

Arthroplasty in veterans: Analysis of cartilage, bone, serum, and synovial fluid reveals differences and similarities in osteoarthritis with and without comorbid diabetes

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Abstract—Osteoarthritis patients with diabetes who receive total knee arthroplasty are more vulnerable to complications, including aseptic loosening and need for revision surgery. To elucidate mechanisms related to arthroplasty failure in diabetes, we examined serum and synovial fluid markers as well as collagen crosslinks in bone and cartilage of 20 patients (10 with diabetes, 10 controls without) undergoing this procedure. Hemoglobin A1c, body mass index, bone alkaline phosphatase, leptin, osteocalcin, and pyridinium were analyzed along with tissue content of the crosslinks hydroxylslypyridinoline, lysylpyridinoline, and pentosidine. Pentosidine levels in tissue specimens from diabetic subjects were higher than in control subjects. Osteocalcin levels negatively correlated with hydroxylslypyridinoline levels in cartilage. Osteocalcin levels also negatively correlated with pentosidine levels in cartilage, but only in subjects with diabetes. This study suggests potential metabolic mechanisms for arthroplasty failure in patients with diabetes.

Key words: arthroplasty, bone, cartilage, collagen, diabetes, leptin, osteoarthritis, osteocalcin, pentosidine, veterans.

INTRODUCTION

In adults, total knee arthroplasty (TKA) is the second most common surgical procedure performed by orthopedic surgeons. Dramatic improvement in both function and pain relief have made this procedure desirable for many patients with end-stage osteoarthritis (OA). With

the population of adults 65 and older expected to double by 2050, rates for TKA in this country are expected to rise significantly [1]. Long-term follow-up studies have documented the durability of this intervention, with expected implant survival rates of greater than 15 years [2]. Still, 3 percent failure rates are observed each year as a result of noninfectious etiologies such as component loosening [1]. Revision TKA is associated with lower functional outcome and higher complication rates than primary intervention. Significant healthcare costs are associated with this procedure; the average charge in the United States for revision TKA is \$73,696 [1]. Not only

Abbreviations: AGE = advanced glycation end product, BAP = bone alkaline phosphatase, BMI = body mass index, DM = diabetes mellitus, ELISA = enzyme-linked immunosorbent assay, HbA1c = hemoglobin A1c, HFBA = heptafluorobutyric acid, HP = hydroxylslypyridinoline, HPLC = high-performance liquid chromatography, LP = lysylpyridinoline, OA = osteoarthritis, OCN = osteocalcin, PYD = pyridinium, RAGE = receptor for AGE, TKA = total knee arthroplasty, VA = Department of Veterans Affairs.

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is the revision procedure associated with higher costs than a primary TKA, but it is also associated with longer lengths of hospitalization, up to 6.6 days on average. This equates to projected hospital costs exceeding \$2 billion by 2030 [1].

Diabetes mellitus (DM) is one of numerous factors that have been implicated in higher rates for revision arthroplasty [3–6]. The revision rate among those with diabetes is up to 9 times higher in some studies [3,5]. The issue of revision arthroplasty is amplified within the Department of Veterans Affairs (VA) population, where the incidence of DM (20.0%) is higher relative to that of the U.S. general population (5.5%) [7]. Reasons behind DM-related arthroplasty failures include superficial and deep surgical infections [3,8], higher joint stiffness and aseptic loosening rates [2,5,9], and dramatic effects on the musculoskeletal system [10], including alteration of bone mineral density [11].

DM effects on bone mineral density have been attributed to decreased mechanical qualities of the bone matrix rather than bone mass [12–13]. Cadaveric studies have shown that increased concentration of nonenzymatic glycation products (also called advanced glycation end products [AGEs]) within the collagen network of bone correlate with many bone mechanical properties, including ultimate strength, yield strength, and fracture toughness [14]. Nonenzymatic modifications of collagen are also significantly increased in bone from DM animal models [13,15]. Furthermore, the products of these nonenzymatic glycation events, AGEs, increase stiffness and brittleness of articular cartilage [16]. Articular cartilage is therefore more prone to mechanical damage with DM, a problem compounded by the increasing age and body mass index (BMI) of the U.S. and veteran populations. Additionally, one study of rats with DM reports a decrease in the immature forms of a different class of collagen crosslinks that mature to the forms called hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) [13]. HP and LP are created by posttranslational modification resulting in inter- and intramolecular collagen crosslinks [17]. These crosslinks are beneficial in that they stabilize the collagen matrix and, thus, increase tissue mechanical strength [18–19]. Thus, DM could potentially increase harmful crosslinks while decreasing beneficial crosslinks.

Bone remodeling is a continuous process mediated by a constant interaction between osteoblasts and osteoclasts and directly affects bone mechanics. This process is associated with the release of several biochemical markers (“biomarkers”) in the blood stream. Osteocalcin (OCN)

has been demonstrated to be an indicator of bone formation [20–22], and previous studies have shown decreased baseline levels among DM patients [23]. Further, one study analyzing the differences in these levels among patients undergoing TKA showed statistically higher OCN levels at 12 months postoperative in those patients with potentially unstable fixation of the tibial component [24]. Levels of another biomarker, leptin, correlate to obesity. Several studies have demonstrated the effects of leptin on articular cartilage [25–28]. One study in particular found that leptin had a detrimental effect on chondrocyte proliferation and induced production of interleukin-1 beta and metalloproteinases that mediate the destructive process on articular cartilage in OA [27]. Furthermore, leptin has been identified as a potent inhibitor of bone formation via a pathway in the central nervous system [29].

This study explored the hypothesis that diabetes status is related to bone and joint homeostasis in order to better understand the molecular mechanisms affecting orthopedic surgical outcomes among those with comorbid diabetes. Specifically, this study’s goal was to measure and analyze circulating and local biomarkers as well as tissue collagen crosslinks in the following two groups: (1) veterans diagnosed with diabetes and receiving total knee joint replacement (i.e., TKA), and (2) a reference group of veterans not diagnosed with diabetes but also receiving TKA. The ultimate goal is to use new knowledge of the molecular effects of DM status to improve arthroplasty outcomes for this population.

METHODS

Overview

To accomplish our objectives, we used a cross-sectional, prospective design to recruit 20 patients scheduled for TKA at the Denver VA Medical Center to donate a preoperative blood sample and the tissue wastes from their planned surgeries. The blood and tissues were processed in the research laboratory to measure diabetes-, obesity-, and OA-relevant molecules as described subsequently. The differences between groups and the relationships between variables were analyzed.

Subjects

The inclusion criteria were male surgical patient, aged 45 to 80, from the Denver VA Medical Center, and with radiographic and clinical evidence of end-stage OA.

Exclusion criteria were rheumatoid arthritis, osteoporosis on bisphosphonate therapy, Paget disease or other metabolic bone diseases, renal impairment (serum creatinine >1.3), drug therapies including antiretrovirals and anti-convulsants known to influence bone metabolism, and receipt of steroids (either intraarticular or oral) within the last 12 months. Twenty subjects were enrolled: ten patients with a diagnosis of diabetes and ten control patients without this diagnosis. In this report, the two study groups are designated as “DM” or “non-DM,” respectively. Subjects were enrolled consecutively without regard to race/ethnicity. Demographic and clinical data (hemoglobin A1c [HbA1c], albumin, creatinine, electrolytes, and liver function tests) were collected from chart review.

Sample Collection

Venous blood samples were collected from subjects at the time of their preoperative appointment (typically 2 weeks before surgery). After centrifugation, the serum was frozen in aliquots and stored at -80°C for later analysis. All subjects underwent hybrid TKA with press-fit femoral and patellar components and a cemented tibial component.

When the knee joint was opened during the surgery, all synovial fluid was collected without dilution and stored at -80°C for later analysis. From one of the subjects, no synovial fluid was available (“a dry joint”). The data of this subject, therefore, were omitted from statistical analyses using synovial fluid data.

Standard femoral and tibial cuts were made in preparation for arthroplasty. These tissues were placed in labeled sterile bags, passed out of the sterile field, and then held at 4°C for sample dissection (described below) within 1 hour of surgery.

Analysis of Fluid Samples

Biomarkers for obesity (common to type 2 DM) and bone formation/resorption (bone remodeling is necessary for implant fixation following arthroplasty) were analyzed in serum and synovial fluid. Concentrations of leptin, bone alkaline phosphatase (BAP), OCN, and pyridinium (PYD) were measured in samples that had been subjected to only one freeze-thaw cycle. The specific enzyme-linked immunosorbent assays (ELISAs) used were commercially available kits (Quidel Corporation; San Diego, California, catalog numbers: TE1016, 8012, 8002, and 8019, respectively). Manufacturer’s instructions were followed. Sam-

ples were run neat; no sample had concentration values outside the standard curve for any of the four biomarkers. Concentrations were determined from a standard curve of calibrators that were provided in each kit and plotted as recommended (e.g., linear, quadratic). All samples were measured in duplicate. For the PYD assay, samples were filtered before analysis with a 30 kD molecular weight cut-off filter as provided in the kit.

Analysis of Joint Tissue Samples

Tissue samples (5 mm in diameter) were cored from the tibial cuts. One core from the central one-third of the lateral tibial plateau was used for this study. Bone and cartilage were separated and then hydrolyzed in 6 N HCl, 18 h, at 108°C . Diluted and filtered samples were analyzed for levels of the collagen crosslinks pentosidine, HP, and LP by using high-performance liquid chromatography (HPLC) following the collagen crosslink protocol of Bank et al. [30]. Filtered samples were diluted 1:5 in 10 percent acetonitrile and 0.05 percent heptafluorobutyric acid (HFBA) and then loaded to a Gemini-NX C-18 column (Phenomenex; Torrance, California) fitted to a programmable gradient HPLC system (model 126, Beckman-Coulter; Fullerton, California). The autosampler, gradient pump, and data analysis were controlled by 32 Karat Workstation software v. 5.0 (Beckman Coulter, Inc; Brea, California). Samples were separated with 20 min of solvent containing 24 percent methanol and 0.15 percent HFBA, 10 min of solvent containing 40 percent methanol and 0.05 percent HFBA, and 10 min of solvent containing 75 percent acetonitrile and 0.1 percent HFBA. The column was equilibrated in 24 percent methanol and 0.15 percent HFBA for at least 12 min between sample runs. Collagen crosslink peaks were measured with a programmable fluorescence detector (model FP1520, JASCO; Easton, Maryland). The detection wavelengths for HP and LP were 295 nm excitation and 400 nm emission (0–26 min) and those for pentosidine were 328 nm excitation and 378 nm emission (after 26 min). Crosslink concentration was calculated from a standard curve of five dilutions of a calibrator containing all three crosslinks. Purified calibrators HP and LP were purchased from Quidel Corporation, while purified pentosidine was purchased from Dr. L. Sayre, Case Western Reserve University (Cleveland, Ohio).

All crosslink concentrations were normalized to collagen concentration, which was estimated from hydroxyproline concentration as measured by HPLC. To measure hydroxyproline, an aliquot of the above diluted sample

was further diluted 1:50 and then derivatized with 9-fluorenylmethyl chloroformate and separated on the same system as described, following the amino acid protocol of Bank et al. [31]. This protocol used the following three solutions: (1) 20 mM citric acid and 5 mM tetramethylammonium chloride, pH 2.85; (2) 20 mM sodium acetate and 5 mM tetramethylammonium chloride, pH 4.5; and (3) 100 percent acetonitrile. The gradient elution profile was as follows: from 0 to 11.5 min a gradient of 75 percent (1)/25 percent (3) to 60 percent (1)/40 percent (3), at 13 min switch directly to 64 percent (2)/36 percent (3), from 13.1 to 18 min a gradient of 64 percent (2)/36 percent (3) to 62 percent (2)/38 percent (3), from 18 to 25 min a gradient of 62 percent (2)/38 percent (3) to 30 percent (2)/70 percent (3), from 25 to 30 min a gradient of 30 percent (2)/70 percent (3) to 25 percent (2)/75 percent (3), at 32 min a switch directly to 75 percent (1)/25 percent (3) and then end at 45 min. The column was equilibrated in 75 percent (1)/25 percent (3) for at least 12 min between sample runs. Amino acid peaks were measured with the same fluorescence detector using 254 nm excitation and 630 nm emission for the entire protocol. Hydroxyproline concentration was calculated from a standard curve of five dilutions of an amino acid standard made from collagen hydrolysate (Sigma-Aldrich #H1007; St. Louis, Missouri). The conversion of hydroxyproline to collagen concentration is 285 mol hydroxyproline per mol collagen and is based on the constant concentration of hydroxyproline in fibrillar collagen [32].

For interpreting the data, note that HP (measured in cartilage and bone by HPLC) and PYD (measured in serum and synovial fluid by ELISA) are similar molecules but represent different biological entities. HP is the actual collagen crosslink that was a part of the collagen matrix at the time the tissue was obtained. PYD, on the other hand, is the crosslink that has been released from the collagen matrix and enters the circulation during tissue degradation before blood draw. Thus, PYD in the circulation represents collagen breakdown.

Data Analyses

Data were analyzed statistically with SigmaPlot/Systat, v. 11.2 (Systat Software, Inc; San Jose, California). Data were tested for normality with the Shapiro-Wilk normality test as well as for equal variance. Statistical analyses to detect differences between the non-DM and DM groups were performed with the unpaired, two-tailed test (normal data) or the Mann-Whitney rank sum test

(non-normal data). The strengths of associations between variables were determined with the Pearson product moment correlation test. For significant correlations between variables, multiple linear regression analysis was used to test associations after adjusting for potential confounders. Statistical significance for all tests was declared a priori at $p < 0.05$.

RESULTS

Comparison of Variables Between Control Group (non-DM) and Diabetes Group (DM)

All subjects were veterans. The two groups were of similar age, race/ethnicity, and BMI, and all were male (**Table 1**). All subjects were being treated for late-stage knee OA with total joint arthroplasty. The difference in mean preoperative HbA1c was statistically significant ($p = 0.003$). None of the non-DM subjects had ever been diagnosed with diabetes. Nine subjects of the DM group were diagnosed with type 2 DM and one subject with type 1.

To test whether differences existed between groups for levels of AGEs, we measured the biomarker for AGEs (pentosidine) in bone and in cartilage (**Figure 1**). The bone from the DM group had 32 percent higher levels of pentosidine than the bone from the non-DM group ($p = 0.05$). While the DM group appeared to have 21 percent higher levels of cartilage pentosidine than the non-DM group, this difference was not statistically significant ($p = 0.07$). The power of the performed test (0.316) was below the desired power of 0.800, suggesting a lower likelihood of detecting a difference in bone if one actually exists.

We also tested the two other collagen crosslinks (HP and LP). No differences were found between groups for HP level in either bone or cartilage (data not shown). The LP crosslink was not detected in two cartilage samples (previous studies have demonstrated that LP levels are normally very low for cartilage [33]); however, for the samples that did yield detectable levels of LP and also for bone, no differences were found between groups (data not shown).

Correlations Between Biomarkers and Joint Tissue Collagen Crosslinks

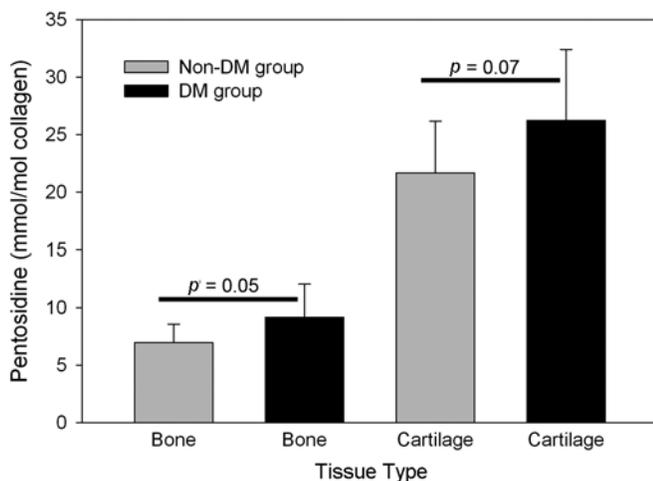
We next analyzed data to determine whether biomarkers and other variables associated with DM (HbA1c), obesity (BMI, leptin), and bone synthesis/turnover (BAP, OCN, and PYD) were correlated to joint tissue collagen

Table 1.Demographics and clinical characteristics of veterans scheduled for total knee arthroplasty. Data presented as % (*n*) unless otherwise noted.

Demographic	Total (N = 20)	Non-DM (n = 10)	DM (n = 10)	<i>p</i> -Value
Age (yr), Mean ± SD	61.4 ± 8.6	59.9 ± 10.3	63.0 ± 6.7	0.43
Sex: Male	100 (20)	100 (10)	100 (10)	
Race/Ethnicity				
African American/Black	10 (2)	10 (1)	10 (1)	
Asian American	0 (0)	0 (0)	0 (0)	
Non-Hispanic Caucasian/White	80 (16)	80 (8)	80 (8)	
Native American	5 (1)	0 (0)	10 (1)	
Hispanic/Latino Puerto Rican	0 (0)	0 (0)	0 (0)	
Hispanic/Latino Mexican	0 (0)	0 (0)	0 (0)	
Hispanic/Latino Other	5 (1)	10 (1)	0 (0)	
BMI (kg/m ²), Median (IQR)	29.41 (27.84–31.69)	28.14 (26.92–30.20)	30.37 (28.98–33.58)	0.054
HbA1c				
Mean ± SD	5.91 ± 1.09	5.25 ± 0.55	6.58 ± 1.10	0.003
Range	4.10–8.60	4.10–5.80	4.90–8.60	

Note: Statistical analysis to detect differences between the non-DM and DM groups were performed for age, BMI, and HbA1c. These data were tested for normality (Shapiro-Wilk normality test) and equal variance. If data passed for normality, mean is reported along with SD and *p*-value using unpaired, two-tailed test. If not, median is reported along with IQR and *p*-value using Mann-Whitney rank sum test.

BMI = body mass index, DM = diabetes mellitus, HbA1c = hemoglobin A1c, IQR = interquartile range (25%–75%), SD = standard deviation.

**Figure 1.**

Pentosidine content of bone and cartilage tissue obtained from total knee arthroplasty. Black bars represent diabetes mellitus (DM) group, and light gray bars represent non-DM group.

crosslink molecules (pentosidine, HP, and LP). The levels of variables were obtained from medical records (HbA1c and BMI), measured by ELISA in blood serum and synovial fluid (leptin, OCN, and PYD) or measured by HPLC (pentosidine, HP, and LP). The strengths of association

between measures (Pearson product moment) are displayed in **Table 2**.

Not surprisingly, the two markers for obesity (BMI and leptin) were strongly correlated to each other regardless of whether leptin was measured in the serum or the synovial fluid. Note, however, that a less strong and non-significant correlation was found when the non-DM group was examined separately (**Table 2**).

Next, correlations between serum and synovial fluid were tested for each of the four biomarkers measured by ELISA in both fluids (**Table 2** and **Figure 2**). A very strong correlation ($r > 0.900$) was found between serum and synovial fluid leptin (**Table 2** and **Figure 2(a)**). This correlation was significant regardless of whether the groups were examined together or separately. For OCN, a strong correlation between the two fluids was found only in the DM group (**Table 2** and **Figure 2(b)**). No correlations were found between the two fluids for either BAP or PYD (**Figure 2(c)** and **(d)**), indicating that these two metabolites are processed differently and that these biomarkers may be appropriate for systemic analysis (i.e., in serum) but not necessarily for local articular joint analysis (i.e., in synovial fluid). One explanation may be that these metabolites pass from the synovial fluid to the greater circulation quite quickly. Another would be the influence of tissue source; for example, a greater contribution from a nonlocal bone

source in the serum sample compared with the local synovium source in the synovial fluid sample [34].

Table 2.

Pearson product correlations between measures. These tests were used to identify relationships between variables without designating which is explanatory and which is response variable.

Factor 1	Factor 2	All Subjects	Non-DM Only	DM Only
BMI	Serum Leptin	$r = 0.750$; $p < 0.001$	$r = 0.580$; $p = 0.08$	$r = 0.841$; $p = 0.002$
BMI	Synovial Fluid Leptin	$r = 0.798$; $p < 0.001$	$r = 0.495$; $p = 0.14$	$r = 0.919$; $p < 0.001$
Serum Leptin	Synovial Fluid Leptin	$r = 0.924$; $p < 0.001$	$r = 0.960$; $p < 0.001$	$r = 0.912$; $p < 0.001$
Serum OCN	Synovial Fluid OCN	$r = 0.425$; $p = 0.07$	$r = 0.080$; $p = 0.82$	$r = 0.735$; $p = 0.02$
Bone Pentosidine	Cartilage Pentosidine	$r = 0.718$; $p < 0.001$	$r = 0.575$; $p = 0.08$	$r = 0.695$; $p = 0.03$
Serum OCN	Cartilage HP	$r = -0.474$; $p = 0.03$	$r = -0.089$; $p = 0.81$	$r = -0.579$; $p = 0.08$
Synovial Fluid OCN	Cartilage HP	$r = -0.566$; $p = 0.01$	$r = -0.835$; $p = 0.003$	$r = -0.502$; $p = 0.17$
Serum BAP	Cartilage HP	$r = -0.398$; $p = 0.08$	$r = 0.472$; $p = 0.17$	$r = -0.643$; $p = 0.04$
Synovial Fluid OCN	Cartilage Pentosidine	$r = -0.309$; $p = 0.20$	$r = 0.112$; $p = 0.76$	$r = -0.835$; $p = 0.005$
Synovial Fluid OCN	Bone Pentosidine	$r = 0.0303$; $p = 0.90$	$r = 0.655$; $p = 0.04$	$r = -0.464$; $p = 0.23$
Serum Leptin	Bone HP	$r = 0.538$; $p = 0.01$	$r = 0.700$; $p = 0.02$	$r = 0.466$; $p = 0.17$
Synovial Fluid Leptin	Bone HP	$r = 0.568$; $p = 0.01$	$r = 0.599$; $p = 0.07$	$r = 0.503$; $p = 0.17$

Note: Pearson product correlation coefficients (r) that were found significant ($p \leq 0.05$) are displayed in bold font.

BMI and HbA1c values were taken from patient medical records. Leptin, OCN, BAP, and PYD were measured by enzyme-linked immunosorbent assay, and pentosidine and HP were measured by high-performance liquid chromatography.

BAP = bone alkaline phosphatase, BMI = body mass index, DM = diabetes mellitus, HbA1c = hemoglobin A1c, HP = hydroxyllysylpyridinium, OCN = osteocalcin, PYD = pyridinium.

Regarding the joint tissue collagen crosslinks, cartilage pentosidine and bone pentosidine were strongly correlated to each other (**Table 2**). Correlations were significant for all subjects together and for the DM group considered alone but did not reach significance for the non-DM group considered alone.

The cartilage HP crosslink was negatively correlated to both serum and synovial fluid OCN, with some variation in strength of correlation depending on whether groups were tested together or separately (**Table 2** and **Figure 3(a)** and **(b)**).

In some tests, the correlations for the non-DM group were opposite those of the DM group. A moderately strong and negative correlation ($r > -0.600$) was found between serum BAP and cartilage HP for the DM group, but not for the non-DM group, which appeared to have a (nonsignificant) positive correlation (**Table 2** and **Figure 4(a)**). The correlation between synovial fluid OCN and cartilage pentosidine was strong and negative for the DM group but was not significant for the non-DM group (**Table 2** and **Figure 4(b)**). Conversely, the correlation between synovial fluid OCN and bone pentosidine was strong and positive for the non-DM group but was not significant for the DM group (**Table 2** and **Figure 4(c)**).

DM and obesity variables correlated with some biomarkers and crosslinks depending on DM status. Both

serum and synovial fluid leptin were correlated to bone HP, but not significant for the DM group tested separately (**Table 2** and **Figure 5(a)** and **(b)**). Also of note, but not reaching significance, was a positive association between HbA1c and serum PYD for the DM group ($r = 0.594$, $p = 0.07$), but no suggestion of a relationship for the non-DM group ($r = 0.350$, $p = 0.32$). An inverse relationship between serum OCN and HbA1c was suggested by data in the DM group only, but this was not significant ($r = -0.466$, $p = 0.17$). The non-DM group suggested an opposite effect ($r = 0.507$, $p = 0.13$). No such relationship was found between synovial fluid OCN and HbA1c (data not shown).

Models for Relationships Between Synovial Fluid Biomarkers and Joint Tissue Collagen Crosslinks in Osteoarthritis

Because local (synovial fluid) levels of some analytes were correlated with pentosidine and/or with HP, we next sought to determine whether these were independent factors by using multiple regression analysis. Since age and BMI are risk factors for knee OA, these factors were included in the models.

As shown in **Table 3**, synovial fluid OCN was an independent factor associated with collagen crosslinks ($p < 0.05$). Model 1 revealed that synovial fluid OCN of the indexed knee was an independent factor for cartilage

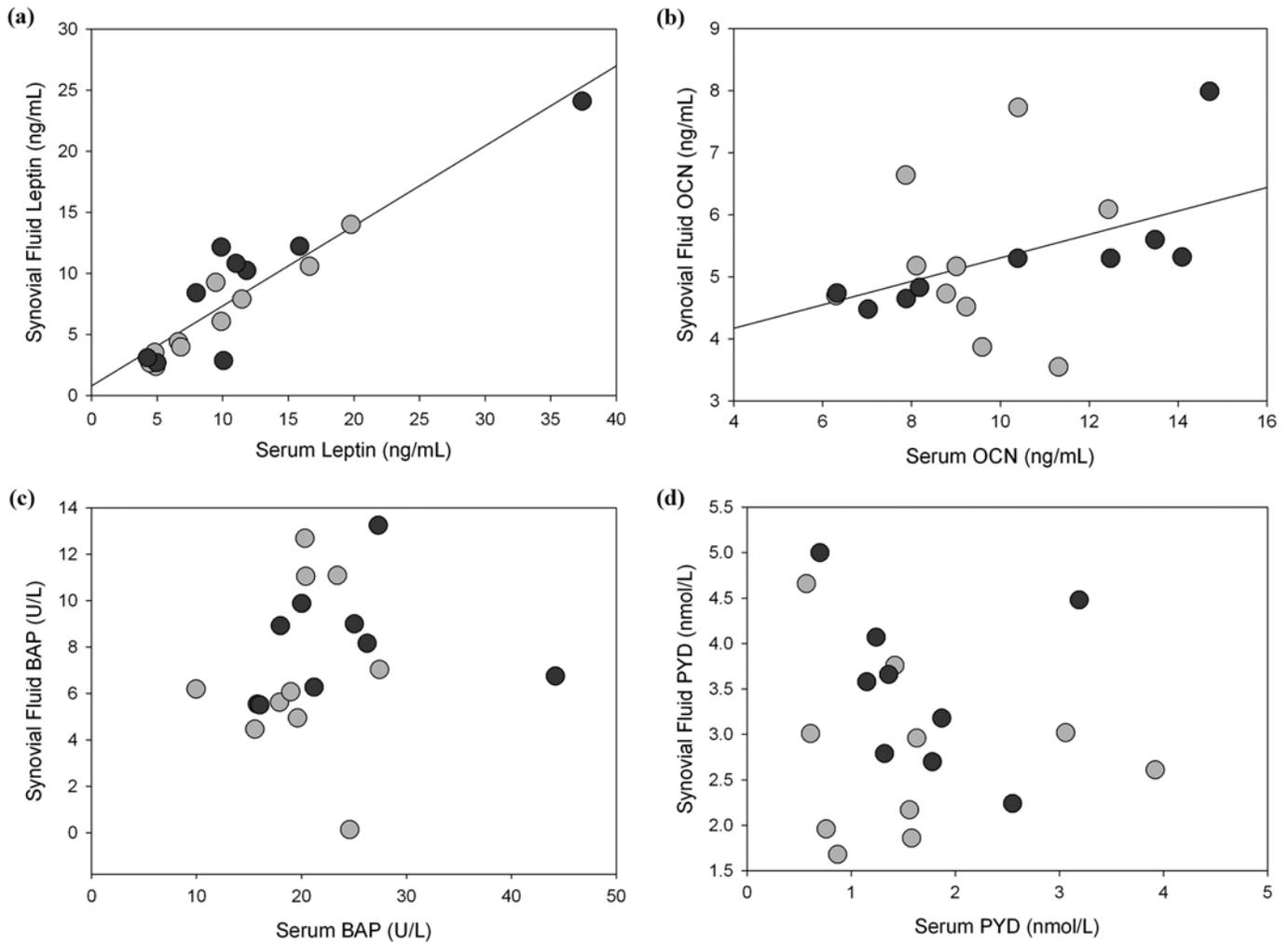


Figure 2.

Correlation, if any, between serum and synovial fluid levels of four biomarkers. (a) Leptin levels in serum were correlated to those in synovial fluid. (b) Osteocalcin (OCN) levels in serum were correlated to those in synovial fluid. (c) Bone alkaline phosphatase (BAP) levels in serum were not correlated to those in synovial fluid. (d) Pyridinium (PYD) levels in serum were not correlated to those in synovial fluid. Black filled circles represent diabetes mellitus (DM) group, and light gray filled circles represent non-DM group. Regression line is indicated with black line.

pentosidine in OA subjects diagnosed with DM. Model 2 revealed that synovial fluid OCN of the indexed knee was an independent factor for cartilage HP regardless of DM status.

The correlation between synovial fluid leptin and bone HP was also tested by regression analysis. However, because of the correlation between leptin and BMI, BMI was not included in the leptin model. Also, because of skewness, data were log transformed before analysis. Model 3 (Table 3) revealed that synovial fluid leptin of the indexed knee was an independent factor of age for subchondral bone HP in OA subjects regardless of DM status.

All other potential models based on findings presented in Table 2 found no significant independent biomarker variables (data not shown). However, some models were underpowered, indicating a lower likelihood of detecting a difference when one actually existed; thus, a lack of significance should be interpreted cautiously.

DISCUSSION

This pilot study examined diabetes, obesity, and cartilage/bone metabolism variables and uncovered interac-

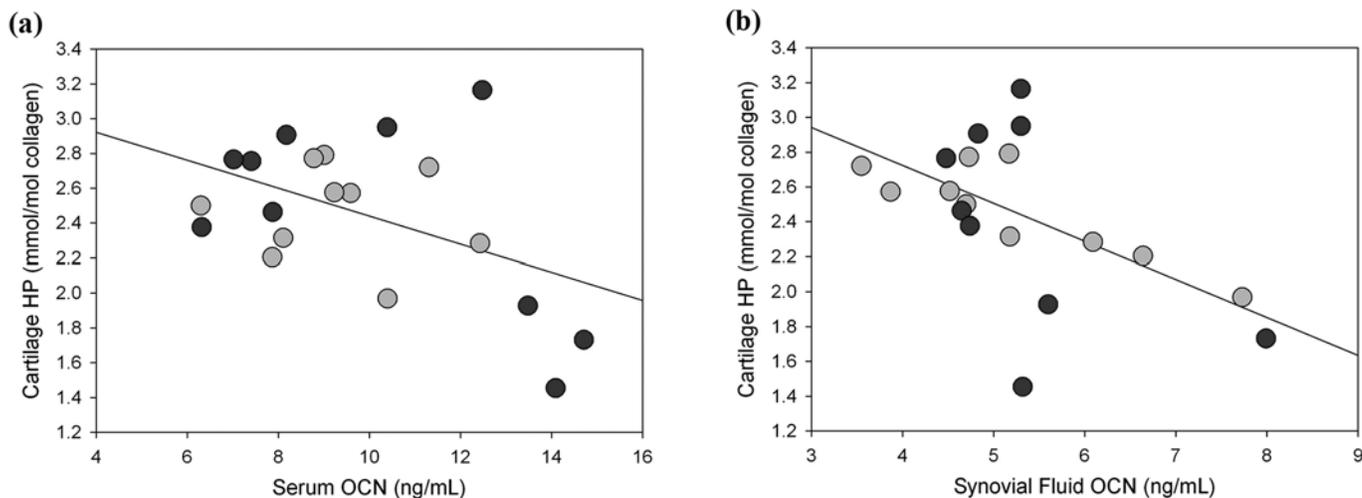


Figure 3.

Correlations between osteocalcin (OCN) and cartilage hydroxylysylpyridinoline (HP). (a) OCN levels in serum were correlated to cartilage HP. (b) OCN levels in synovial fluid were correlated to cartilage HP. Black filled circles represent diabetes mellitus (DM) group, and light gray filled circles represent non-DM group. Regression line is indicated with black line.

tions relevant to bone and joint health that are different in an OA population based on DM status, as well as interactions that are similar in an OA population regardless of DM diagnosis.

The main difference between the DM and non-DM groups is the level of the AGE, pentosidine, in joint tissues. This difference is not surprising, considering high levels of AGEs have been demonstrated in other tissues of DM subjects and animal models of DM [35–37]. Higher levels of other AGEs would be a problem in OA because they lead to increased inflammation due to signaling via the receptor for AGE (RAGE) [38]. Although pentosidine is not itself a ligand for RAGE, when pentosidine is increased in cartilage or bone, tissue brittleness increases and tissue function decreases [13,16]. That DM OA subjects differ from non-DM OA subjects in regard to pentosidine is further supported by Model 1, in which the level of OCN in the synovial fluid of the OA knee is inversely correlated to pentosidine levels in the cartilage of the same knee (after adjusting for age and BMI), but only in DM subjects.

OCN is produced by mature osteoblasts and osteocytes as well as by chondrocytes in OA cartilage and is considered a biomarker for bone formation [22,39–40]. A recent perspective article from Clemens and Karsenty presents the hypothesis that higher OCN is associated with improved glucose metabolism, including both increased insulin secretion from the pancreas and

increased insulin sensitivity in the peripheral tissues, and that leptin plays a role as a negative regulator of this mechanism [41]. In several human studies of the effect of DM on osteoporosis, a disease of pathological bone metabolism, serum OCN is negatively correlated to HbA1c and other indicators of glucose metabolism [42–45]. Our study also finds this inverse correlation between serum OCN and HbA1c. However, multiple regression analysis (adjusting for age and BMI) finds no relationship, though one should note that this model was underpowered.

Interestingly, a different collagen crosslink, HP, is also inversely correlated to synovial fluid OCN. HP is considered a “mature” crosslink since it forms an estimated 7 to 30 days after collagen synthesis [46]. However, this relationship (Model 2) applies regardless of DM status and may represent HP loss with increasing OCN in OA. Although decreased HP is undesired, this effect (β coefficient of Model 2) is much lower than the OCN effect for pentosidine (β coefficient of Model 1). Bone HP appears to be influenced by synovial fluid leptin (Model 3). This model should be interpreted cautiously. BMI is highly correlated to leptin, and age was a “near” significant factor in this slightly underpowered model. However, the β coefficient for age is extremely low compared with leptin; thus, even if significant, age would have a very low impact on bone HP. If HP concentration is normalized to total collagen concentration (as is standard for HPLC measurement

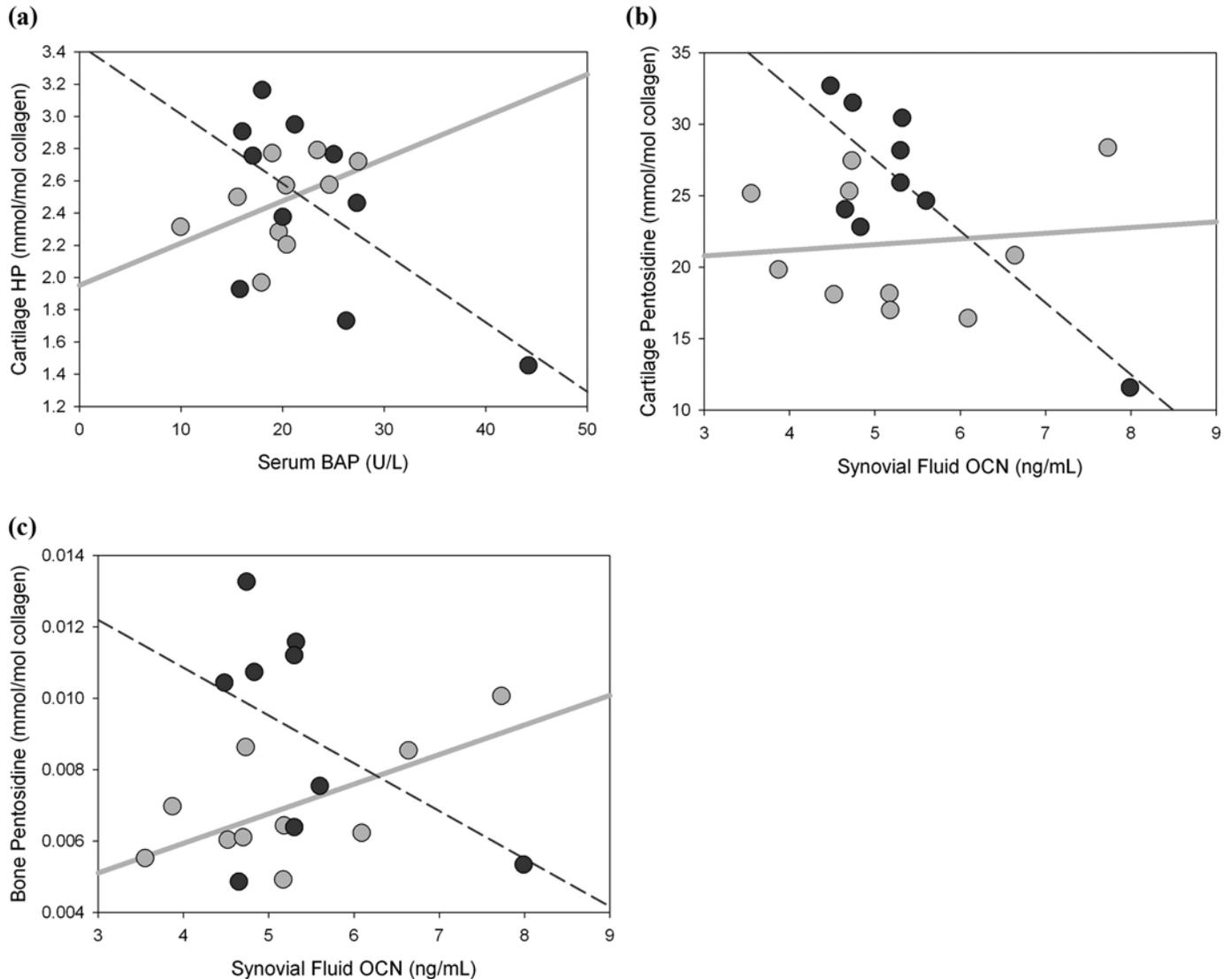


Figure 4.

Correlations between biomarkers and collagen crosslinks that differed between diabetes mellitus (DM) and non-DM groups. **(a)** Bone alkaline phosphatase (BAP) levels in serum were positively correlated to cartilage hydroxylslypyridinoline (HP) in non-DM group, but negative in DM group. **(b)** Osteocalcin (OCN) levels in synovial fluid were not correlated to cartilage pentosidine in non-DM group, but were negatively correlated to cartilage pentosidine in DM group. **(c)** OCN levels in synovial fluid were not correlated to bone pentosidine in non-DM group, but were negatively correlated to bone pentosidine in DM group. Black filled circles represent DM group, and light gray filled circles represent non-DM group. Regression lines are indicated with dashed black line for DM group and solid gray line for non-DM group.

of HP), then periods of higher collagen turnover, as seen in OA, may decrease overall HP concentration. All subjects of this study experienced late OA in the indexed knee; thus, the inverse relationship between the bone biomarker OCN and cartilage HP seems plausible. However, unlike in cartilage, this relationship was not statistically significant for bone HP. This lack of sensitivity is primarily due

to the very low amounts of HP in bone compared with cartilage (data not shown, first reported by Eyre et al. [32]).

Overall, our data are consistent with the hypothesis that higher OCN is correlated to better health, even in peripheral tissues. Other indirect support comes from a study by Berry et al., in which OCN is negatively correlated to OA cartilage loss as measured by magnetic resonance imaging [47]. In this and other studies, however,

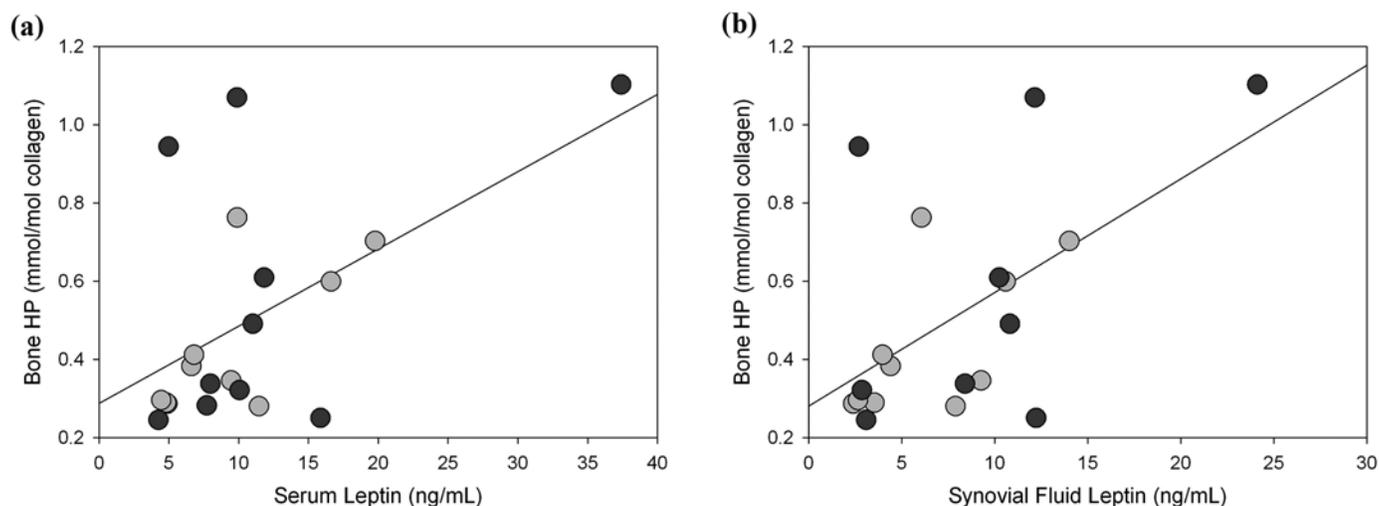


Figure 5.

Correlations between leptin and bone hydroxylysylpyridinoline (HP). (a) Leptin levels in serum were correlated to bone HP. (b) Leptin levels in synovial fluid were correlated to bone HP. Black filled circles represent diabetes mellitus (DM) group, and light gray filled circles represent non-DM group. Regression line is indicated with black line.

DM subjects may or may not have been excluded, but their numbers in the study population and the effect of DM status on outcome measures have not been reported

Table 3.

Multiple linear regression models for collagen crosslinks. These tests were used to verify association between single independent or explanatory variable (OCN, leptin) and response or dependent variable (pentosidine, HP), when controlling for other explanatory variables (age, BMI).

Dependent Variable	Independent Variables	Coefficient (β)	Standard Error	<i>p</i> -Value
Model 1: <i>n</i> = 9 DM				
Cartilage Pentosidine	Age	0.122	0.217	0.60
	BMI	-0.522	0.290	0.13
	OCN (synovial fluid)	-4.750	1.090	0.007
Model 2: <i>n</i> = 19 non-DM and DM				
Cartilage HP	Age	0.00395	0.0143	0.79
	BMI	0.0153	0.0258	0.56
	OCN (synovial fluid)	-0.232	0.0989	0.03
Model 3: <i>n</i> = 19 non-DM and DM				
log Bone HP	Age	-0.00972	0.00538	0.09
	log Leptin (synovial fluid)	0.315	0.147	0.048

Note: Pentosidine and HP were measured by high-performance liquid chromatography. Age and BMI values were taken from patient medical records. Synovial fluid OCN, BAP, and leptin were measured by enzyme-linked immunosorbent assay.

These data were tested for normality (Shapiro-Wilk) and for constant variance.

For model 1, adjusted $R^2 = 0.742$, power of performed test with $\alpha = 0.050$: 0.969.

For model 2, adjusted $R^2 = 0.203$, power of performed test with $\alpha = 0.050$: 0.755.

For model 3, adjusted $R^2 = 0.279$, power of performed test with $\alpha = 0.050$: 0.790.

BMI = body mass index, DM = diabetes mellitus, HP = hydroxylysylpyridinium, OCN = osteocalcin.

first study to specifically examine and propose effects of DM on cartilage pentosidine involving OCN. We propose that OCN is correlated to improved molecular function in

[47–52]. This may explain why little consensus exists as to whether OCN is positively, negatively, or not correlated to OA presence or severity. To our knowledge, ours is the

DM joint tissues but that the exact mechanisms are not fully clear at this time.

In the OCN-glucose metabolism hypothesis [41], leptin is proposed as a negative factor. In bone formation,

as leptin increases, OCN production (tied to osteoblast function) decreases. Leptin metabolism, coincidentally, is also cautiously proposed by others as affecting OA [53]. High leptin levels as found in obesity are proposed to have hormonal effects in the development and/or progression of OA [54–55]. In our study, Model 3 shows local (i.e., synovial fluid) levels of leptin to be a significant factor in subchondral bone HP levels, with age as a potential minor but nonsignificant factor. This suggests that leptin is a positive factor for bone collagen function regardless of DM status, although these data cannot determine a cause-and-effect relationship, particularly since BMI did not significantly differ between groups. Leptin effects on human bone have shown both an osteogenic role and an antiosteogenic role [56–59]. Nonetheless, the majority of these studies have been performed only in women and further study needs to be done to understand fully the relationship between leptin and bone and cartilage. In agreement with other studies [60–61], we find no relationship between these biomarkers in either fluid (data not shown).

The goal of this study was to examine the effect of DM on cartilage and bone metabolism in order to form hypotheses regarding DM-OA and regarding arthroplasty outcomes for those with DM. Patients with comorbid DM appear to receive total joint replacement at a higher rate than non-DM patients, and DM patients aged 46 to 55 have arthroplasty and revision arthroplasty at nearly double the rate of non-DM patients [62]. Our study demonstrates the biomarker for AGEs, pentosidine, is expressed in higher levels in the bone and cartilage of DM patients. The way in which AGEs affect bone and joint metabolism is not entirely clear. We propose that in DM-OA cartilage, AGEs increase inflammation, leading to greater and/or faster tissue degeneration, while greater pentosidine crosslinking leads to brittle articular cartilage that poorly absorbs physiological loading. Since AGEs may decrease osteoblast function of bone formation [63–67], we further propose that following arthroplasty, DM-OA bone is less capable of the bone remodeling and bone formation required for osseointegration of the prosthesis. Two mechanisms that would hinder osseointegration are (1) excessive pentosidine crosslinking that would inhibit collagen degradation needed for remodeling [68–69], and (2) hyperglycemia-mediated decrease in osteoblast cell differentiation and function [66,70]. Impair-

ment of these processes could ultimately lead to aseptic loosening requiring revision surgery.

There are limitations to our study. We have only examined patients enrolled for surgery to treat severe OA pain and disability. This study has examined the joint tissues from a specific population set that was similar in race/ethnicity distribution in the U.S. veteran population, which in the 2001 National Survey of Veterans is reported as 84.8 percent White, 8.8 percent Black or African American, and fewer percentage other races and as 4.5 percent Spanish, Hispanic, or Latino ethnicity [71]. Therefore, our findings can only be directly extrapolated to this specific population. The small sample size decreases the power of this study, and the single sex of this cohort may further limit the generalization of these findings. The DM subjects of this study have largely well-controlled glucose levels (mean HbA1c of 6.6), and the BMI of this group is not significantly different from controls. Larger differences are likely to be seen in the more general population of DM patients, as several studies have identified significant differences in arthroplasty outcomes in DM patients with poorly controlled glucose [2–3,8,72]. Another limitation is that although all blood draws were in the morning, they did not follow subject fasting, which has recently become the standard for clinical studies of biomarkers. The biomarker levels in synovial fluid have been normalized to volume rather than another variable such as total protein; this may have increased variability across subjects. Also, our measurement of biomarkers at a single time point may not accurately reflect ongoing tissue metabolism, which likely had been also influenced by repair processes occurring in late OA.

Finally, we would like to note that, at least anecdotally, the effect of DM status appears more strongly related to one's diagnosis rather than one's present HbA1c level. One DM subject in our study brought his HbA1c down to the normal range before surgery. However, the pentosidine values for both his cartilage and bone are in the range of the DM group, not the non-DM group. Considering the long half-life of fibrillar collagens, including type II (cartilage) and type I (bone), the persistence of glycemic effects on collagen crosslinks is reasonable. An alternative explanation would be the presence of "metabolic memory" as described by Ceriello et al. [73–74]. Metabolic memory suggests that DM complications persist even after glycemic control is regained. This persistence is demonstrated in a DM zebrafish study of bone (fin) regeneration where 2

weeks or more of streptozotocin-induced hyperglycemia followed by pancreatic regeneration (and return of normoglycemia) still results in impaired bone repair [75]. An analogous metabolic memory effect on human bone repair would have implications for osseointegration following arthroplasty even in those patients who have successfully regained healthy HbA1c levels.

CONCLUSIONS

Ours is the first study to consider recent hypotheses in the understanding of glucose metabolism as potentially relevant to arthroplasty success in OA patients with diabetes. The present study demonstrates an inverse relationship between the levels of local synovial fluid OCN and the levels of pentosidine/AGEs in OA joint tissues. The implications of these findings are that impaired bone healing in those with diabetes should be further studied at the molecular and tissue levels so that hypotheses regarding potential interventions may be tested. Furthermore, the detrimental effects of pentosidine and other AGEs to bone repair may indicate that control of glycemia before surgery would be beneficial not only to increase perioperative safety but also to avoid impairment of bone remodeling following arthroplasty. Clinicians should be aware that with regard to bone healing, current blood glucose levels may be less important than long-term glyce-mic control.

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