Low Bone Mineral Density and Its Predictors in Type 1 Diabetic Patients Evaluated by the Classic Statistics and Artificial Neural Network Analysis

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OBJECTIVE—To investigate factors associated with bone mineral density (BMD) in type 1 diabetes by classic statistic and artificial neural networks.

RESEARCH DESIGN AND METHODS—A total of 175 eugonadal type 1 diabetic patients (age 32.8 ± 8.4 years) and 151 age- and BMI-matched control subjects (age 32.6 ± 4.5 years) were studied. In all subjects, BMI and BMD (as Z score) at the lumbar spine (LS-BMD) and femur (F-BMD) were measured. Daily insulin dose (DID), age at diagnosis, presence of complications, creatinine clearance (ClCr), and HbA_{1c} were determined.

RESULTS—LS- and F-BMD levels were lower in patients (-0.11 ± 1.2 and -0.32 ± 1.4, respectively) than in control subjects (0.59 ± 1, $P < 0.0001$, and 0.63 ± 1, $P < 0.0001$, respectively). LS-BMD was independently associated with BMI and DID, whereas F-BMD was associated with BMI and ClCr. The cutoffs for predicting low BMD were as follows: BMI <23.5 kg/m^2, DID >0.67 units/kg, and ClCr <88.8 mL/min. The presence of all of these risk factors had a positive predictive value, and their absence had a negative predictive value for low BMD of 62.9 and 84.2%, respectively. Data were also analyzed using the TWIST system in combination with supervised artificial neural networks and a semantic connectivity map. The TWIST system selected 11 and 12 variables for F-BMD and LS-BMD prediction, which discriminated between high and low BMD with 67 and 66% accuracy, respectively. The connectivity map showed that low BMD at both sites was indirectly connected with HbA_{1c} through chronic diabetes complications.

CONCLUSIONS—In type 1 diabetes, low BMD is associated with low BMI and low ClCr and high DID. Chronic complications negatively influence BMD.

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S everal studies reported a reduction of bone mineral density (BMD) in patients with type 1 diabetes (1). Potential pathogenic mechanisms of type 1 diabetes–related bone damage may include hyperglycemia (2), autoimmune inflammation (3), increased marrow adiposity through increased peroxisome proliferator–activated receptor-γ activity (4,5), hypoinsulinemia and hypoamylinemia (6), a deficit of IGF-I (7) and vitamin D (8), and the nonenzymatic glycosylation of type 1 collagen with subsequent formation of advanced glycation end products (9).

In some studies, the early-onset and long duration of the disease has played a role in the type 1 diabetes–associated bone loss (10–14), whereas in other studies, chronic diabetes complications predicted the high risk of fractures in type 1 diabetic patients (12–14). These discordances are probably due to the nonhomogeneous samples studied (i.e., evaluating eugonadal and hypogonadal together). Moreover, osteoporosis is a multifactorial disease in which different factors and environments interact in nonlinear biological mechanisms, which probably need a special mathematical approach, such as artificial neural networks (ANNs), to be understood.

Because ANNs are artificial adaptive systems, able to modify their internal structure in relation to a function objective, they are particularly suited for solving nonlinear problems. The ability to learn in an adaptive way (i.e., extracting from the available data the information needed to gather a specific task and to generalize the acquired knowledge) makes the ANN model a powerful tool for data analysis (15). Although ANNs have been shown to improve the predictive value of classic statistics in many areas of medicine (16), no studies have investigated the ability of ANNs in predicting low bone mass in type 1 diabetes.

The aim of the current study was to investigate, in a homogeneous sample of type 1 diabetic patients, the factors associated with low BMD by using ANNs and the classic statistic approach.

RESEARCH DESIGN AND METHODS

Patients

This cross-sectional study was performed in the Republic Clinical Hospital of Medical Rehabilitation (Minsk, Belarus) and the First Minsk City Clinical Hospital (Minsk, Belarus). From 2001 to 2007, 300 consecutive type 1 diabetic patients were evaluated. A total of 175 patients between the ages of 18 and 53 years were enrolled. The exclusion criteria were as follows: presence of severe chronic kidney disease; history of diseases and syndromes, other than type 1

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diabetes, associated with low BMD (i.e., hyperthyroidism, hyperparathyroidism, intestinal malabsorption, and inflammatory bowel disease); intake of drugs that influence bone metabolism, pregnancy, and lactation period; and menopause or menstrual abnormalities. A total of 151 age-, sex-, and BMI-matched healthy subjects served as control subjects.

Methods
In all patients, height, weight, BMI, the presence and the degree of diabetes complications (neuropathy, retinopathy, and nephropathy), HbA_{1c}, and BMD by dual-energy X-ray absorptiometry were assessed.

Diabetic neuropathy evaluation was based on symptoms, quantitative sensory testing (temperature, vibration, and pressure perception), and quantitative motor testing (patellar and ankle reflexes). On the basis of fundoscopic examination, diabetic retinopathy was categorized as nonproliferative, preproliferative, and proliferative (17). Diabetic nephropathy was assessed by measuring microalbumin in 24-h urine (normal values <30 mg/day) at the enrollment and after 3–6 months. Microalbuminuria and macroalbuminuria were diagnosed if albumin excretion rate was between 30 and 300 or >300 mg/day, respectively (17).

Blood samples were taken in the morning after a 10-h fast before the insulin injection, centrifuged, and stored at −70°C until analysis. HbA_{1c} and serum creatinine were measured by high-performance liquid chromatography with a D10 autoanalyzer (Bio-Rad, Hercules, CA) and by a HITACHI 911 analyzer (Roche Diagnostics, Mannheim, Germany), respectively. Creatinine clearance (ClCr) was calculated with the Cockcroft-Gault equation (17). BMD was assessed by dual-energy X-ray absorptiometry (Sophos L-XRA; Sopha Medical, Buc, France) at the spine (L_2–L_4) and at the femur (coefficient of variation for both <1.5%) and was expressed as SD units (Z values) in relation to the age-matched reference population.

Statistical analysis
Classic statistics.
Statistical analysis was performed by the SPSS version 17.0 statistical package (SPSS, Chicago, IL). The results are expressed as means ± SD. Categorical and continuous variables were compared by the χ^2 test and one-way ANOVA or Bonferroni test (as appropriate), respectively.

The associations between variables were tested by either Pearson or Spearman correlation, as appropriate. Multivariate linear regression analysis assessed the association between lumbar spine (LS-BMD) and femur (F-BMD) BMD (expressed as Z score, dependent variable) and the following independent variables: BMI, age at diabetes diagnosis, daily insulin dose (DID), HbA_{1c}, ClCr, and the sum of the chronic complications of diabetes. These variables were chosen since they were found to be associated with BMD by bivariate correlations. By receiver operating characteristic (ROC) analysis, the cutoff values of the BMD predictors at any site were established. For each patient, the presence/absence of each predictor and a total predictor risk factor score (RFSc) for low BMD then was calculated by summing the number of the predictors. P values <0.05 were considered significant.

ANN analysis.
We applied supervised ANNs to develop a model able to predict with the maximal possible accuracy two separate targets as follows: low LS- and F-BMD (yes/no). The input variables for each model were as follows: age (years), female/male, height (cm), weight (kg), BMI (kg/m^2), diabetes duration (years), age at diabetes diagnosis (years), DID (units/kg), HbA_{1c} (%), neuropathy (presence/absence), nephropathy (presence/absence), nephropathy grade, serum creatinine ($\mu mol/L$), ClCr (mL/min), retinopathy (presence/absence), and retinopathy (grade).

Data analysis was performed using a resampling system (TWIST by Semeion Research Center). This system consists of an ensemble of two previously described systems, training and testing algorithm and input selection (18), based on a genetic algorithm, the genetic doping algorithm, developed at Semeion Research Center (19) and previously applied in different medical contexts (20). After this processing, the most significant features for the classification were selected, and the training and testing set were created with a function of probability distribution similar to the one that provided the best results in the classification. Two series of supervised multi-layer perceptrons, with four hidden units, were then used for the classification task for each modeling target with a classic training and testing procedure as follows: training on subset A involving approximately half of the records and blind testing on subset B, involving the other half of the records and then training on subset B and testing on subset A. Results are given for each experiment (A vs. B and B vs. A) in terms of sensitivity, specificity, global accuracy, and ROC curves and for the average of cost functions as well. The final ANNs, which were trained and tested on the new dataset generated by the TWIST system, are "virgin" and operate independently and blindly from each other and from the TWIST system.

Semantic connectivity map.
An existing mapping method (21) was used to graphically highlight the most important links among variables, using the Auto Contractive Map algorithm, which is a special kind of ANN able to find the consistent patterns and/or systematic relationships and hidden trends and associations among variables. After the training phase, the weights developed by Auto Contractive Map are proportional to the strength of associations between the variables. The weights are then transformed in physical distances. Variable couples for which connection weights are higher become nearer, and vice versa. A simple mathematical filter represented by a minimal spanning tree is applied to the distances matrix, and a graph is generated. This step allows observation of connection schemes among variables and detecting variables acting as "hubs" as being highly connected. This matrix of connections, as detailed by Buscema and Grossi (21), preserves nonlinear associations among variables and captures connection schemes among clusters.

RESULTS—The general characteristics of diabetic patients and the control group are presented in Table 1. Age, BMI, and distribution of males and females were comparable between patients with diabetes and control subjects. Type 1 diabetic patients had a lower BMD than control subjects at both sites: spine and femur. The prevalence of low LS-BMD and F-BMD (Z score BMD less than or equal to −1.0) was higher in diabetic patients than in control subjects.

Because the spine and femur are constituted by different types of bone (prevalently trabecular and cortical, respectively), we evaluated the impact of type 1 diabetes on the spine and femur separately. All patients with diabetes were divided into groups on the basis of a Z score BMD less than or equal to −1.0 (low BMD) or more than −1.0 at both the spine and femur. Patients with diabetes with low LS-BMD and patients with low F-BMD showed lower BMI (22.7 ± 3.4 and 23.0 ± 3.3 kg/m^2, respectively) and higher DID (0.9 ± 0.3 and 0.8 ± 03 UI/kg, respectively) than their counterparts with normal

Table 1—*Clinical characteristic of type 1 diabetic patients and control subjects*

	Type 1 diabetic patients	Control subjects	*P*
n	175	151	
Age (years)	32.8 ± 8.4 (18–53)	32.6 ± 4.5 (20–45)	0.77
BMI (kg/m^2)	24 ± 3.7 (17.8–37.9)	24.3 ± 3.8 (19.0–40.8)	0.47
Males/females	71/104 (40.6/59.4)	66/85 (43.7/56.3)	0.57
Diabetes duration (years)	13 ± 8.4 (0.5–44)	—	—
Age at diabetes diagnosis (years)	19.8 ± 9.2 (1–44)	—	—
DID (units/kg)	0.7 ± 0.2 (0.1–1.5)	—	—
HbA$_{1c}$ (%)	8.3 ± 1.16 (4.4–12.3)	—	—
ClCr (mL/min)	97.24 ± 31.7 (28.1–223.57)	—	—
LS-BMD (*Z* score)	−0.11 ± 1.2 (−3.49 to 5.04)	0.59 ± 1.0 (−2.2 to 3.6)	<0.0001
Low LS-BMD (*n*)	46 (26.3)	14 (9.3)	<0.0001
F-BMD (*Z* score)	−0.32 ± 1.4 (−3.9 to 6.26)	0.63 ± 1.0 (−2.6 to 3.5)	<0.0001
Low F-BMD (*n*)	58 (33.1)	12 (7.9)	<0.0001
Neuropathy (yes/no)	111/64 (63.4/36.6)	—	—
Retinopathy (yes/no)	91/84 (52/48)	—	—
Nephropathy absent	89 (50.9%)	—	—
AER 30–300 mg/day	56 (32%)	—	—
AER > 300 mg/day	30 (17.1%)	—	—

Data are means ± SD (range) or *n* (%) unless otherwise indicated. *Z* score: SD units (*Z* values) in relation to our age-matched reference population. AER, albumin excretion rate.

BMD (BMI 24.4 ± 3.7 kg/m^2, *P* = 0.005, and 24.5 ± 3.8 kg/m^2, *P* = 0.013, respectively; DID 0.7 ± 0.2 UI/kg, *P* = 0.0001, and 0.7 ± 0.2 UI/kg, *P* = 0.006, respectively). Patients with low F-BMD but not patients with low LS-BMD showed a younger age at diabetes diagnosis (17.3 ± 8.7 years) and lower ClCr (88.1 ± 31.3 mL/min) and tended to have higher HbA$_{1c}$ (8.6 ± 1.1%) than control subjects (age at diabetes diagnosis 21 ± 9.2 years, *P* = 0.013, ClCr 101.8 ± 31.3 mL/min, *P* = 0.007, and HbA$_{1c}$ 8.2 ± 1.2%, *P* = 0.052). Age, prevalence of males and females, duration of diabetes, and prevalence of complications were not different among the different groups.

In the whole group of patients, both the LS- and F-BMD *Z* score was directly associated with age (*r* = 0.32, *P* = 0.001, and *r* = 0.2, *P* = 0.008, respectively), age at diabetes diagnosis (*r* = 0.15, *P* = 0.044, and *r* = 0.27, *P* = 0.001, respectively), and BMI (*r* = 0.3, *P* = 0.001, and *r* = 0.27, *P* = 0.001, respectively) and was inversely associated with DID (*r* = −0.3, *P* = 0.001, and *r* = −0.19, *P* = 0.014, respectively). F-BMD showed a positive correlation also with ClCr (*r* = 0.22, *P* = 0.004) and a negative correlation with HbA$_{1c}$ (*r* = −0.16, *P* = 0.036), which in turn was directly associated with DID (*r* = 0.22, *P* = 0.004). Disease duration was not correlated with LS- and F-BMD.

The presence of each diabetic chronic complication separately taken was not

associated with both LS- and F-BMD. However, in each patient, the sum of the complications was inversely associated with LS-BMD (*r* = −0.158, *P* = 0.036) and F-BMD (*r* = −0.169, *P* = 0.025).

By multivariate regression, the independent associations of the sum of chronic complications, BMI, age at diabetes diagnosis, DID, HbA$_{1c}$, and ClCr with LS- or F-BMD were tested. Both LS- and F-BMD were confirmed to be positively associated with BMI (β = 0.25, 95% CI 0.03–0.13, *P* = 0.002, and β = 0.16, 0.01–0.12, *P* = 0.04, respectively). LS- but not F-BMD was confirmed to be negatively associated with the DID (β = −0.21, 95% CI −1.88 to −0.3, *P* = 0.007), whereas F-BMD but not LS-BMD was positively associated with ClCr (β = 0.18, 0.01–0.01, *P* = 0.019). LS- and F-BMD were not associated with age at diabetes diagnosis, HbA$_{1c}$, and sum of complications.

By ROC analysis, we determined the best cutoff of the statistically significant factors in predicting low (less than or equal to −1.0) LS- or F-BMD. We found that BMI ≤23.5 kg/m^2, DID >0.67 units/kg, and ClCr <88.8 mL/min were the best predictors of low LS- or F-BMD. After that, for each patient, the presence/absence of each risk factor and a total RFSc for low BMD was calculated by summing the number of the risk factors. For each unit of RFSc increase, the risk of having low BMD was twofold increased (odds ratio 2.0, 95% CI

1.43–2.99, *P* = 0.000). In the presence of an RFSc = 0, the probability of having a normal BMD was 84.2% (negative predictive value 84.2%, sensitivity 95.8%, accuracy 48.6%), whereas in the presence of an RFSc = 3, the probability of having a low BMD was 62.9% (positive predictive value 62.9%, specificity 87.4%, accuracy 64%); in the presence of an RFSc ≤1 or ≤2, the negative predictive value, positive predictive value, sensitivity, specificity, and accuracy for predicting low BMD were identical (55.3, 72.2, 53.1, 74, and 62.3%, respectively).

Through the TWIST system, we established a discrete possibility to predict the status of having or not having a low BMD. For both low LS- and F-BMD, the following variables were selected: age, height, weight, and BMI. For low F-BMD, the selected variables were as follows: presence of neuropathy, absence and grade of nephropathy, serum creatinine, ClCr, and absence of retinopathy; for low LS-BMD, the selected variables were both sexes, diabetes duration, DID, absence of neuropathy, presence and grade of nephropathy, and presence of retinopathy.

Sensitivity, specificity, overall accuracy, and ROC values pertinent to F-BMD target and LS-BMD target are reported in Table 2 together with other information relative to experiments performed.

Artificial neural networks were found easier to predict low F-BMD rather than

Table 2—*Predictive accuracy of back propagation ANN in relation to LS-BMD/F-BMD target*

ANN	Records in testing (n)	Low BMD (n)	Normal BMD (n)	Sensitivity (%)	Specificity (%)	Overall accuracy (%)	Area under the curve
Lumbar spine							
FF_Bp 4 BA	101	35	66	74.29	59.09	66.69	0.688
FF_Bp 4 AB	74	23	51	56.52	78.43	67.48	0.631
Mean				65.4	68.76	67.08	0.66
Femur							
FF_Bp 4 BA	88	25	63	48	79.37	63.68	0.595
FF_Bp 4 AB	87	21	66	61.9	74.24	68.07	0.726
Mean				54.95	76.8	65.88	0.66

FF_Bp 4 AB, feed forward back propagation ANN with four hidden units: results obtained in sequence: training on subset A and testing on subset B; FF_Bp 4 BA, feed forward back propagation ANN with four hidden units: results obtained in sequence: training on subset b and testing on subset A.

low LS-BMD class (65.4 vs. 54.95%, respectively; $P < 0.01$) and normal LS-BMD rather than normal F-BMD class (76.8 vs. 68.76% respectively; $P < 0.01$). The overall accuracy rate was not significantly different (area under the curve ROC = 0.66 for both targets). We tried to develop analog-predictive models with stepwise logistic regression, but because of the high nonlinearity and small sample size, the results were disappointing. No consistent model was generated.

Through the connectivity map (Fig. 1), we established the connections between the studied variables and the condition of low F-BMD and LS-BMD. The presence of retinopathy was the principle variable of the whole system that was directly connected with HbA_{1c}, with the presence of nephropathy and neuropathy connected with HbA_{1c} separately. The cluster formed by HbA_{1c} and the presence of diabetes complications then was connected with low LS- and F-BMD. Age at diagnosis, diabetes duration, BMI, or ClCr was not directly connected with low BMD at both sites. On the other hand, age at diagnosis, diabetes duration, BMI, and DID were connected with HbA_{1c} separately.

CONCLUSIONS—In the current study, we found that BMD is reduced in type 1 diabetes. From classic statistics, low trabecular (spine) and cortical (femur) BMDs were associated with high DID and low ClCr, respectively. Low BMI was associated with both low trabecular and cortical BMD. The analyses with ANNs added the presence of diabetes complications, through poor metabolic control, to the list of contributors of low BMD in type 1 diabetes.

Several studies suggested potential mechanisms responsible for low bone mass in type 1 diabetes, such as the inflammatory state, the local levels of insulin-like growth factors, the amylin deficit, and the nonenzymatic glycation of type 1 collagen with subsequent advanced glycation end product formation (6). However, almost all previous studies have relatively small and nonhomogeneous samples (i.e., evaluating eugonadal and hypogonadal subjects together). In addition, because osteoporosis is a multifactorial skeletal disease, the link between BMD and the risk factors may be hardly understandable using the classic statistics.

In this study, the factors associated with low BMD in type 1 diabetes were investigated in a large and homogeneous sample (all eugonadal individuals) using ANNs, which are considered particularly suited for solving nonlinear problems (15).

In the current study, after adjusting for the confounding factors, the age at diabetes diagnosis was not associated with BMD. This result is in contrast to some previous data showing the young onset of diabetes as a risk factor in adult life, possibly because of a low bone mass peak (7,22). This discordance may be
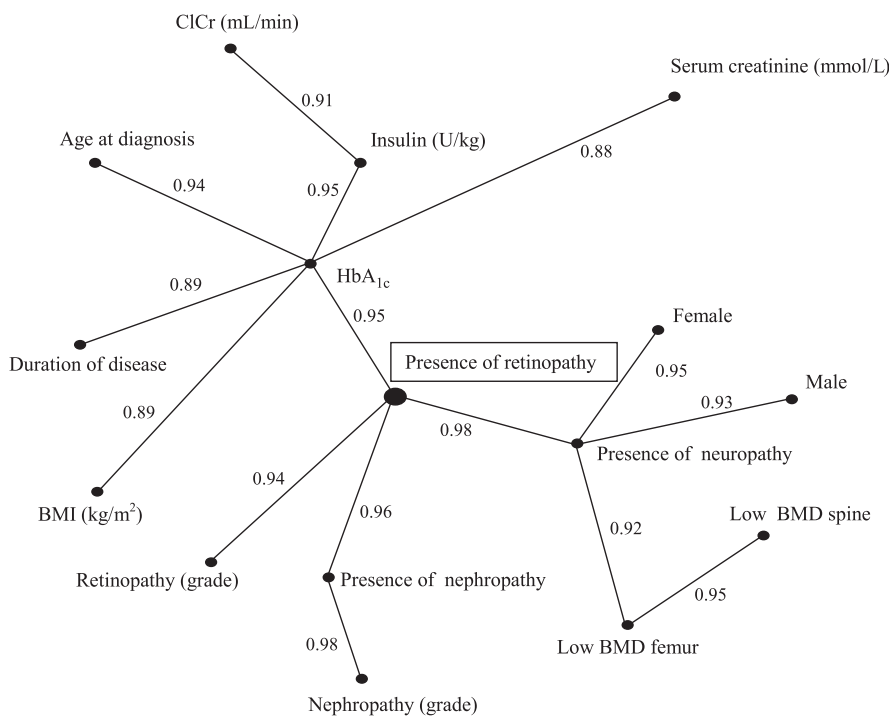

Figure 1—*Semantic connectivity map of studied variables. The figures on the connections represent the strength of the link on a 0–1 scale. Low BMD femur, presence of low F-BMD; Low BMD spine, presence of low LS-BMD. Males and females are both connected to neuropathy but with a different conditional probability. The link for females is stronger (0.95) than it is for males (0.93).*

attributed to the different inclusion criteria of the current study with respect to the previous ones.

On the other hand, we found that both LS- and F-BMD were independently associated with BMI and that patients with low BMD were thinner than individuals with a normal BMD. Because body composition and lean mass may influence bone density, this finding suggests a possible link between BMD and muscle mass in type 1 diabetic patients.

Insulin is considered an anabolic agent for bone (6), and, therefore, one should expect BMD to increase with the increase of DID. On the contrary, in our study, patients with diabetes with low BMD had higher DID, which was independently associated with low LS-BMD. This result is in accordance with previous data, showing that large amounts of insulin had an independent deleterious effect on bone in type 1 diabetes (22). However, in poorly compensated patients with diabetes, the increased levels of inflammation markers and oxidative stress were shown to be inversely associated with BMD (23). The present finding of a correlation between DID and HbA$_{1c}$ suggests that the need of high insulin dose may reflect the presence of a more severe disease (i.e., a more pronounced inflammatory milieu), leading per se to bone damage (6). This hypothesis is supported by the connectivity map of the ANN (Fig. 1), showing that DID was strictly connected with HbA$_{1c}$ and then with low BMD, although through diabetes complications.

In keeping with previous studies (10,11,23,24), HbA$_{1c}$ levels were associated with BMD after adjusting for confounders. However, we measured HbA$_{1c}$ only at enrollment. Because HbA$_{1c}$ levels are representative of the metabolic status of the last 3 months, we could not exclude that, during the previous years in the individual subject, the metabolic status was different and therefore not reliably mirrored by the current HbA$_{1c}$ levels. Indeed, in those studies demonstrating the influence of metabolic control on BMD, HbA$_{1c}$ was also measured during the previous years of disease (10,11,23,24). However, the lack of a correlation between BMD and HbA$_{1c}$ may also depend on a nonlinear relationship between these variables, hardly detectable by the classic statistics. The connectivity map of ANN showed that HbA$_{1c}$ was connected with low BMD through the link with the diabetes complications (Fig. 1).

The chronic diabetes complications per se have been suggested to predict low BMD in type 1 diabetes. The reduced visual function and the presence of diabetic neuropathy may predispose patients to low physical activity, which, in turn, may cause bone loss (12,14). The presence of diabetic nephropathy with negative calcium balance and reduced vitamin D level was reported to be an early indicator of osteopenia in type 1 diabetes (13). However, because complications are the result of the chronic exposure to high blood glucose of target organs, the finding of an association between chronic complications and low BMD may also reflect the effect of chronic hyperglycemia on bone.

Finally, previous data suggested that even a mild impairment of kidney function may lead to bone loss (25), underlying the importance of estimation of ClCr when evaluating patients for fracture risk. A novel finding of the current study is that in type 1 diabetic patients, ClCr levels were independently associated with low F-BMD, showing the importance of mildly reduced kidney function for bone loss at the femur in type 1 diabetes.

Another novelty of our study is that we elaborated a RFSc (composed by ROC analysis–derived cutoffs for BMI, ClCr, and DID for low BMD). In the presence of RFSc = 0, the probability to have a normal bone mass is 84.2%; therefore, measuring BMD may not be necessary. On the contrary, in the presence of an RFSc = 3, the probability to have a low BMD is 62.9%, and the measurement of BMD might be considered, particularly if the chronic complications are present, as shown by ANNs (Fig. 1). The finding that the accuracy for predicting low BMD is similar for the classic statistics (64%) and ANNs (67%) suggests that other variables, not considered in our analyses, may be responsible for low BMD in type 1 diabetes.

The current study had several limitations. First, the cross-sectional design did not allow us to assess causality but only association between variables. Second, we did not measure bone turnover markers, which would have possibly helped us to better understand the mechanism of reduced BMD. Finally, the current study did not investigate the mechanisms for bone damage in type 1 diabetes.

A final comment is inherent to ANNs and their pros and cons. For the most part, most ANNs have few probabilistic underpinnings, unlike their more statistical or Bayesian counterparts in the more general field of machine learning. This result can be an advantage but also a problem, depending on the context. A significant part of using ANNs is the training process. Training is an iterative process, where the data are repeatedly presented to the network, and training incrementally improves the model to match the data more closely. Training is difficult and requires a great deal of technical experience. However, when the underlying relation among the variables on study is complex and there is no reliable a priori statistical model to reference, ANNs can be the ideal solution, since they self-adjust their structure as they learn from their own errors and can simultaneously handle a high number of variables, irrespective of their underlying degree of nonlinearity.

In conclusion, the current study confirms that, in type 1 diabetes, BMD is reduced. Moreover, it shows that low BMI and the presence of chronic diabetes complications are associated with both LS- and F-BMD, whereas high insulin dose and low ClCr are associated with low LS- and F-BMD, respectively.

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