Absorptiometry X-Ray (DXA, Hologic-Discovery, Hologic Inc., Waltham, Massachusetts, USA). The CV *in vivo* was 1.40% (L2-L4 column), 2.9% (femoral neck) and 2.5% (total hip).

5. Bone histomorphometry and biomechanical study

Microstructural analysis of the biopsies was carried out using computerized microtomography (micro-CT), with SkyScan 1172, 100 kV, 1.3 MPixels. The entire sample was scanned to reconstruct the images and used for the quantitative and qualitative analysis of the trabecular bone microstructure. To analyze this microstructure, microtomography equipment software (CTAn 1.7.0.5) was used. The quantitative variables were: bone volume fraction (BV/TV), specific bone surface (BS/BV), bone surface density (BS/TV), trabecular thickness (Tb.Th), trabecular number), Trabecular pattern factor (Tb.Sp), trabecular pattern factor (Tb.Sp), trabecular pattern factor (Tb.Sp), anisotropy degree (DA), and structure-index structures.

The samples were subjected to a mechanical mono-axial compression test until rupture so as to evaluate the elastic-plastic mechanical properties of the biopsy (Microtest EM1/10/FR/m) at a constant loading speed and using a load cell of 1 kN or 10 kN, once the force-displacement curve was obtained Young's elastic modulus (E), the hardness (u), the maximum supported voltage (σ), the maximum force reached (F), the stiffness (S) and the energy required to fracture (U).

6. Statistical Analysis

The variables were analyzed for normal distribution by the Kolmogorov-Smirnov test. Student's t-test was performed to determine statistically significant differences between the two groups.

The SPSS version 22.0 package for Windows (IBM Corp., Armonk, New York, USA) was used for the statistical management of results. In all cases, the level significance was considered as $p < 0.05$. Data are expressed as mean ± SD.

Results

The anthropometric characteristics and BMD of the hip, femoral neck and spine are shown in Table 1. Both groups were similar in age, weight and lifestyle. Absolutely expressed BMD values and T-score of hip, femoral neck and spine were lower in the normal weight group, obtaining the greatest difference in hip T-score of -2.87±0,84 in subjects with normal weight and -1.67±1.07 in subjects with obesity although these differences were not statistically significant.

The FRAX® 10-year risk of major fracture and hip fracture was lower in obese patients than in patients with a BMI <25 kg/m², although it was not statistically significant.

The biochemical analysis of parameters related to bone metabolism is shown in Table 2. No differences were observed between the two study groups. It should be noted that vitamin D levels were found below 20 ng/mL in almost all patients studied independent of patients' BMI.

Microstructural indices show differences in the microarchitecture of spongy bone between both groups (Figure 1). The group of obese subjects presented higher BV/TV (p=0.015), BS/TV (p=0.015), Tb.Th (p=0.04) and Tb.N (p=0.007). In addition, they have less trabecular separation Tb.Sp (p=0.038) and lower values of Tb.Pf (p=0.015) and SMI (p=0.012). Indicating all this a better bone microstructure in the obese osteoporotic subjects compared to those who presented normal weight.

The biomechanical parameters studied (Figure 2) confirm a higher resistance of the trabecular bone in obese patients compared to subjects with normal weight. The obese group showed a greater rigidity, both in the stiffness due to the structural characteristics (p=0.029), and due to bone material properties: Young's modulus (p=0.01), maximum tension (p=0.036) and maximum force reached (p=0.034). In addition, the energy required to fracture the obese osteoporotic bone is twice that in subjects with normal weight, although this difference did not reach statistical significance.

Discussion

The effect of obesity on bone tissue is still unclear. Although it is known that obese women have reduced the volume of cortical bone and increased the volume of the trabecular bone[19], there are little data on the repercussion in the microstructure and/or biomechanics of the bone of obese people.

Our results indicate a positive effect of body weight on parameters of microarchitecture and biomechanics in trabecular bone. Although both study groups have presented hip fracture, with similar BMD values, bone quality characteristics are better in the obese group than in normal weight individuals.

The micro-CT study of the trabecular bone biopsies of the femoral head from obese fractured patients indicates that they present a greater amount of bone, in relation to the total body volume, greater bone density and greater number of trabeculae, and that these are wider. At the

same time, we also noted that the trabeculae have less separation between them. All these microstructural values correlate with the values obtained in the biomechanical studies in which we observe how the trabecular bone of the obese patients present a greater rigidity and a greater model of Young than the group of subjects with a BMI <25 kg/m^2.

We consider that this effect on the microarchitecture may be due to two fundamental facts: hormonal and/or mechanical factors. At the hormonal level, the increase in the aromatization of estrogens to androgens in adipose tissue leads to a decrease in sex hormones bound to globulin, a greater transformation of the adrenal hormones to peripheral estrones and hyperinsulinemia, which has a mitogenic effect on osteoblasts[21]. In addition, these patients, when carrying greater weight, have a greater mechanical activity on bone that may also stimulate osteogenesis[19,21-23].

The positive effect of body weight on bone tissue also leads us to question whether fatty tissue, muscle or both, to a greater or lesser extent, are responsible for these results. If it is the greater amount of fat or muscle tissue that is responsible for this skeletal beneficial effect, we cannot be sure, as we do not have data on hormone composition or serum levels of adipokines and myokines for these subjects.

Our results do not agree with those of other authors that indicate a worse bone microstructure of the femur in obese subjects[24]. However, these authors do not present results of bone biopsies but the microstructural values are evaluated by DXA. Recently, Shen et al., in a similar study, have not found such differences concluding that the adipose tissue can interfere in the values obtained since by DXA, the soft tissues that surround the bone, can give an erroneous reading in the measurement of the area Bone and therefore the amount of bone mineral content[25,26].

Obesity is associated with increased bone mass and a reduced risk of hip fractures. However, other fractures such as those of the ankle or humerus have a higher incidence in obese persons[27]. In our case, BMD was comparable in both study groups, the likelihood of fracture according to FRAX® was also similar between subjects with normal weight and obese, but we must consider that in all our hip fracture patients, there was no higher incidence of previous fractures in obese people than those of normal weight.

Our obese patients have slight increases in PTH levels and lower vitamin D and P1NP levels. In healthy post-menopausal women, PTH values correlate positively with BMI[19] and low levels of vitamin D are described. These lower levels of vitamin D have been attributed to a greater absorption of this hormone by the adipocytes[28] and to lower solar exposure, due to the more limited mobility of obese subjects[29]. On the other hand, it has been reported that obese patients tend to have less bone remodeling activity. It is unknown whether this is due to the effect of other diseases associated with obesity such as type 2 diabetes mellitus, among others, and/or by the effect of adipokines and myokines on remodeling[21,29].

Table 1. Anthropometric characteristics, FRAX® and BMD (mean ± standard deviation)

	Normal weight	Obese	p
Gender (♂/♀)	3/4	2/7	
Age (years)	78±9	79±7	
Height (cm)	157±9	153±8	
Weight (kg)	55.07±9.7	81.53±12.4	
BMI (kg/m^2)	22.1±2.4	33.8±3.6	0.000
10 year risk of major fracture (FRAX®)	16.5±9.8	13.8±12.0	
10 year risk of hip fracture (FRAX®)	11.8±8.3	7.6±9.8	
BMD femur neck (gHA/cm^2)	0.456±0.16	0.52±0.09	
BMD hip (gHA/cm^2)	0.589±0.10	0.759±0.17	
T-score neck	-3.47±1.36	-3.02±0.64	
T-score hip	-2.87±0.84	-1.67±1.07	
BMD column (gHA/cm^2)	0.81±0.05	0.86±0.85	
T-score column	-2.57±0.67	-2.17±0.64	

Table 2. Biochemical values (mean ± SD)

	Normal weight	Obese
Glucose (mg/dL)	98.33±33.5	129.5±32.5
HbA1c (%)	5.7±1.4	6.0±0.8
IGF-1 (ng/mL)	75.16±42.7	42.37±12.51
PTH (pg/mL)	46.5±32.3	76.4±46.7
β-CrossLaps (µg/mL)	0.8±0.5	0.7±0.3
P1NP (ng/mL)	89.4±57.5	54.1±36.5
Calcium (mg/dL)	9.6±0.5	9.1±0.1
Phosphorus (mg/dL)	3.6±1.1	2.9±0.4
Total alkaline phosphatase (U/L)	229.8±28.2	211.37±67.5
25-hydroxivitamin D (ng/mL)	12.9±9.1	9.3±4.6

Our study has several limitations: the main one is the sample size, which is relatively small, but the data obtained from microstructure and biomechanics, which were the objectives of our study, are quite forceful and statistically strong. We do not have serum levels of adipokines and hormones derived from the fatty tissue in order to relate them to the microstructural and biomechanical parameters.

In conclusion, we can say that measurements of trabecular bone biopsies from the femoral head indicate that obese patients have better biomechanical properties and better bone microarchitecture than patients with normal weight, showing a beneficial effect of body weight on bone quality.

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Conflict of interests: The authors declare that there is no conflict of interests regarding this research paper.

Ethics: This study was approved by the Institutional Ethics Committee (HUV Macarena, Seville, Spain) and informed consent was obtained from each participant.

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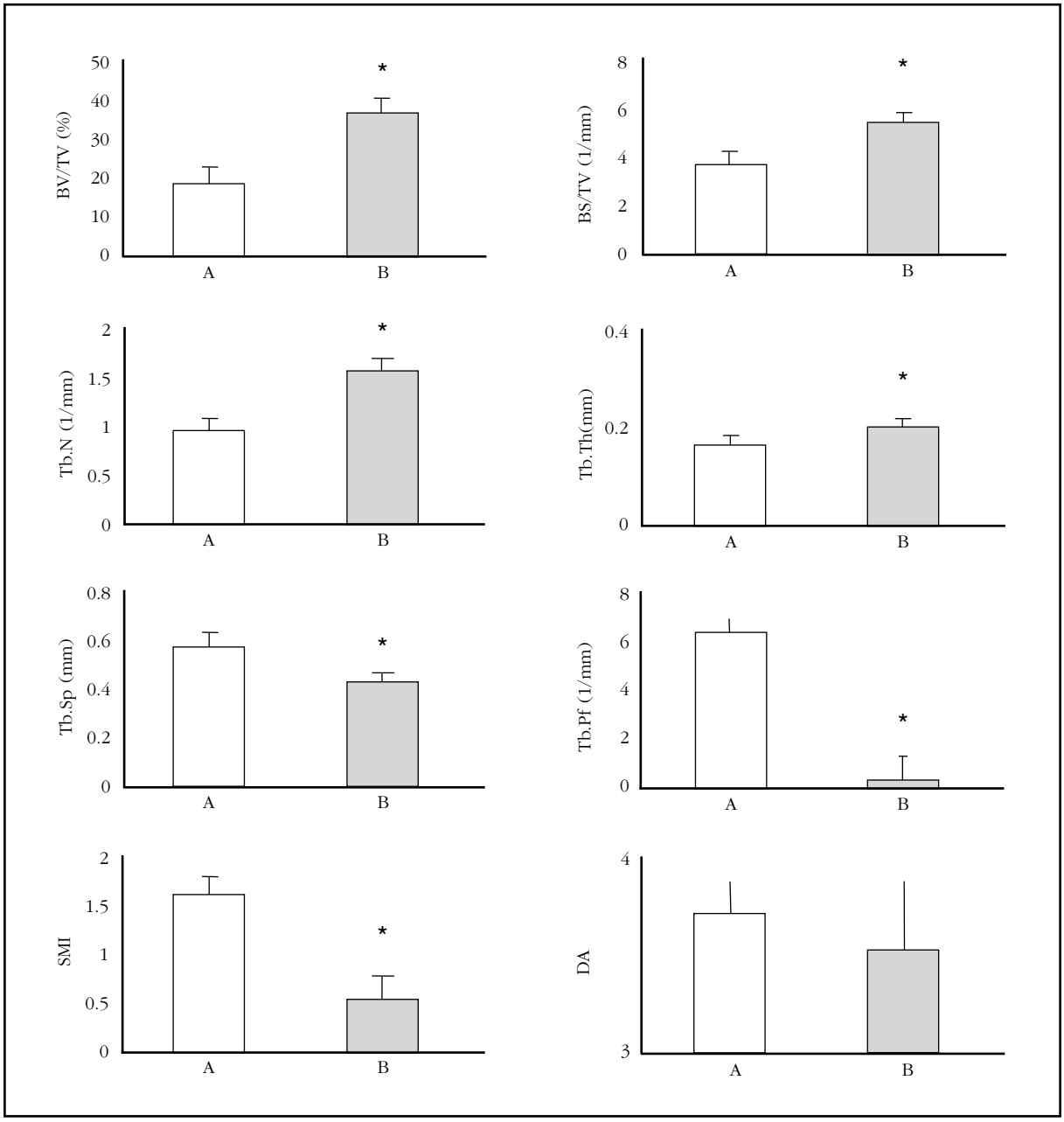
Figure 1. Comparison of structural parameters between patients with normal weight (A) and obese (B)



BV/TV: bone volume fraction; BS/BV: bone specific surface; BS/TV: bone surface density; Tb.Th: trabecular thickness; Tb.N: trabecular number; Tb.Sp: trabecular separation; DA: degree of anisotropy; SMI: Structural model index; Tb.Pf: trabecular connectivity.
Values are expressed as mean ± SD. *statistically significant values.

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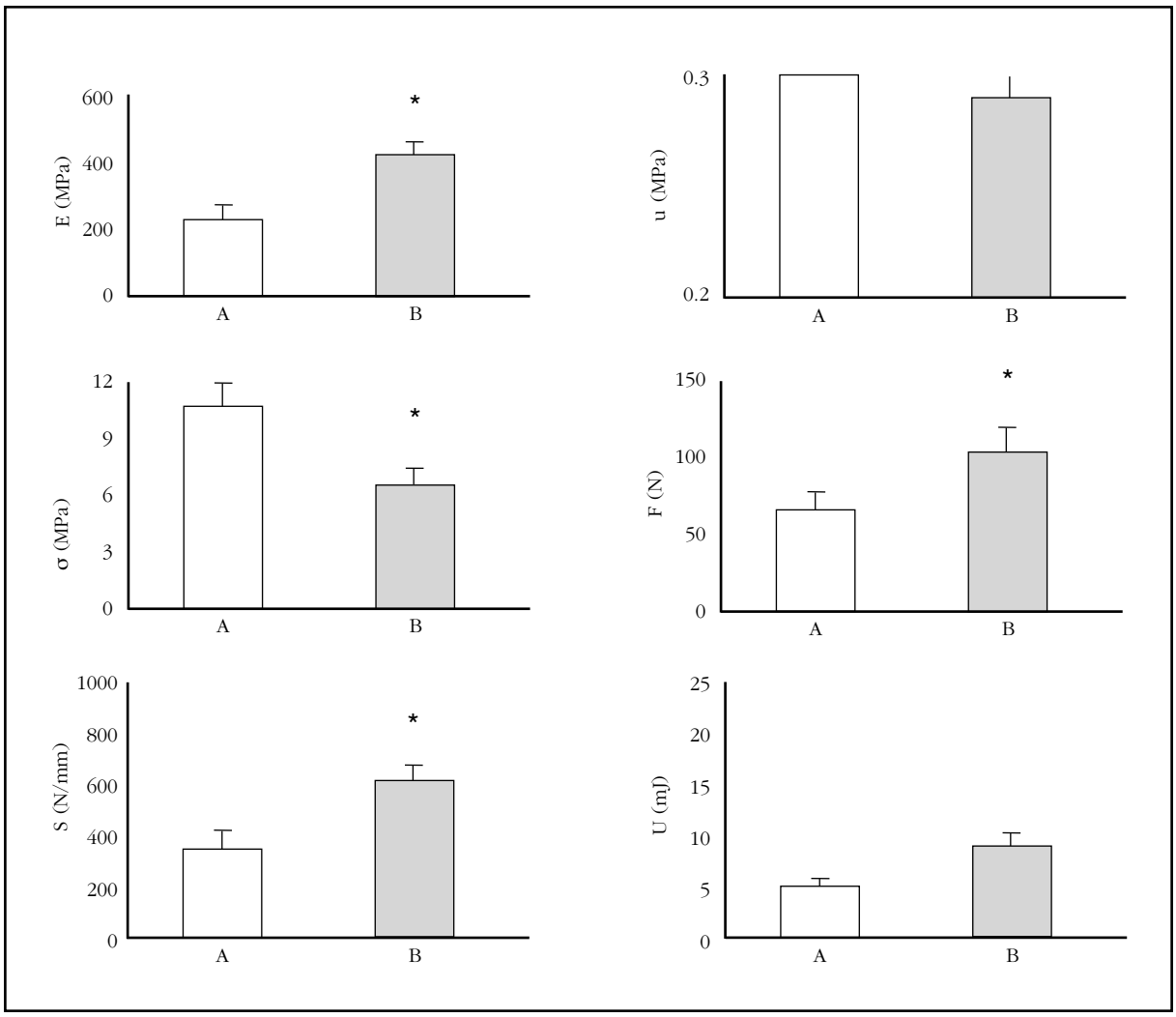
Figure 2. Comparison of biomechanical parameters between the two study groups: normal weight subjects (A) and obese (B)



The parameters represented are: Young's elastic modulus (E); hardness (u); maximum supported voltage (σ); maximum force reached (F); stiffness (S); energy needed to fracture (U).

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